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| 1<br>2   | Recommendations for measuring whisker movements and locomotion in mice with sensory, motor and cognitive deficits.   |  |  |  |  |  |
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| 22   |  |  |  |  |  |  |
| 23   | Highlights   |  |  |  |  |  |
| 24   | <ul> <li>Mice move their whiskers during tactile exploration.</li> </ul>   |  |  |  |  |  |
| 25   | <ul> <li>We measure whisker movements and locomotion in 9 mouse strains.</li> </ul>  |  |  |  |  |  |
| 26   | <ul> <li>Genotype, background strain, sex and source breeder affected whisker movements.</li> </ul>  |  |  |  |  |  |
| 27   | • 8 of the 9 models showed differences in whisker movements, compared to controls.   |  |  |  |  |  |
| 28   | <ul> <li>We recommend a standardized protocol to measure whisker movements.</li> </ul>   |  |  |  |  |  |
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## 38 Abstract

- 39 **Background :** Previous studies have measured whisker movements and locomotion to characterise
- 40 mouse models of neurodegenerative disease. However, these studies have always been completed
- 41 in isolation, and do not involve standardized procedures for comparisons across multiple mouse
- 42 models and background strains.
- New Method : We present a standard method for conducting whisker movement and locomotion
   studies, by carrying out qualitative scoring and quantitative measurement of whisker movements
   from high-speed video footage of mouse models of Amyotrophic Lateral Sclerosis, Huntington's
- 46 disease, Parkinson's disease, Alzheimer's disease, Cerebellar Ataxia, Somatosensory Cortex
- 47 Development and Ischemic stroke.
- 48 **Results :** Sex, background strain, source breeder and genotype all affected whisker movements. All
- 49 mouse models, apart from Parkinson's disease, revealed differences in whisker movements during
- 50 locomotion. R6/2 CAG250 Huntington's disease mice had the strongest behavioural phenotype.
- 51 Robo3R<sup>3-5</sup>-CKO and RIM-DKOSert mouse models have abnormal somatosensory cortex development
- 52 and revealed significant changes in whisker movements during object exploration.
- 53 **Comparison with Existing Method(s) :** Our results have good agreement with past studies, which
- 54 indicates the robustness and reliability of measuring whisking. We recommend that differences in
- 55 whisker movements of mice with motor deficits can be captured in open field arenas, but that mice
- 56 with impairments to sensory or cognitive functioning should also be filmed investigating objects.
- 57 Scoring clips qualitatively before tracking will help to structure later analyses.
- 58 **Conclusions:** Studying whisker movements provides a quantitative measure of sensing, motor
- 59 control and exploration. However, the effect of background strain, sex and age on whisker
- 60 movements needs to be better understood.
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  Keywords: rodent, sensorimotor, barrel cortex, animal behaviour, neurodegeneration, disease models, transgenic, mouse models
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### 72 Introduction

73 Studies on laboratory mice (Mus musculus) have contributed significantly to our understanding of 74 human biology and health (Fox et al., 2006; Morse, 2007; Perlman, 2016), and mouse models are a 75 powerful tool for research in biomedical science. This is mainly due to their genetic similarities to 76 humans (Waterston et al., 2002), the ability to create transgenic, knock-out, and knock-in varieties, 77 as well as the ease and relatively low expense of keeping and breeding them (Burns et al., 2015). 78 However, the use of mice in biomedical research needs to take account of, and perhaps even 79 advantage of, the differences between mice and humans (Perlman, 2016). Modern rodents and 80 primates are thought to have diverged from a common ancestor around 85 million years ago 81 (Springer and Murphy, 2007; Perlman, 2016). It is not, therefore, surprising that there are many 82 genetic, physiological and behavioural differences between humans and mice. One of the striking 83 features of mice is their prominent facial whiskers, or vibrissae. Whisker-brain connections are 84 regularly studied by neuroscientists as a model system for sensory processing (Diamond et al., 2008; 85 Kleinfeld et al., 2006). Indeed, the arrangement of whiskers on each side of a mouse's face are 86 mimicked in physical topographic structures that can be seen throughout the brain in brainstem, 87 thalamus and layer IV of somatosensory (Barrel) cortex (Waite, 1995; Petersen, 2007; Feldmeyer et 88 al., 2013). Sensory processing of whisker signals in the cortex is likely to be involved in 89 environmental exploration and object discrimination tasks (Kleinfeld et al., 2006; Grant et al., 2012; 90 Hong et al., 2018).

91 As well as being an important tactile sensory system, whiskers also move. Indeed, mice can move 92 their whiskers up to 25 times per second, in a process called whisking, which is amongst the fastest 93 movements that mammals can make (Mitchinson et al., 2011). In addition to making simple, cyclic 94 movements, mice can also alter the timing, spacing and positioning of their whiskers to maximise 95 sensory information (Carvell and Simons, 1990; Grant et al., 2009; Mitchinson et al., 2007; 2011). For 96 example, when contacting an object, rodents reliably and robustly reduce the spread of their 97 whiskers, to bunch them up, increasing the number of whiskers that contact the object (Grant et al., 98 2009). Whisker movements also show contact-induced asymmetry, as the whiskers contralateral to 99 the contact are positioned more forward to increase whisker contact, and the whiskers ipsilateral to 100 the contact are positioned more backward to enable light touches against the surface (Mitchinson et 101 al., 2007; 2011). Therefore, as well as a model of sensory processing, whiskers are also a good 102 system for the study of motor control and exploratory behaviours. Moreover, the positioning and 103 orienting of whiskers have been linked to the focussing of attention (Arkley et al., 2014; Huet et al., 104 2014; Mitchinson and Prescott, 2013; Towal and Hartmann, 2006), which suggests that the study of 105 whisker movement might be used to measure cognitive function.

106 As whisker movements of mice are precisely controlled and can be accurately measured, they can 107 provide quantitative behavioural measures of sensory processing, motor control, exploration and, 108 possibly cognition. Indeed, changes in whisker movements have been observed in mouse models of 109 motor disorders, including Amyotrophic Lateral Sclerosis (Grant et al., 2014) and Huntington's 110 disease (Garland et al., 2018) during filming in a simple, open field arena. Moreover, whisker movement analysis revealed a behavioural phenotype in Huntington's disease R6/2 CAG250 and Hdh 111 112 CAG250 knock-in mice at an earlier age than any other behavioural measure (Garland et al., 2018). 113 Whisker movements are also altered in mouse models of anxiety (Grant et al., 2016) and Alzheimer's 114 disease (Grant et al., 2018). Grant et al. (2018) utilised three tests for studying whisker movements 115 in an Alzheimer's disease mouse model: object exploration, sequential object exploration and 116 tunnel-running; however, only the object exploration task revealed significant differences in whisker movements in the transgenic (5xFAD) mice. Garland et al. (2018) compared whisker movements 117

- among three different mouse models of Huntington's disease only in open field arenas. However, it
- 119 is not clear whether experimental approaches should differ between mouse models of motor
- 120 disorders, compared to disorders of sensory processing or cognitive functioning. Indeed, so far,
- 121 studies of whisker movements have been conducted in isolation, and there have been no formal
- 122 comparisons or recommendations for measuring whisker movements in different mouse models.
- 123 This paper will present a standard procedure for conducting whisker movement studies for the first
- 124 time, by carrying out both qualitative scoring and quantitative measurement of high-speed video
- 125 footage of whisker movements. We provide data on locomotion speeds and whisker movements in
- selected mouse models with motor disorders: Amyotrophic Lateral Sclerosis (SOD1<sup>G93A</sup>),
- 127 Huntington's disease (R/62 CAG250), Parkinson's disease (SNCA-OVX); Ischemic Stroke (MCAO, a
- sensory and motor model); Sensory alterations in somatosensory cortical map development: RIM DKO Sert, Robo3R<sup>3-5</sup>-cKO; Cerebellar Ataxia (Reeler B6c3Fe, which also have altered sensory cortica)
- 129 DKO Sert, Robo3R<sup>3-5</sup>-cKO; Cerebellar Ataxia (Reeler B6c3Fe, which also have altered sensory cortical 130 map development); and Cognitive functioning: Alzheimer's disease (3xTg-AD, 5xFAD). We measured
- 131 whisker movements of mice in open-field environments for all mouse models, and investigated
- 132 object exploration in a sub-set of models. After presenting our findings, we make recommendations
- for conducting standardized whisker movement studies in the future, in order to ensure robust
- 134 comparisons between mouse models.
- 135

# 136 Methods

- 137 Mouse models
- 138 Whisker movement and locomotion speed data are presented from nine mouse models, including
- 139 Amyotrophic Lateral Sclerosis (SOD1<sup>G93A</sup>), Huntington's disease (R/62 CAG250), Parkinson's disease
- 140 (SNCA-OVX), Stroke (distal middle cerebral artery occlusion (MCAO)), Cerebellar Ataxia (Reeler
- 141 B6C3Fe), Cortical Development Disorders (RIM-DKO Sert, Robo3R<sup>3-5</sup>-cKO) and Alzheimer's disease
- 142 (3xTg-AD, 5xFAD). Data were collected from laboratories around the world (University of Sheffield,
- 143 University of Cambridge, University of Oxford, University of Nottingham, University of Goettingen,
- 144 INSERM, Dalhousie University, respectively). Details of genetic lines and controls can be found in the
- references in the first column of Table 1, husbandry information for the individual mice can be found
- 146 in Appendix 1 and a description of the MCAO surgery can be found in Appendix 2. All animals had
- 147 food and water *ad libitum* (apart from the MCAO mice, see Appendix 1 for more details).
- 148 Mice were removed from their home cage for 5-10 minutes, placed in a custom test apparatus for 149 filming and then returned to their home cage. Each mouse was tested one to three times in total,
- 150 but only once per day. All procedures were approved by ethical committees at the local institutions
- 151 (University of Sheffield, University of Cambridge, University of Oxford, University of Nottingham,
- 152 University of Goettingen, INSERM, University of Dalhousie, Manchester Metropolitan University),
- and carried out in accordance with regulations issued by the UK Home Office (at UK institutions), EU
- 154 (at University of Goettingen and INSERM) and the Canadian Council of Animal Care (at University of
- 155 Dalhousie).
- 156 Animals were selected when they were likely to show strong behavioural phenotypes, these were
- 157 late stage in the disease process in transgenic mice and soon (2 weeks) after the middle cerebral
- 158 artery occlusion (MCAO) surgery in stroke mice. In stroke, we also included a later date from surgery
- 159 (1 month) when most deficits have usually recovered. Across labs, there were preferences for
- 160 studying different genders, depending on ease of handling and disease progression. The genders
- 161 tested can be seen in Table 1. Where possible, both males and females were tested, but this was

- 162 only possible for R6/2 CAG250 and Rim-DKOSert models, and their controls. All transgenic animals
- 163 were compared to age-matched non-transgenic controls, apart from MCAO mice which were
- 164 compared to sham controls (see Kuraoka et al., (2009) for more details), and SNCA-OVX mice which
- 165 were compared to  $\alpha$ -synuclein null ( $\alpha$ -syn null) mice (see Janezic et al. (2013) for more details). The
- 166 number of mice, and hence number of clips, varied between models due to the availiability of the
- animals. This may have impacted some of the results when sample sizes were smaller, such as for
   the RIM-DKO Sert, MCAO 2-week sham and 5xFAD mice. Sample sizes can be observed in Table 1.
- the kine-bko sett, meho z-week shall and ski kb linee. Sample sizes can be observed in Table 1.

169 Each mouse model was coded as to whether it would be likely to reveal sensory, motor or cognitive

- symptoms (for further evidence, refer to the discussion section). Considering these likely symptoms,
- the mice were tested to examine their whisker movements during locomotion (for all mouse
- models), and during object exploration (for Reeler B6C3Fe, RIM-DKOSert, Robo3R<sup>3-5</sup>-cKO, 3xTg-AD,
   5xFAD).
- 173 174
- 175 **Table 1: Information about mouse models, controls and sample sizes**. The mouse column contains
- 176 the mouse model name, the reference containing genetic line information, and then underneath the
- 177 background strain in italics. Source breeders are indicated in square brackets: J: Jackson, JJ: Janvier,
- and CR: Charles River. Transgenic: Tg, Non-transgenic: NTg, α-synuclein null: α-syn null, Middle
- 179 cerebral artery occlusion (Stroke surgery): MCAO, Sham stroke surgery: Sham, Horizontal gaze palsy
- 180 with progressive scoliosis: HGPPS, female: f, male: m Mouse models were coded for likely sensory,
- 181 motor or cognitive symptoms, evidence for which is presented more in the discussion.

| Mouse  | Model system                                       | Symptoms                       | Sample size<br>and gender              | Age                         | No. clips<br>collected | No. Clips<br>(locomotion) | No. Clips<br>(whisker<br>movements) |
|--|--|--------------------------------|--|-----------------------------|------------------------|---------------------------|-------------------------------------|
| <b>SOD1<sup>G93A</sup></b><br>(Mead et al., 2011)<br><i>C57BL/6J</i> [J]               | Amyotrophic<br>Lateral<br>Sclerosis                | Motor                          | 7 Tg f<br>8 NTg f                      | 120 days                    | 133                    | 81 Tg<br>44 NTg           | 76 Tg<br>45 NTg                     |
| <b>R6/2 CAG250</b><br>(Morton et al., 2009)<br><i>C57BL/6J x CBA</i> [J]               | Huntington's<br>disease                            | Motor,<br>Cognitive,<br>Social | 8 Tg m<br>8 Tg f<br>7 NTg m<br>8 NTg f | 16-20<br>weeks              | 522                    | 106 Tg<br>104 NTg         | 123 Tg<br>107 NTg                   |
| <b>SNCA-OVX</b><br>(Janezic et al., 2013)<br><i>C57BL/6 [CR]</i>                       | Familial and<br>sporadic<br>Parkinson's<br>disease | Motor                          | 9 Tg f<br>6 α-syn null<br>f            | 18-19.5<br>months           | 150                    | 71 Tg<br>47 NTg           | 63 Tg<br>40 NTg                     |
| MCAO 1 month<br>(Kuraoka et al., 2009;<br>Appendix 2)<br><i>C57BL/6J</i> [ <i>CR</i> ] | Ischemic stroke                                    | Sensory,<br>Motor              | 12 MCAO m<br>12 Sham m                 | 1 month<br>post-op<br>Adult | 268                    | 129 MCAO<br>127 Sham      | 120 MCAO<br>123 Sham                |
| MCAO 2 weeks<br>(Kuraoka et al., 2009;<br>Appendix 2)<br>C57BL/6J [CR]                 | Ischemic stroke                                    | Sensory,<br>Motor              | 5 MCAO m<br>2 Sham m                   | 2 weeks<br>post-op<br>Adult | 96                     | 50 MCAO<br>25 Sham        | 52 MCAO<br>23 Sham                  |

| <b>Reeler (B6C3Fe)</b><br>(Falconer, 1951; Guy<br>et al., 2014)<br><i>B6C3Fe [J]</i>                    | Cerebellar<br>Ataxia,<br>Somatosensory<br>Cortex<br>Development | Sensory,<br>Motor               | 7 Tg m<br>6 NTg m                      | 7-9<br>months  | 75  | 84 Tg<br>72 NTg   | 56 Tg<br>61 NTg  |
|---|---|---------------------------------|--|----------------|-----|-------------------|------------------|
| <b>RIM-DKO Sert</b><br>(Narboux-Nême et al.,<br>2012)<br>129Sv x C57BL/6 [J]                            | Somatosensory<br>Cortex<br>Development                          | Sensory                         | 3 Tg m<br>2 Tg f<br>3 NTg m<br>2 NTg f | 6-9<br>months  | 59  | 29 Tg<br>30 NTg   | 27 Tg<br>30 NTg  |
| <b>Robo3R<sup>3-5</sup>-cKO</b><br>(Renier et al., 2010)<br><i>C57BL/6 [JJ]</i>                         | Somatosensory<br>Cortex<br>Development,<br>HGPPS                | Sensory,<br>Motor               | 6 Tg m<br>6 NTg m                      | 1-5<br>months  | 228 | 109 Tg<br>112 NTg | 61 Tg<br>41 NTg  |
| <b>3xTg-AD</b><br>(Stover et al., 2015)<br><i>B6129SF2</i> [J]  | Familial<br>Alzheimer's<br>disease                              | Motor,<br>Cognitive             | 8 Tg f<br>6 NTg f                      | 17 months      | 222 | 53 Tg<br>52 NTg   | 53 Tg<br>133 NTg |
| <b>5xFAD</b><br>(Oakley et al., 2006;<br>O'Leary et al., 2017;<br>2018b)<br><i>C57BL/6J x SJL/J [J]</i> | Familial<br>Alzheimer's<br>disease                              | Sensory,<br>Motor,<br>Cognitive | 3 Tg f<br>4 NTg f                      | 12.5<br>months | 80  | 24 Tg<br>26 NTg   | 29 Tg<br>30 NTg  |

### 183 Experimental Procedures

184 The procedure for testing was identical in each laboratory, using a custom portable set-up (Grant et 185 al., 2014). Mice were placed in a transparent, Perspex, rectangular arena (20 x 30 x 15 cm), which 186 was lit from below by a bright, infra-red light box (either custom made, or LEDW-BL-400/200-SLLUB-187 Q-1R-24V, PHLOX) (Figure 1a). Mice were filmed from above using a digital high-speed video camera 188 (Phantom Miro ex2, or Photron Fastcam) recording at 500 frames per second with a shutter-speed 189 of 1 ms (and resolution of 640x480 or 1024x1024 pixels, respectively) (Figure 1a). In most cases, the 190 arena was an open field, with no objects present. However, for models likely to show sensory or 191 cognitive effects (Reeler B6C3Fe, RIM-DKOSert, Robo3R<sup>3-5</sup>-cKO, 3xTg-AD, 5xFAD) an object (either a 192 Perspex cube or small yo-yo) was also placed within the arena to promote exploration. Multiple 193 video clips were collected opportunistically (by manual trigger) when the animal moved into the 194 camera's field of view. Each clip was around one second long. 6-16 clips were collected from each 195 animal over a 5-10 minute period (see Table 1 for the number of clips collected). All clips were 196 selected and trimmed to a portion of footage based on selection criteria developed by Grant et al., 197 (2014). These criteria were: i) the mouse was clearly in frame; ii) both sides of the face were visible; 198 and iii) the head was level with the floor (no extreme pitch or yaw). While data was collected from 199 multiple labs, all videos were analysed and statistics conducted by only one laboratory (at 200 Manchester Metropolitan University), to ensure consistency. Instructions for the experimental 201 procedures, as well as the subsequent video analyses can be found in Appendix 3.

### 202 Qualitative Whisker Scores

203 To assess the general whisker movements and exploratory strategies, clips of just the transgenic and

- 204 MCAO animals were scored based on a system developed by Grant et al. (2012; 2017, Appendix 3).
- All the transgenic and MCAO animals were scored for whisking in each included clip. In particular if

- 206 the animal was not whisking (0), doing only retractions (1), only protractions (2) or retractions and
- 207 protractions (3). When an object was present, for Reeler B6C3Fe, RIM-DKOSert, Robo3R<sup>3-5</sup>-cKO,
- 208 3xTg-AD, 5xFAD mice, clips where the mouse had contacted the block were also scored for contact-
- 209 induced asymmetry (CIA) and spread reduction. Contact-induced asymmetry (CIA) was given a score
- for being absent (0), increased contralateral protraction (1), reduced ipsilateral protraction (2) and
- both increased contralateral protraction and reduced ipsilateral protraction (3). Spread reduction
- was scored as absent (0) or present (1), when the whisker spread decreased following a contact. We
- also scored whether head turning asymmetry (Mitchinson et al., 2011; Towal and Hartmann, 2006)
  and look-ahead behaviours (Arkley et al., 2014) were present (1) or absent (0); however, these
- 215 behaviours were not very common and there were no significant differences observed, so they were
- 216 removed from further analyses.

# 217 Quantitative analysis of locomotion and whisker movements

218 For guantitative analysis of whisker movements and locomotion, clips were further selected, based 219 on two more criteria (developed in Grant et al., 2014): i) the whiskers were not in contact with a 220 vertical wall; and ii) the mouse was clearly moving forward. We wanted to assess here how the 221 whiskers move during forward locomotion without any object contact, as whisker movements are 222 significantly controlled and altered during object exploration. In each included clip, the mouse was 223 tracked using the Automated Rodent Tracker (ART) (Hewitt et al., 2018). This used image analysis to 224 locate the mouse nose tip and centroid, to calculate locomotion speed (from the yellow trace in 225 Figure 1b). A ruler was filmed at the start of each episode of data collection to enable a calibrated 226 measure of locomotion speed in metres per second. The ART whisker detector was validated by 227 manually inspecting the overlaid video footage to check the position of the nose tip and centroid 228 point (Figure 1b). The number of videos included in the locomotion analysis can be seen in Table 1, 229 and was a total of 1397 clips.

230 The snout and whiskers of the mouse were detected using ARTv2 (the Automated Rodent Tracker, 231 version2), as part of the LocoWhisk software package (Gillespie et al., 2019). This is the first time 232 that this software has been demonstrated on a dataset. The whisker detector automatically found 233 the orientation and position of the snout, and the whisker angles (relative to the midline of the 234 head) of each identified whisker (Figure 1b). ARTv2 is only a whisker detector, therefore, it does not 235 maintain the identity of the whisker between frames (i.e. tracking); rather, in all whiskers that are 236 detected a mean angle is approximated from each frame. Whisker angles refer to the angle that the 237 whiskers made with the mid-line of the nose and head, giving that larger angles represent more 238 forward-positioned whiskers. If whiskers are occluded (such as by whisker crossing) the software will 239 not detect that whisker; therefore, the number of whiskers detected can vary from frame to frame, 240 with a total of 2-12 whiskers detected in each frame (with around 10-12 whiskers being usual, 5-6 on 241 each side). Whisker detection was validated by manually inspecting the software annotations 242 overlaid on to the video frames and a total of 1291 clips, each of around 0.5 seconds in length, were 243 included in the entire analysis. Clips were only included when the mouse was not contacting a wall 244 or object within the arena. The number of videos included in the whisker movement analysis can be 245 seen in Table 1.

Mean whisker angle was calculated by taking a mean of all the detected whiskers on each side, on a frame by frame basis (Figure 1c). The following variables were calculated from the mean whisker angles: mean angular position (the average whisker angle), amplitude (2V2\* the standard deviation of whisker angles, to approximate the range of whisker movements), asymmetry (the difference in whisker angles between the left and right sides), and the mean angular retraction and protraction speeds (calculated as the average velocity of all the backward (negative) and forward (positive) whisker movements, respectively). For mean angular position, amplitude, and whisker speeds, the
values for right and left whisker measurements were averaged (mean) to give only one value per
clip.

255 Statistical Considerations

256 Locomotion was not normally distributed, and therefore, a Mann Whitney U Test was used, with 257 mouse type (transgenic or MCAO vs. controls) as the grouping (between) variable. A significance 258 level of 0.05 was used. The quantitative whisker variables were compared using a MANOVA. The 259 effect of background strain (C57BL/6, 129Sv x C57BL/6, C57BL/6 x SJL/J, C57BL/6 x CBA, B6C3Fe, 260 B6129SF2) and source breeder (Jackson, Janvier (with Jackson mouse) and Charles River) were 261 investigated by grouping whisker variables by background strain and source breeder. As each mouse 262 model was collected from a different lab (apart from the 5xFAD and 3xTg-AD mice, Appendix 1), the 263 effect of lab could not be analysed. Whisker variables were then grouped by mouse genotype (transgenic or MCAO vs. controls) and mouse background (comparing: SOD1<sup>G93A</sup>, R6/2 CAG250, 264 SNCA-OVX, MCAO 1 month, MCAO 2 weeks, Reeler (B6C3Fe), RIM-DKOSert, Robo3R<sup>3-5</sup>-cKO, 3xTg-AD 265 and 5xFAD mice, including all mice: transgenic, MCAO and controls). A Bonferroni correction was 266 267 applied, so p was significant at values less than 0.01. Individual ANOVAs were conducted to examine 268 the effect of genotype in each mouse model, as each strain and mouse model appeared to be very different. To investigate sex differences, a multivariate ANOVA compared mouse type (transgenic vs 269 270 controls) as well as sex (male and female) for all the whisker variables. A Bonferroni correction was 271 applied, so p was significant at values less than 0.01. For the qualitative whisker scores, a Kruskal 272 Wallis test was used, with whisking, CIA and spread reduction scores grouped by each mouse model. 273 A significance value of p<0.05 was used. Results were plotted as mean bar charts with standard error 274 bars. Significant results are indicated on figures with an asterisk (\*).

275

## 276 Results

## 277 Quantitative Whisker Scores in control mice: effect of background strain and source breeder

278 Background strain (MANOVA: F(25,3495)=22.481, p<0.001) and source breeder (MANOVA:

- 279 F(10,1392)=62.342, p<0.001) significantly affected whisker positions and movements in the control
- 280 mice (Figure 2). This was apparent in all whisker variables measured, including mean angular
- 281 positions (strain: F(5,706)=126.688, p<0.001, breeder: F(2,706)=441.786, p<0.001), amplitude
- 282 (strain: F(5,706)=25.323, p<0.001, breeder: F(2,706)=11.744, p<0.001), retraction speed (strain:
- 283 F(5,706)=8.170, p<0.001, breeder: F(2,706)=64.885, p<0.001), protraction speed (strain:
- 284 F(5,706)=14.846, p<0.001, breeder: F(2,706)= 98.699, p<0.001) and asymmetry (strain:
- 285 F(5,706)=6.112, p<0.001, breeder: F(2,706)= 4.781, p=0.009). Relationships were relatively variable
- among the background strains, source breeders and whisker variables, with the most variation
- observed in the mean angular position variable (Figure 2a). For example, the B6C3Fe (Reeler),
- 288 C57BL/6 x CBA (R6/2 CAG250) and 129Sv x C57BL/6 (RIM-DKO Sert) mice all had significantly
- 289 different mean angular positions from each other as well as all the other background strains
- 290 (C57BL/6, C57BL/6 x SJL/J, B6129SF2). Mice from different source breeders also all had significantly
- 291 different mean angular positions (Figure 2a), protraction speeds (Figure 2d) and retraction speeds
- 292 (Figure 2e) from one another. For example, the Janvier lab mice (JJ) had the lowest values of mean
- angular position values, retraction speeds and protraction speeds, compared to the Jackson lab mice
- (J), which had medium values, and the Charles River mice (CR), which had high values for all. As the
   majority of mice had a background strain of C57BL/6 (those with white bars in figure 2), just these

- 296 mice were then looked at further to see if whisker positions and movements significantly varied in
- individual models with the same background strain. Indeed, mice with the same background strain
- 298 had significantly different whisker movements and positions, including mean angular position (F(2,
- 299 332)=48.035, p<0.001), retraction speed (F(2,332)=33.092, p<0.001), protraction speed (F(2,
- 300 332)=22.324, p<0.001) and asymmetry (F(2,332)=3.171, p=0.043), but not amplitude (F(2,
- 301 332)=0.858, p=0.425), For example, SOD1<sup>G93A</sup> mice and MCAO control mice at 1 month of age all had
- different mean angular position values from each other and all the other mouse models with a
   C57BL/6 background strain (in white in Figure 2a, including MCAO 2 weeks, SNCA-OVX and Robo3R<sup>3-</sup>
- 304 <sup>5</sup>-cKO mice). This suggests that the host lab might also have an effect on whisker positions and
- 305 movements. Because background strain, source breeder and lab had such a significant effect on
- 306 quantitative whisker measurements in the control mice, the transgenic and MCAO mice were
- 307 compared only to their matched controls, and not within different models (see Quantitative analyses
- 308 below).
- 309 Qualitative Whisker Scores in Transgenic and MCAO mice
- 310 The qualitative scores of whisking showed that all the mice fully whisked, apart from R6/2 CAG250
- mice (U(586)=486.3, p<0.001) (Figure 3a), which had significantly reduced whisking scores,
- 312 compared to the other transgenic mice. The R6/2 CAG250 mice only moved their whiskers forward
- 313 with protractions, and did not make full retraction movements, giving a score of 2 for their whisker
- 314 movements. This can also be seen in the example traces in Figure 6b.
- 315 In the whisker scores taken during object exploration, the Robo3R<sup>3-5</sup>-cKO mice performed contact-
- induced asymmetry against the object more often than the other transgenic mouse models,
- 317 including Reeler B6C3Fe, RIM-DKOSert, 3xTg-AD and 5xFAD mice (Figure 3b) (U(209)=58.04,
- p<0.001). Often the asymmetry was also more pronounced in the Robo3R<sup>3-5</sup>-cKO mice, compared to
- the other mice too; indeed, the example in Figure 4 shows the Robo3R<sup>3-5</sup>-cKO mice (Figure 4 middle)
- 320 with more pronounced contralateral protractions than the non-transgenic mice (Figure 4 left). The
- 321 RIM-DKO Sert mice performed spread reduction against an object less often than the other
- transgenic mouse models, including Reeler B6C3Fe, Robo3R<sup>3-5</sup>-cKO, 3xTg-AD and 5xFAD mice (Figure
- 323 3c) (U(214)=28.34, p<0.001). In fact, visually, the RIM-DKO Sert mice often froze following an object
- 324 contact and did not move their whiskers for a time. This behaviour was not captured by our
- measurement and scoring system, but can be seen in Figure 4 (RIM-DKO Sert mice, right), where the
- 326 whiskers freeze against an object, and show no evidence of spread reduction (compare with Figure
- 327 4, control non-transgenic mouse, left).
- 328 Quantitative analysis of locomotion and whisker movements
- 329 The individual mouse models were variable, with mouse background strain (including transgenic,
- 330 MCAO and controls) having more of an effect on whisker movements overall (MANOVA:
- F(45,6410)=33.155, p<0.001, medium effect size:  $\eta^2 p=0.189$ ), than being transgenic (MANOVA:
- 332 F(10,255)=1.944, p=0.030, small effect:  $\eta^2 p=0.008$ ). An example of which can be seen in Figure 5b,
- 333 where the Robo3R<sup>3-5</sup>-cKO transgenic and non-transgenic mice both had significantly lower mean
- angular position values than any other mouse model. Such a large variation in whisker movements
- between mouse models further justifies comparing only transgenic and MCAO mice with their age-
- 336 matched controls.
- 337 The SOD1<sup>G93A</sup> mice had significantly slower locomotion speeds than the non-transgenic controls
- 338 (U(124)=980, p<0.001) (Figure 5a). However, both the transgenic Robo3R<sup>3-5</sup>-cKO and 5xFAD mice had
- 339 significantly faster locomotion speeds than their non-transgenic controls (Robo3R<sup>3-5</sup>-cKO:

- 340 U(220)=4083, p<0.001, 5xFAD: U(49)=772, p<0.001) (Figure 5a). The R6/2 CAG250, Reeler (B6C3Fe),
- 341 RIM-DKO Sert and 5xFAD mice all had their whiskers positioned further forward, with significantly
- higher mean angular positions, than their non-transgenic controls (R6/2 CAG250: F(1,228)=22676,
- 343 p<0.001, Reeler: F(1,15)=6.904, p=0.010, RIM-DKO Sert: F(1,53)=53.222, p<0.001, 5xFAD:
- F(1,57)=14.450, p<0.001) (Figure 5b). However, both the MCAO (2 week) and 3xTg-AD mice had their
- 345 whiskers positioned further back, with significantly lower mean angular positions than their sham
- 346 and non-transgenic controls (MCAO (2 weeks): F(1,73) = 101.525, p<0.001, 3xTg-AD: F(1,184) =
- 53.443, p<0.001) (Figure 5b). Examples of whisker traces can be seen in Figure 6.
- 348 All the MCAO and transgenic mice had similar whisker amplitudes (Figure 5c) and asymmetry values
- 349 (Figure 5d) to their sham and non-transgenic controls; apart from the SOD1<sup>G93A</sup> mice, who had larger
- amplitude whisks than the non-transgenic mice (F(1,119) = 6.491, 0=0.010), and the MCAO (1
- 351 month) mice, which had larger whisker asymmetry than the sham mice (F(1,241)=7.399, p=0.007)
- (Figure 5c and d). Many transgenic mice had significantly slower whisker speeds than their non transgenic controls (Figure 5e and f). This included the SOD1<sup>G93A</sup>, R6/2CAG-250 and Robo3R<sup>3-5</sup>-cKO
- mice for protraction speed (SOD1<sup>G93A</sup>: F(1,119) = 10.826, p<0.001, R6/2CAG-250: F(1,228) = 22.070,
- 355 P<0.011, Robo3R<sup>3-5</sup>-cKO: F(1,100) = 35.587, p<0.001), and R6/2CAG-250, Robo3R<sup>3-5</sup>-cKO and 3xTg-AD
- 356 mice for retraction speed (R6/2CAG-250: F(1,228) = 21.306, p<0.001, Robo3R<sup>3-5</sup>-cKO: F(1,100) =
- 44.613, p<0.001, 3xTg-AD: F(1,184) = 8.875, p=0.005). Examples of whisker traces can be seen in
- 358 Figure 6.
- 359 Sex differences

360 There were no differences in whisker movements between male and female transgenic and non-

- transgenic RIM-DKO Sert mice (p>0.01). However, there were significant differences in protraction
- 362 and retraction speeds, between male and female transgenic and non-transgenic R6/2 CAG250 mice
- 363 (Protraction speed: F(1,226)=21.069, p<0.001, Retraction speed: F(1,226)=31.681, p<0.001). In
- 364 particular, the female non-transgenic mice had significantly faster protraction and retraction speeds
- than the female transgenic and all male mice (Figure 7a and b).
- 366

# 367 Discussion

368 Whisking is an ethologically important sensing behaviour that is mediated by a complex set of 369 circuits, involving large parts of the rodent brain (Diamond et al., 2008, Prescott et al., 2011). Thus, 370 whisker movements were significantly affected in many of the mouse models that we tested here, 371 compared to their non-transgenic controls. The Huntington's disease R6/2 CAG250 mice showed the 372 biggest differences in whisker movements that could be seen in both the qualitative scoring and quantitative tracking. The Robo3R<sup>3-5</sup>-cKO and RIM-DKO Sert mice showed differences in exploratory 373 374 strategies during object exploration, when compared to the Reeler, 3xTg-AD and 5XFAD transgenic mice. Specifically, the Robo3R<sup>3-5</sup>-cKO revealed stronger contact-induced asymmetry than the other 375

- transgenic mice, and the RIM-DKO Sert did not show spread reduction. We suggest that making
- 377 precise measurements of whisker movements is important for capturing motor declines, but
- 378 qualitatively scoring whisker movements during object exploration is sufficient for capturing some
- 379 sensory deficits. While taking many different measurements of whisker movements can capture a
- 380 range of behaviours and motions, measuring mean angular position and whisker speeds will capture
- 381 most of the differences in background strain, source breeder, genotype and sex.

#### 383 Motor Disorders

384 Measuring whisker movements and locomotion in mice with motor deficits has previously been

conducted in mouse models of Amyotrophic Lateral Sclerosis (SOD1<sup>G93A</sup>) (Grant et al., 2014) and

Huntington's disease (R 6/2, Q175 and HdH knock-in models) (Garland et al., 2018). SOD1<sup>G93A</sup> mice

are a model of Amyotrophic Lateral Sclerosis and have been well- studied using behavioural tests

- (Bucher et al. 2007); they have splayed hind limbs and impaired gait from around 90 days (Heiman Patterson et al., 2005). We show here that 120 day-old SOD1<sup>G93A</sup> mice had significantly slower
- 390 locomotion speeds, as well as larger mean whisking amplitudes than the non-transgenic mice (Figure
- 5a and c), in agreement with Grant et al. (2014). However, we also found that SOD1<sup>G93A</sup> mice had
- 392 significantly slower protraction speeds than the non-transgenic mice (Figure 5e). Grant et al., (2014)
- 393 also found slower protraction speeds in SOD1<sup>G93A</sup> mice, compared to non-transgenic mice, but this
- 394 was not significant in their data. They also found significantly faster retraction speeds in SOD1<sup>G93A</sup>
- 395 mice compared to non-transgenics, which we did not find here (Figure 5f).

396 The R6/2 mouse model of Huntington's disease exhibit skeletal muscular atrophy and

neuromuscular junction abnormalities (Ribchester et al., 2004), with both muscle pathology and

- motor function decline contributing to motor deficits in these mice (Garland et al. 2018). Motor
- deficits in these mice can be observed in rotarod tasks, balance beam tests and gait analyses (Carter
- 400 et al., 1999; Menalled et al., 2003; Morton et al., 2009; Pallier et al., 2009); however, these tasks
- 401 often lack sufficient sensitivity to characterise disease progression, hence the benefit of measuring a
- 402 highly quantitative behaviour, such as whisker movements. We found that the R6/2 mice had larger
- 403 angular positions and slower retraction speeds than the non-transgenic mice (Figure 5b and f and
- 404 Figure 6a), in agreement with Garland et al., (2018). We found that R6/2 mice also had slower
- 405 protraction speeds than non-transgenic mice (Figure 5e), which was also found by Garland et al.,
- 406 (2018), but was not significant. They also found a significant reduction in whisker amplitude in R6/2
- 407 mice compared to non-transgenic controls, which we did not see in our data (Figure 5c).

One might expect to observe similar results in a Parkinson's disease model, compared to a late-stage
 Huntington's disease model. However, we did not observe any significant whisker movement or
 locomotion differences in the SNCA-OVX mice compared to the α-synuclein null controls. However,

- 411 the R6/2 CAG250 model is a relatively severe model, whereas the SNCA-OVX model is much more
- 412 subtle. The SNCA-OVX model recapitulates disease-relevant levels of alpha-synuclein and
- 413 progressively develops motor phenotypes with the onset of rotarod impairment at 18-months. On
- the other hand, the R6/2 CAG250 mouse model shows considerable motor impairment by 18 weeks
- as measured by grip strength. Hdh knock-in Huntington's mice are often considered to be a better
- 416 genetic constrict of Huntington's disease, and have a subtler behavioural phenotype (Jin et al.,
- 417 2015). Hdh CAG250 knock-in mice also do not have late-stage differences in whisker movements,
- 418 but reveal early changes in whisker amplitude protraction speeds and retraction speeds as early as
- 419 10 weeks old (Garland et al., 2018). Therefore, perhaps testing SNCA-OVX mice at younger ages
  420 might reveal more differences in whisker movements. SNCA-OVX mice at 18 months of age have
- 421 been found to have motor deficits in the rotarod task, and reduced stride length (Janezic et al.,
- 421 been found to have motor deficits in the rotarod task, and reduced stride length (Janezic et al., 422 2013) so perhaps an accompanying celt exclusion with the length (Janezic et al.,
- 422 2013), so perhaps an accompanying gait analysis might also usefully compliment this work, such as
- 423 using the LocoWhisk arena (Grant et al., 2018; Gillespie et al., 2019).
- 424 Stroke
- 425 The MCAO stroke model has functional motor and sensory impairments, such as deficits in the
- 426 parallel bar crossing and maze exploration test (Nedermann et al. 2007). While the whisker
- 427 movements of mice with stroke (MCAO) has not specifically been measured before, some studies

428 have referred to whisker-use in these animals. The forelimb placing test (Schallert et al., 2000) 429 involves detecting the edge of a table with a forelimb, after touching the edge with the vibrissae. It is 430 thought to be affected following damage to sensorimotor cortex, striatum, spinal cord and 431 related sensorimotor structures, such as in stroke models. The cross-midline test (Woodlee et al., 432 2005) also explores placement of forelimbs following whisker touch, and MCAO animals do not place 433 their forelimb if the whisker touch is ipsilateral to the limb. Nedelmann et al. (2007) observed that 434 one of their MCAO rats (1-6 days post-surgery) lacked any whisker movements on one side, which 435 would cause whisker asymmetry in our experiment. Indeed, we observed significantly higher values 436 of whisker asymmetry in the MCAO mice 1 month post-surgery, compared to sham controls (Figure 437 5d). We also observed an increase in mean angular position in MCAO mice 2 weeks post-surgery 438 (Figure 5b, Figure 6c), compared to sham controls. The model used here was the distal MCAO model, 439 which produces only a cortical lesion and is a less severe model of stroke than the proximal MCAO, which induces a cortical and subcortical infarct. It is better for animal welfare but deficits are often 440 441 hard to detect in this model (Rossell et al., 2013). Therefore, seeing deficits at one month post-lesion 442 is very promising, and whisker analysis may allow this model to be utilized more for studies that 443 require functional outcome measures. The lesion in the model is mainly in sensory cortex (Appendix 444 2), therefore, the mice are likely to have both sensory and motor deficits. Perhaps MCAO mice will 445 reveal alterations in whisker movements during object exploration, as well as in the open field

446 arena, which should be explored in future work.

## 447 Somatosensory cortex development models

448 As the cortex is likely to play a key role in object exploration and discrimination (Kleinfeld et al., 449 2006), investigating whisker movements during object exploration is likely to capture the most 450 salient changes in mice with somatosensory developmental alterations. Indeed, we see significant differences in exploratory behaviour in the Robo3R<sup>3-5</sup>-cKO and RIM-DKO Sert mice compared to 451 452 other mouse models (Figure 3 and 5). While we demonstrate here that exploratory whisking during 453 contact can be described by a simple scoring system, it is possible to also quantitatively measure 454 behaviours such as whisker spread and whisker asymmetry before and during contact, as per Grant 455 et al. (2009). This might be important in future studies that may wish to examine the extent of 456 exploratory whisker disruption.

457 The Robo3R<sup>3-5</sup>-cKO mice have impacted performance on Rotarod tasks and reduced locomotion 458 speeds (Renier et al. 2010). They have disrupted VPM thalamic (barreloid) and cortical (barrel) maps, 459 such that two functional whisker maps exist in these areas, and receive input from both sides of the 460 face (Renier et al., 2017; Gaspar and Renier, 2018). We observed that contact-induced asymmetry 461 was particularly prevalent (Figure 3b) and strongly appeared (Figure 4) in these mice, compared to 462 other transgenic mice. This might be due to abnormal bilateral sensory processing in these animals (Renier et al., 2017). As well as sensory deficits, Robo3R<sup>3-5</sup>-cKO mice have revealed motor deficits in 463 the rotarod task (Renier et al., 2010); we observed slower protraction and retraction whisker speeds 464 465 (Figure 5e and f), but faster locomotion speeds in these mice (Figure 5a), compared to non-466 transgenic controls.

RIM-DKO Sert mice have impaired balance by weaning and can have difficulties maintaining upright
postures (Karst et al., 2011). They have a 67% reduction in thalamic projections in to barrel cortex
(Narboux-Nême et al., 2012), which is likely to impact sensory processing. We observed that they
often froze following a contact, and were not able to reduce their whisker spread, so did not bunch
their whiskers on to an object following a contact (Figure 4). We also observed that they had larger
mean angular positions (Figure 5b, Figure 6e) than their non-transgenic controls. Reeler mice also
have an altered cortex, specifically having disrupted layers in cerebellum and cortex (Falconer, 1951,

Wagener et al., 2016; Guy and Staiger, 2017), but cortical topography often remains intact (Wagener
et al., 2016) with lemniscal fibers successfully innervating their target columns (Guy and Staiger,
2017; Wagener et al., 2016). Like the RIM-DKO Sert mice, Reeler mice have impaired balance and
difficulties maintaining posture, they can also have tremors from 2 weeks of age (Podhorna and
Didriksen, 2004). We did not observe any changes in these mice during object exploration but, like
the RIM-DKO Sert mice, we also observed an increase in whisker angular position, compared to their
non-transgenic controls (Figure 5b, Figure 6d).

481 While we have mainly referred to sensory alterations in these mice, cortical areas are also involved 482 in sensorimotor processing through a variety of nested loops (Kleinfeld et al., 2006). Therefore, 483 changes in somatosensory areas are likely to affect both sensing and motion in mice with cortical 484 alterations. Much research in whisker sensing is focussed on the somatosensory cortex, particularly 485 the barrel cortex; however, there remains a major challenge understand the effect of barrel cortex 486 architecture on awake, freely moving, behaving animals (Brecht, 2007). Genetic manipulations of 487 mice have allowed us to alter barrel cortex architecture, especially in terms of incoming axons, 488 neural activity and molecular patterning (Gaspar and Renier, 2018), and we have shown that the 489 whiskers of these mice can be imaged to measure their precise movements and exploration 490 strategies. Measuring whisker movements during object exploration and measuring mean angular 491 position in an open field arena could be applied to many transgenic mouse models, in order to 492 understand more about sensory processing in these animals, and the role of the cortex in whisker 493 touch.

## 494 Alzheimer's disease

495 Measuring whisker movements and locomotion in mice with impacted cognitive functioning has 496 previously been conducted in mouse models of Huntington's Disease (Garland et al., 2018) and 497 Alzheimer's disease (5xFAD) (Grant et al., 2018). Our results show that the 5xFAD female Alzheimer's 498 disease mice at 12.5 months had significantly higher mean angular positions than the non-transgenic 499 mice (Figure 5b), which was also observed in female 7 month-old 5xFAD mice in Grant et al. (2018). 500 The 5xFAD mice have sensory deficits, in both olfaction (Roddick et al., 2014; 2016) and hearing 501 (O'Leary et al. 2017) tasks. Whisker movement alterations in 5xFAD mice might also be linked to 502 sensory deficits. While we did not observe any differences in whisker movements during object 503 exploration, 5xFAD mice show a reduction of inhibitory interneurons in Layer IV of the whisker barrel 504 cortex, which leads to changes in vibrissae-related behaviour that include a lack of whisker barbering 505 in the home cage and an avoidance of enclosed spaces. These behaviours dissipate when the 506 whiskers are trimmed and Flanigan et al. (2014) suggested that 5xFAD mice might have over-507 sensitive vibrissae. We do demonstrate that 5xFAD mice have altered whisker angles compared to 508 non-transgenic mice, but are not able to associate this with increased sensitivity of the whiskers.

509 The 5xFAD mice also have both motor (O'Leary et al. 2018a; 2018b) and cognitive deficits (Roddick 510 et al. 2014; Gür et al. 2019). O'Leary et al. (2018a) found that 5xFAD mice, at 9-10 months of age, 511 showed reduced body weight, reduced rearing in the open-field and impaired performance on the 512 rotarod compared to wild-type controls. At 12-13 months, 5xFAD mice showed reduced locomotor 513 activity on the open-field, and impaired balance on the balance beam. We also show here that the 514 female 5xFAD mice at 12 months of age had slower locomotion speeds in an open-field arena, 515 compared to the non-transgenics (Figure 5a), which supports the finding that they have locomotor 516 impairments from 9 months of age (Bhattacharya et al., 2014; O' Leary et al., 2018a; 2018b). 517 Whisker impairments have been found to be present even earlier in female 5xFAD mice, from 7 518 months of age (Grant et al., 2018).

519 The other Alzheimer's disease model (3xTg-AD mice) also have impacted performance on sensory 520 (vision: King et al. 2018; olfaction: Roddick et al. 2016), motor (Stover et al. 2015; Garvock-de 521 Montbrun et al. 2019) and cognitive tasks (Stevens et al. 2015; Gür et al. 2019; Fertan et al. 2019). 522 We found that the 3xTg-AD mice had lower mean angular positions (Figure 5b) and retraction 523 speeds (Figure 5f), compared to their non-transgenic controls. Why angular position would show the 524 opposite pattern, and other variables appear significant in the 3xTg-AD mice compared to the 5xFAD 525 mice is unclear (compare Figure 6g and h). However, it might be due to the differences in disease 526 mechanism and pathologies between the two models (Guzmán et al., 2014). The 3xTg mice do not 527 show the clear age-related motor dysfunction that is observed in the 5xFAD mice, and have even 528 been found to have an enhanced motor phenotype at 6 months of age (Stover et al., 2015; Garvock-529 de Montbrun et al. 2019). They often perform better than non-transgenic mice on rotarod tasks 530 (Stover et al., 2015; Blanchard et al., 2010; Chen et al., 2013; Garvock-de Montbrun et al. 2019), and have longer stride lengths (Stover et al., 2015). However, we did not observe any significant changes 531 532 in locomotion, even at 17 months of age, and the 3xTg-AD mice had slower whisker movements 533 overall, compared to non-transgenic mice.

## 534 Other effects

535 We observed a large variation in whisker movements between the mouse models, even in the 536 control mice (Figure 2, Figure 5). This variation had a larger effect on whisker movements than being 537 transgenic (Effect sizes: Mouse background strain:  $\eta^2 p=0.189$ ; Genotype:  $\eta^2 p=0.008$ ). For example, 538 non-transgenic and transgenic Robo3R<sup>3-5</sup>-cKO mice had the lowest mean angular positions overall, 539 compared to all other mice (Figure 5b). The highest mean angular position was observed in sham 540 stroke mice, 2 week post-surgery (Figure 5b), which accounts for the significant difference between 541 the sham and MCAO mice. There is no real reason why these control mice have significantly more 542 forward positioned whiskers than any other mice, and care has to be taken that observed 543 differences are from the disorder and not as a result of individual or other differences. Certainly, the 544 effect of background mouse strain and source breeder on whisker movements has not previously 545 been explored. We found that background strain and source breeder both affect whisker 546 movements in control mice. All the mice featured in this study also had different ages, were from 547 different labs, and had different sample sizes in each group (due to availability), so we are not able 548 to systematically compare them (Table 1). It is likely that husbandry procedures and enrichment will 549 have an effect on whisker movements, as the development of whisker movements are likely to be 550 experience-dependent (Grant et al., 2012); individual husbandry details for the mice can be found in Appendix 1. Age is also likely to have an effect, due to age-related and experience-dependent effects 551 552 (Grant et al., 2012). Collecting samples from many mice in different labs will help us to understand 553 the variation of whisker movements, as well as ensuring that videos and set-ups are comparable 554 between each experiment. It is likely that holding and husbandry conditions, background strain, 555 source breeder, age and sex will all impact whisker movements and exploratory behaviours.

556 Indeed, we also observed an effect of gender in the R6/2 CAG250 mice, specifically that the non-557 transgenic females had faster protraction and retraction speeds than the non-transgenic males, and transgenic males and females (Figure 7). RIM-DKO Sert mice did not reveal any significant 558 559 differences between males and females. No studies have systematically explored sex differences in 560 whisker movement in different strains of mice or rats, however, Grant et al. (2018) found that 5xFAD 561 Alzheimer's disease mice revealed significant sex differences, with female mice moving their whiskers much more than the males overall. Of course, there are many sex differences in mouse 562 563 models, in general (Jonasson, 2005; Schellinck et al., 2010; Roddick et al., 2014; 2016; Kane et al. 2018; Gür et al., 2019), and often females are affected more by disease pathologies (Bhattacharya et 564

al., 2014). A systematic exploration of sex differences in healthy mouse strains needs to be
 conducted before whisker measurements become a standard behavioural test.

567 Other outcomes can also arise as part of breeding mice. For example, some 5xFAD and wildtype 568 mice have retinal degeneration and are completely blind (Chang et al., 2002; Dalk and Graw, 2005) 569 as a result of the retinal degeneration gene (Pde6brd1) in the SJL/J background strain, (Chang et al., 570 2002). C57BI6N mice also show some retinal degeneration (Mattapallil et al., 2012). Rats and mice 571 with retinal degeneration have been shown to move their whiskers more than sighted animals 572 (Arkley et al., 2014; Grant et al., 2018), and Grant et al. (2018) showed that sex differences and 573 retinal degeneration both affected whisker movements more than being 5xFAD transgenic. Indeed, 574 measuring whisker movements is so precise, that we are able to pick up many differences in 575 movements, and we can observe differences in mice with certain backgrounds and genotypes. This 576 might mean that a mouse with the same transgene might produce a different behavioural 577 phenotype if they have a different background strain, as background strain affects whisker 578 movements. It is important to understand any behavioural variation that may arise in mouse

579 models, and multi-lab studies such as this is good place to start describing behavioural variations.

### 580 Recommendations

581 That our results are mainly in agreement with other studies (Grant et al., 2014; Garland et al., 2018;

582 Nedelmann et al., 2007) indicates that measuring whisker movements are a robust and repeatable

583 way to capture motor deficits. Indeed, it is highly quantitative and also quick to capture videos,

taking only 5-10 minutes per mouse. Measuring whisker movements can also reveal earlier motor

585 deficits than in locomotion tasks, which has already been demonstrated in R6/2 CAG250, HdH

586 CAG250 knock-in and 5xFAD mice (Garland et al., 2018; Grant et al., 2018). However, so far, whisker

587 movement data has been collected by only one laboratory; more laboratories would have to 588 conduct data collection in order to truly test the robustness of using whisker measurements to

589 characterise sensory, motor and cognitive disorders. Collecting more whisker movement data from

- 590 mouse models of other disorders in open field experiments would also usefully complement this
- 591 dataset.

592 As whisker movements are very fast and the whiskers themselves are very small, it is important to 593 review the video footage and make sure that findings from the whisker traces are robust and 594 evidenced clearly by the videos. We suggest that qualitative scoring should be carried out before 595 undertaking tracking on the videos. This can clearly identify large changes in whisker movements 596 (such as in the R6/2 CAG250 mice in Figure 3a and Figure 6b) and also demonstrate whether 597 contact-related measures need to be extracted (such as comparing spread or asymmetry before and 598 during contact, as detailed in Grant et al. (2009)). As in most behaviour studies, significant effects 599 should also be accompanied by example video stills or whisker traces that further demonstrate the 600 effect. Indeed, the experimenter needs to ensure that significant effects are truly caused by being 601 transgenic and not by gender or individual differences (such as in the sham stroke mice in Figure 5b). 602 We suggest that open field arenas are suitable for measuring the whisker movements of mice with 603 motor disorders, but filming whisker movements during object exploration is a useful addition for 604 other mouse models that have sensory, exploratory or cognitive deficits. We also suggest that, 605 where possible, both genders should be considered to incorporate sex differences. Accordingly, we 606 suggest for future studies testing whisker movements in mouse, or even rat, models to follow the 607 methods schematic in Figure 8 (see also Appendix 3 for more details on testing and tracking).

Overall, we demonstrate here that, with a number of caveats, measuring whisker movements canprovide precise, quantitative descriptions of motor and exploratory behaviour in many mouse

- 610 models. The variety of mouse models available can capture a range of diseases and disorders, as well
- as provide careful manipulations of brain structure and circuitry. Measuring whisker movements in
- 612 mouse models could help us to better understand disease progression and design new treatments. It
- also has the capacity to provide us with a more in-depth understanding of sensory processing.
- 614

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#### 862 FIGURES



Figure 1: Experimental data collection and video analysis. a) filming set-up, showing the Perspex
box and high-speed video camera. The yellow box indicates the field of view, and corresponds to b)
which shows an example video clip of a mouse. ARTv2 LocoWhisk software automatically locates the
mouse centroid (red point, yellow line), nose tip (Red point, blue line) and whiskers (coloured lines).
c) The coloured lines of the whiskers make an angle with the head, termed whisker angle. The mean
whisker angles of each side are shown here for the left (blue) and right (red) whiskers.



873 Figure 2: Summary of the quantitative whisker movement data for control mice to examine

background strains, for a) mean angular position, b) amplitude, c) asymmetry, d) protraction speed
and e) retraction speed. Bars show mean values with standard error bars. Colours and patterns on
the bars correspond to the background strain (see key on figure), with white bars corresponding to a
C575L/6 background strain, and grey and black bars corresponding to various C575L/6 crosses.
Letters above the bars correspond to source breeders: J: Jackson, JJ: Janvier, and CR: Charles River.



Figure 3: Qualitative whisker movement scores. Whisker movement scores for whisking (a),
 contact-induced asymmetry (CIA) (b) and spread reduction (c). Bars show mean values with standard
 error bars. Asterisks show significant values (P<0.05) for the transgenic or MCAO mice, compared to</li>
 each other.



Figure 4: Example video stills of mice exploring a Perspex block, pre-contact (top) and during
 contact (bottom). Control mice (left) show contact-induced asymmetry on the block, and also
 spread reduction. Robo3R<sup>3-3</sup>-CKO mice (middle) show many strong instances of contact-induced
 asymmetry during contact. RIM-DKO Sert mice (right) do not show spread reduction on the block
 during contact.



| 895 | Figure 5: Summary of the quantitative locomotion and whisker movement data for transgenic or      |
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| 896 | MCAO mice (in black) and control mice (in white). For locomotion speed (a), mean angular position |
| 897 | (b), amplitude (c), asymmetry (d), protraction speed (e) and retraction speed (f). Bars show mean |
| 898 | values with standard error bars. Asterisks show significant values (P<0.01) for the transgenic or |
| 899 | MCAO mice compared to the control mice.   |
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Figure 6: Examples of whisker angle values, illustrating differences in whisker movement traces
 between transgenic or MCAO mice (solid black line) and control mice (dashed black line).





Figure 7: Sex differences in the R6/2 CAG250 mice. Female non-transgenic control mice had larger
 protraction (a) and retraction (b) speeds than any of the other mice. Black bars are transgenic and
 white bars are non-transgenic controls. Bars show mean values with standard error bars. Asterisks
 show significant values (P<0.01)</li>

