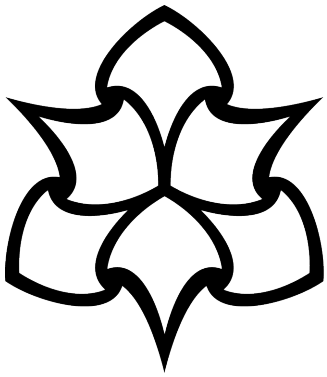

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ORIGINAL ARTICLE

Genes, Brain and Behavior

Abnormal whisker movements in the 3xTg-AD mouse model of Alzheimer's disease

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Abstract

Alzheimer's disease is the most frequent form of dementia in elderly people. The triple transgenic (3xTg-AD) mouse model of Alzheimer's Disease is important in biomedical research as these mice develop both neuropathological and behavioural phenotypes. However, their behavioural phenotype is variable, with findings depending on the specific task, as well as the age and sex of the mice. Whisker movements show motor, sensory and cognitive deficits in mouse models of neurodegenerative disease. Therefore, we examined whisker movements in 3, 12.5 and 17-month-old female 3xTg-AD mice and their B6129S/F2 wildtype controls. Mice were filmed using a high-speed video camera (500 fps) in an open arena during a novel object exploration task. Genotype and age differences were found in mice exploring the arena prior to object contact. Prior to whisker contact, the 3-month-old 3xTg-AD mice had smaller whisker angles compared with the wildtype controls, suggesting an early motor phenotype in these mice. Pre-contact mean angular position at 3 months and whisking amplitude at 17 months of age differed between the 3xTg-AD and wildtype mice. During object contact 3xTg-AD mice did not reduce whisker spread as frequently as the wildtype mice at 12.5 and 17 months, which may suggest sensory or attentional deficits. We show that whisker movements are a powerful behavioural measurement tool for capturing behavioural deficits in mouse models that show complex phenotypes, such as the 3xTg-AD mouse model.

KEYWORDS
Alzheimer's, animal behaviour, disease model, mouse model, neurodegeneration, rodent, sensorimotor, transgenic, vibrissae, whisker

1 | INTRODUCTION

Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder, the most frequent form of dementia in elderly people.[1–3] Mouse models are essential for improving our understanding of the neural and behavioural changes that occur during AD progression, and to develop novel therapeutic targets.[4,5] The triple transgenic (3xTg-AD) mouse model is considered to have high validity as these mice develop both Aβ plaques and tau tangles,[6] as well as show cognitive deficits.[7,8] The 3xTg-AD mice have altered performance on sensory tasks involving vision,[9] olfaction,[10] and touch,[11] as well as motor[12,13] and cognitive

tasks.[5,14–16] The 3xTg-AD mice generally perform worse than their wildtype controls in spatial learning and memory tests.[16,17] They have a complex motor phenotype and have even shown an enhanced motor phenotype at 6 and 16 months of age.[12,13] They show higher frailty measures[18] and have a shorter lifespan than their wildtype background strain. Male 3xTg-AD mice also have a shorter lifespan than females,[19] as well as altered immune function and gene expression.[20]

Behavioural studies have shown quite variable outcomes with these mice, especially in motor and cognitive tasks, such as spatial learning and memory.[12,16] Age, sex, experimental apparatus and test design all impact the performance of 3xTg-AD mice during behavioural tasks.[16,18,21] Therefore, a better understanding of the behavioural manifestations that occur in this model of AD is needed. Measuring whisker movements in mouse models has been suggested as an easy and robust way to capture elements of sensory, motor and cognitive deficits in mice.[11] Such deficits have been shown in mouse models of Amyotrophic Lateral Sclerosis,[22] Huntington's Disease,[23] anxiety,[24] Alzheimer's Disease,[11,25] as well as Cerebellar Ataxia, Somatosensory Cortex Development disorders and Ischemic stroke.[11]

Rodents rely on their whiskers as their primary sense of touch.[26] In addition to their sensory function, whisker movements indicate aspects of motor control; they can move rhythmically to-and-fro in a process called whisking, which occurs up to 25 Hz in mice.[27] Mice also precisely control their whisker movements during object exploration.[28–30] When a mouse contacts an object with their whiskers, they tend to decrease their whisker angles (the angle between the head and whiskers), reduce whisker spread, increase whisker asymmetry and amplitude, and slow whisker speeds.[29–31] These changes allow many whiskers to contact a surface for longer durations, hence increasing the quality of sensory information from whisker contacts.[30] The positioning and focussing of many whiskers onto an object are thought to be associated with attention.[32,33] These whisker movements are disrupted in many mouse models of disease and may be indicative of sensory, motor or cognitive deficits.[11,22,23]

We have previously shown that 17-month-old female 3xTg-AD mice had smaller whisker angular positions and retraction speeds compared with wildtype controls when moving around their environment without object contacts.[11] However, it is important to measure these changes at different age points and during object contact to better understand the deficits in whisker movements in this mouse model. Therefore, the aim of this study is to investigate whisker movements in the 3xTg-AD mouse model at different ages, before and during object exploration. This study was designed based on the recommendations of Simanaviciute et al.[11] as shown in Figure 1. To detect sensory, motor, and/or cognitive deficits in the 3xTg-AD mouse model, we tested for all the suggested steps in Figure 1. We scored whisker movements prior to object contact and during object exploration using both qualitative and quantitative measures in order to detect any deficits in whisking behaviour in the 3xTg-AD mice.

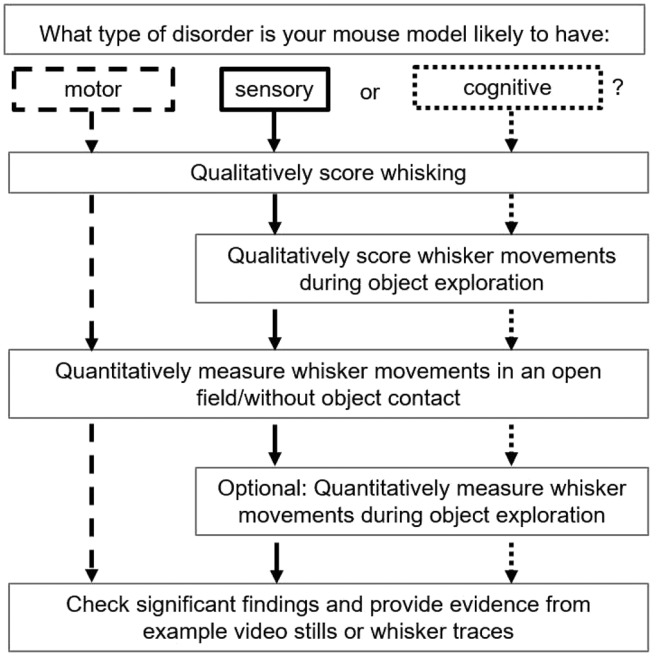

FIGURE 1 Methods schematic for testing mouse models with likely motor, sensory or cognitive symptoms, suggested by our previous research (adapted from Simanaviciute et al.[11]).

2 | MATERIALS AND METHODS

2.1 | Animals

A total of 38 female mice were used in this cross-sectional study: 17 transgenic (3xTg-AD, JAX # 004807) mice (3 at 3 months, 6 at 12.5 months, 8 at 17 months) and 21 wildtype (B6129S/F2 WT, JAX# 101045) mice (8 at 3 months, 7 at 12.5 months, 6 at 17 months). All mice were born in-house at Dalhousie University from breeding pairs purchased from Jackson Laboratory (Bar Harbour, Maine USA). The 3xTg-AD mice were engineered by injecting APPSwe and tauP301L transgenes into single-cell embryos of homozygous PS1M146V knock-in mice. This causes Aβ42 aggregation in the frontal cortex and the hippocampus at around 3 months of age, extracellular plaques in the frontal cortex and the hippocampus at 6 months of age, and hyperphosphorylated tau tangles at 12 months of age.[6] Our study spans these changes by observing mice from 3 to 17 months of age. Due to increased mortality in male mice by 17 months of age,[19] only female mice were included in this study.

Mice were weaned at 21 days of age, their ears were punched for individual identification, and they were housed in same sex groups of 2–4 in 30 × 18 × 12 cm translucent polycarbonate cages with wire lids and microisolator tops. Cages contained woodchip bedding (Fresh Bed, Shaw Resources, NS, Canada) and a 4 × 7 cm PVC tube for enrichment. They were kept in a climate controlled (22°C ± 2°C) vivarium on a reversed 12:12 light: dark cycle with lights off at 09:45 am. All behavioural testing was completed during

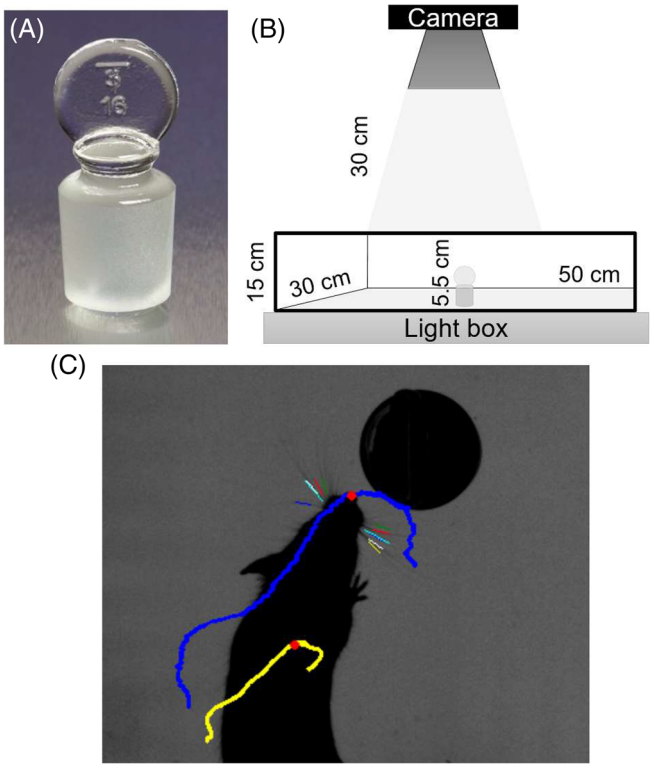

FIGURE 2 Data collection and video analysis. Panel A shows the glass bottle stopper object used in the experiments; Panel B illustrates the filming set-up, the object size and location in relation to the Perspex box, and the distance between the arena and high-speed video camera. The field of view in light grey corresponds to the video still in c) showing an example video clip. ARTv2 LocoWhisk software was used to automatically locate the mouse centroid (red point, yellow line), nose tip (red point, blue line) and whiskers (coloured lines), and detects them on a frame-by-frame basis.

the dark (active) portion of the light: dark cycle. Mice had ad libitum access to Purina Laboratory Rodent Chow #5001 (Agribrand Purina, Strathroy, Ont., Canada) and tap water. Mice were treated in accordance with the regulations set forth by the Canadian Council on Animal Care and the experimental protocol was approved by the Dalhousie University Committee on Animal Care and the local ethics committee at Manchester Metropolitan University.

2.2 | Experimental procedures

For filming whisker movements, mice were placed in a transparent Perspex rectangular arena (30 × 50 × 15 cm) which was lit from below by an infra-red light box (LEDW-BL-400/200-SLLUB-Q-1R-24 V, PHLOX) (Figure 2B). Mice were filmed from above using a digital high-speed video camera (Phantom Miro ex2) recording at 500 frames per second with a shutter-speed of 1 ms and resolution of 640 × 480 pixels. A Pyrex glass bottle stopper (Figure 2A) was placed inside the arena as an object to explore. Multiple 1.6 s video clips (800 frames) were collected opportunistically (by manual trigger) when the mouse moved into the camera's field of view.

2.3 | Video analysis: qualitative whisker scores

Video clips were selected for analysis based on the criteria developed by Grant et al.[22] These criteria were: (i) the mouse was clearly in the frame; (ii) both sides of the face were visible; and (iii) the head was level with the floor (no extreme pitch or yaw). In these clips, *whisking* by mice was scored on a four-point scale from no whisking (0), to only retractions (1), only protractions (2) or both retractions and protractions (3). To qualitatively assess whisker behaviours and exploratory strategies, all of the video clips that met the above criteria were scored based on a system developed by Grant et al.,[31,34] in which *contact-induced asymmetry*, *spread reduction*, and *head turning asymmetry* were measured.[27,35] When the mouse was contacting an object with their whiskers, *contact-induced asymmetry* (CIA) was scored on a three-point scale from absent (0), to showing increased contralateral protraction (1), reduced ipsilateral protraction (2) and both increased contralateral protraction and reduced ipsilateral protraction (3). Object-directed whisker *spread reduction* was scored as absent (0) or present (1) when whisker spread decreased following object contact. *Head turning asymmetry (HTA)* was scored as present (1) or absent (0), during a head turn.

2.4 | Video analysis: quantitative analysis of locomotion and whisker movements

For quantitative analysis of whisker movements and locomotion, video clips were divided into pre-contact (PC) and during object contact (DC). Therefore, the clip selection criteria were amended to also include (i) the mouse must be travelling towards the object in the PC section of the clip; and (ii) the whiskers were only contacting the object and not the vertical arena walls, in the DC section of the clip. In this way, general whisker movements could be assessed for motor behaviour in the PC section of the clip (similar to an open field), and object exploration could be assessed in the DC section of the clip. Only clips that had both considerable PC and DC segments (>0.2 s) were included in this quantitative analysis. The clips were tracked using the Automated Rodent Tracker, version 2 (ARTv2).[36] This used image processing to automatically locate the snout and the centroid of the mouse, for *locomotion speed* calculations (from the yellow trace in Figure 2C). A ruler was filmed at the start of each episode of data collection to enable a calibrated measure of locomotion speed in metres per second.

The whisker detector program (ARTv2) found the orientation and position of the snout, and the whisker angles (relative to the midline of the head) of each identified whisker (Figure 2C). The ARTv2 program is only able to detect whiskers and does not maintain the identity of the whisker between frames (i.e., tracking); rather, a mean angle is calculated from each frame using all detected whiskers. Larger whisker angles represent more forward-positioned whiskers. If a whisker is occluded (such as by whisker crossing) the software will not detect it; therefore, the number of whiskers detected can vary from frame to frame, with a total of

2–12 whiskers detected in each frame (with around 10–12 whiskers being usual, 5–6 on each side). Whisker detection was validated by manually inspecting the software annotations overlaid onto the video frames. From 1 to 12 video clips per mouse were included in data analysis (Supplementary Table 1), resulting in a total of 183 whole clips, all of which contained both PC and DC sections. PC sections ranged from 100 to 600 frames per clip, whereas DC sections ranged from 100 to 625 frames.

Mean whisker angle was calculated by taking the mean of all the detected whiskers on each side, on a frame-by-frame basis (Figure 2C). The following variables were then calculated from the mean whisker angles: *mean angular position* (the average whisker angle), *amplitude* ($2\sqrt{2}$* 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 speed of all the backward (negative) and forward (positive) whisker movements, respectively). For the first time in a mouse model study, whisker spread was also quantified. Mean angular position and spread are considered the two most informative parameters to assess in whisking,[30] thus this was an important quantitative measure to supplement the qualitative scoring of spread reduction. *Spread* was scored as the standard deviation of all tracked whisker angular positions. For mean angular position, amplitude, whisker speed and spread, the mean values for right and left whisker measurements were used to give one value per video clip.

2.5 | Statistical analyses

For all qualitative and quantitative whisker measurements, each variable was compared between wildtype and 3xTg-AD mouse, at each age (3, 12.5, and 17 months). Qualitative scores of whisking behaviours were analysed using the Kruskal–Wallis test with Dunn's post-hoc tests using GraphPad Prism 8 software, as these were on ordinal scales and not normally distributed.

Quantitative measures of the pre-contact (PC) whisker variables were first analysed. Then, the changes in whisker measurements during object exploration were analysed by subtracting the during-contact measures from the pre-contact measures (PC-DC). PC-DC was chosen, rather than DC-PC, as it is more intuitive to identify increases in variables during contact as positive, and reductions as negative; in addition, many of the whisking parameters were expected to be higher in PC. A Linear Mixed-Effects Model was constructed using the package lme4[37] in R Studio to analyse the effect of age and genotype on all PC and PC-DC whisker variables. The model computed *F* tests on the fixed effects of age and genotype and provided *p*-values using a type III ANOVA, as well as interaction effects (although all the interaction effects were not significant and will not be referred to further in the main text, though, see Supplementary Tables 3 and 4 for more detail). Since the mice were filmed repeatedly exploring an object, and every subsequent video clip was different, with the mouse acquiring increasingly more information, each video clip was treated as a within variable, but the degrees of freedom and F-statistics were approximated using a Kenward–Rodger's method.[38] This method takes account of uneven and low sample numbers (such as from the 3-month-old animals). The degrees of freedom were automatically determined to be anywhere between the number of animals and the number of video clips for each particular measurement analysed. A significance value of $p < 0.05$ was used throughout. Significant pairwise comparison results are indicated on all figures with an asterisk (*). The Kenward–Rodger's approximation is the preferred method of approximating degrees of freedom over Satterthwaite's method,[39,40] and of reporting *p*-values over likelihood ratios and Wald *t*-values.[41] It also produces acceptable Type 1 error rates in smaller sample sizes in models fitted with restricted maximum likelihood.[41] We also conducted Satterthwaite's method to approximate *F*-tests and degrees of freedom on the quantitative measures. Significant results identified from this method were less conservative than those calculated by the Kenward–Rodger's approach, therefore, increasing the confidence in our statistical reporting.

3 | RESULTS

3.1 | Qualitative whisker behaviour

The whisking scores from the qualitative measures show that, while all wildtype mice whisked, with median of 3, the 3xTg-AD mice had lower scores with medians of 2–3 ($H [5, 183] = 39.9$, $p < 0.001$; Figure 3A). At 12.5 months ($p = 0.008$) and 17 months ($p < 0.001$) of age the 3xTg-AD mice had significantly reduced whisking scores compared with the age-matched wild types, showing more whisking movements which were only protractions in the 3xTg-AD mice, rather than the protractions and retractions associated with whisking in the wildtype mice. The whisking scores of the 17-month-old 3xTg-AD mice were also significantly lower than those of the 3-month-old 3xTg-AD mice ($p = 0.020$). There were no significant differences in HTA scores between 3xTg-AD and wildtype mice ($H [5, 101] = 6.74$, $p = 0.241$, Figure 3B). During object exploration there were significant differences in spread reduction ($H [5, 183] = 20.6$, $p < 0.001$) and CIA ($H [5, 183] = 26.4$, $p < 0.001$) between 3xTg-AD and wildtype mice. The 12.5-month-old 3xTg-AD mice had significantly lower whisker spread reduction values than their wildtype controls ($p = 0.008$), and these were also lower than the values for 3-month ($p = 0.003$) and 17-month ($p = 0.002$) 3xTg-AD mice (Figure 3C). The CIA scores of the 3-month-old wildtype mice were significantly higher than the age-matched 3xTg-AD mice ($p = 0.008$) and the 12.5-month wildtype mice ($p < 0.001$, Figure 3D). Detailed statistical information for every comparison in qualitative analyses can be found in Supplementary Table 2.

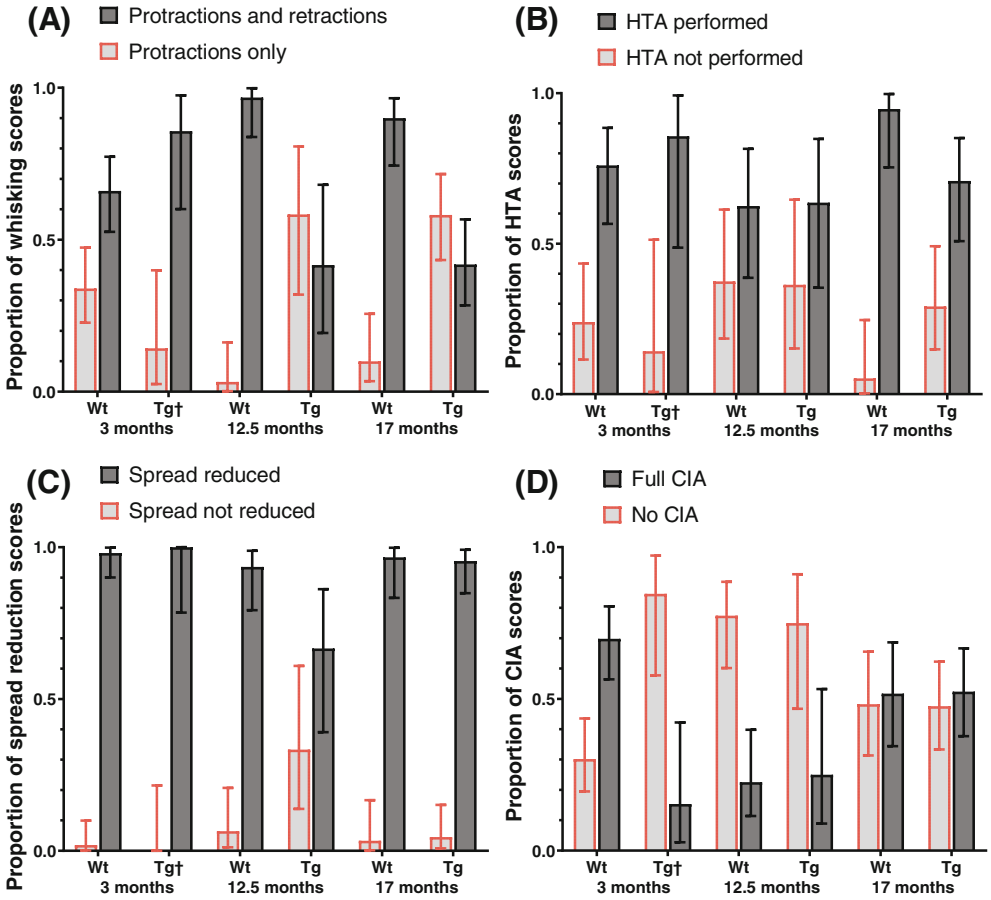

FIGURE 3 Qualitative whisker behaviour scores for (A) whisking, (B) head-turning asymmetry (HTA), (C) spread reduction, (D) contact-induced asymmetry (CIA). The bars indicate the proportion of clips where the behaviour occurred or did not occur, with confidence intervals. † indicates $n = 3$ mice.

3.2 | Pre-contact (PC) quantitative whisker and locomotion movements

For pre-contact whisker amplitude, there were significant main effects of both genotype (F [1, 29.36] = 12.43, p = 0.001) and age (F [2, 27.08] = 4.06, p = 0.029). Specifically, pre-contact whisker amplitude was lower in 3xTg-AD mice than in the age-matched wildtype mice (Figure 4A). Pairwise tests show that these differences were significant in 17-month-old mice (p = 0.013). These differences can also be seen in the pre-contact whisker traces in Figure 6. Furthermore, there was a difference in pre-contact whisker amplitude between 3 and 17-month wildtype mice (p = 0.042) as whisker amplitude increased with age.

For the pre-contact whisker angular position, there were significant main effects of genotype (F [1, 32.82] = 20.38, p < 0.001) and age (F [2, 32.66] = 6.96, p = 0.003). The pre-contact whisker angular position was consistently lower in the 3xTg-AD mice compared with the wildtype mice (Figure 4B), especially at 3 months of age (p = 0.040). These results are supported by the video stills (Figure 5) and the whisker traces (Figure 6), where pre-contact mean whisker angles were lower in the 3xTg-AD mice than the wildtype mice. Wildtype mice at 3 months of age also had larger pre-contact mean angular positions than wildtype mice at 12.5 months (p = 0.038) and 17 months of age (p = 0.006).

In the pre-contact whisker spread, there were significant main effects of genotype (F [1, 32.83] = 10.62, p = 0.003) and age (F 2, [32.67] = 5.61, p = 0.008). However, pairwise tests did not show any significant differences (Figure 4C). There were no significant differences in pre-contact whisker movements in locomotion speed, asymmetry, retraction speed and protraction speed (Supplementary Figure 1). Detailed statistical information for every comparison in PC quantitative analyses can be found in Supplementary Table 3.

3.3 | Contact-related (PC-DC) quantitative whisker and locomotor movements

Both wildtype and 3xTg-AD mice showed robust changes in whisker movements in response to object contact at all ages as indicated by a reduction in locomotion speed (Supplementary Figure 1A), retraction and protraction speeds (Supplementary Figure 1C and D), and an increase in whisker asymmetry (Supplementary Figure 1B) and amplitude (Figure 4A) following an object contact (PC-DC). The whisker traces (Figure 6) show this increase in asymmetry as the left (red) and right (blue) traces separate following object contact in all examples. Since these behaviours were robust in all mice, there were no significant effects of genotype or age in the contact-related (PC-DC) variables of whisker amplitude (Figure 4A), whisker angular position (Figure 4B), locomotion speed, whisker asymmetry, retraction speed and protraction speed (all ps >0.05, Supplementary Figure 1A-D). However, in (PC-DC) whisker spread, there were significant main effects of

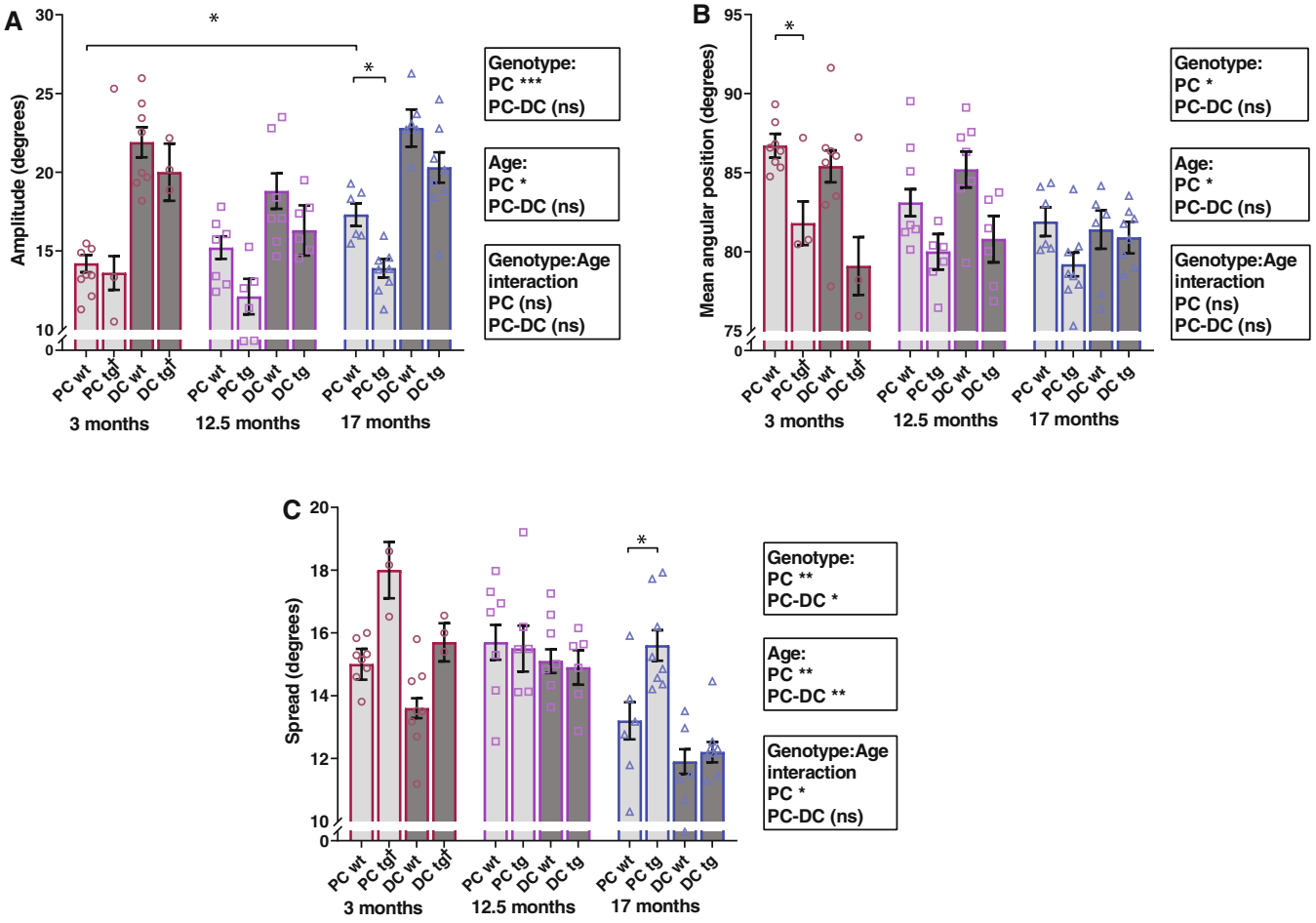

FIGURE 4 Mean angular position, amplitude and spread are affected by genotype and age. All significant differences are between 3xTg-AD and wildtype mice, unless otherwise specified. Panel A: Significant age and genotype effects were found in pre-contact mean angular whisker positions. Pairwise comparisons showed a significant difference in the 3-month age group. Panel B: Significant age and genotype effects were found in pre-contact whisker amplitudes. Pairwise comparisons showed a significant difference in the 17-month age group between 3xTg-AD and wildtype mice, as well as between 3 and 17-month wildtype mice. Panel C: Significant age and genotype effects were found in pre-contact whisker spread. Age and genotype effects were found in contact-related (PC-DC) spread. Pairwise comparisons showed a significant difference in the 17-month age group in (PC-DC) spread as well as between 12.5 and 17-month wildtype mice. The bars indicate the mean values from all the clips (degrees of freedom calculated from a linear mixed-effect model), with standard error bars. Asterisks mark significant values where $p \leq 0.05 = *$, $p \leq 0.01 = **$, $p \leq 0.001 = ***$. Data points show mean values for individual mice, indicated by circles for 3-month mice, squares for 12.5-month mice, triangles for 17-month mice. DC, during contact; PC, pre-contact; PC-DC, contact related behaviours. † indicates $n = 3$ mice.

genotype (F [1, 29.79] = 4.60, p = 0.040) and age (F [2, 28.04] = 6.79, p = 0.004) as (PC-DC) whisker spread was significantly higher in the 3xTg-AD mice than the wildtype mice at 17 months of age (p = 0.041; Figures 4C and 5). There was also a significant difference between 12.5-month and 17-month transgenic mice, with the 17-month transgenic mice reducing their spread more upon contact (p = 0.007) than the 12.5-month mice. Detailed statistical information for every comparison in PC-DC quantitative analyses can be found in Supplementary Table 4.

4 | DISCUSSION

As we hypothesised, the 3xTg-AD mice differed from age-matched wildtype mice in their whisker movements, both prior to and during object exploration. Specifically, we observed significant genotype differences in pre-contact whisking scores, mean angular position and whisking amplitude, as well as during-contact whisker spread, spread reduction scores and contact-induced asymmetry scores. We suggest that these observations may correspond to a whisker motor phenotype in 3xTg-AD mice from 3 months of age and a sensory or attentional deficit, associated with contact-related whisker movements, at 12.5 and 17 months of age.

4.1 | Pre-contact movements

Prior to any object contact, the whisking movements of the 3xTg-AD mice differed from the wildtype mice. The qualitative whisking scores showed that 12.5 and 17-month 3xTg-AD mice did not always make

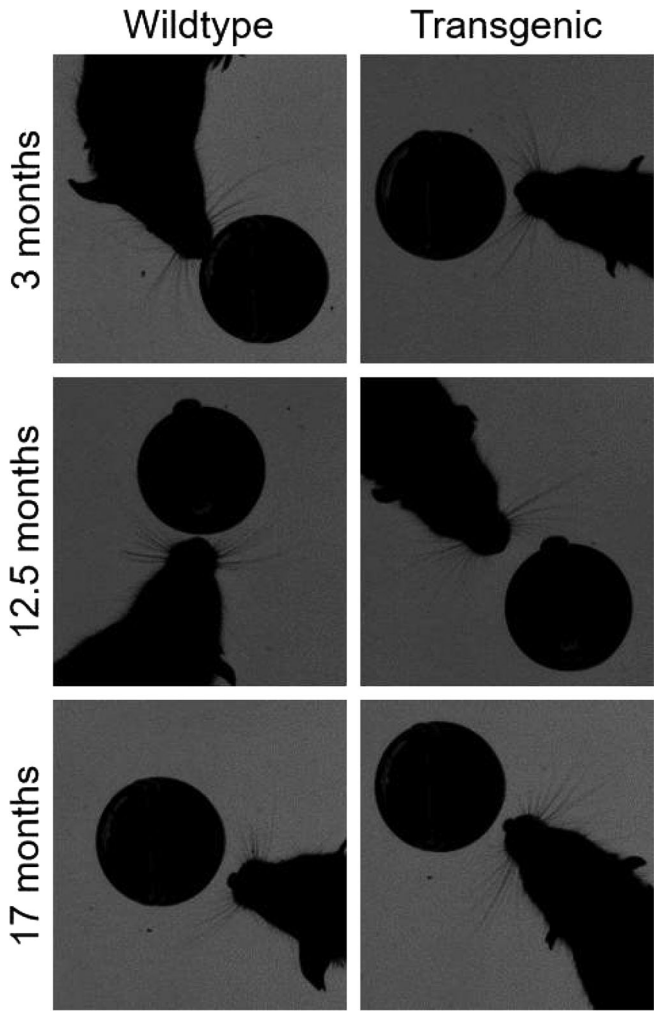

FIGURE 5 Whiskers are more spread out in 3xTg-AD mice during object contact. Video stills of representative mice are shown contacting the object, where whiskers are at maximum protraction. Whiskers of the wildtype mouse are positioned more forward towards the object and less spread out, compared with the 3xTg-AD mouse, especially at 3 and 17 months.

full retraction movements during whisking compared with the wildtypes (Figure 3A). Whisker tracking showed that mean angular positions of 3xTg-AD mice were consistently lower than the wildtype mice, and significantly so at 3 months (Figure 4B). Moreover, pre-contact amplitude was significantly lower in 17-month-old 3xTg-AD mice compared with the wildtypes. These findings suggest the presence of a motor phenotype in 3xTg-AD mice, from perhaps as early as 3 months of age. However, the exact age of this phenotype is unclear from our data and is likely to depend on the exact measure, since it varies between our measures of whisking, whisker angle and amplitude.

The 3xTg-AD mice are known for complex age-related motor abnormalities. The 3xTg-AD mice often perform better than non-transgenic mice in rotarod tasks (Blanchard et al.[42] at 6–7 months; Filali et al.[7] at 12–14 months; Chen et al.[43] at 6 months; Stover et al.[12] at 6 months; Garvock-de Montbrun et al.[13] at 16 months) and have longer stride lengths during locomotion.[12] However, other studies have shown that the stride length,[7,44] walking speed[12,44] and rotarod performance[44,45] can also be unaffected in 3xTg-AD mice. Indeed, locomotion speed was not significantly affected in our mice. However, it is worth noticing that we only measured locomotion speed in several frames as the mouse approached an object, therefore, it is not comparable to the gait analysis or rotarod and balance beam set-ups used by other studies. Some studies have even shown a reduced motor phenotype in 3xTg-AD mice. For instance, Garvock-de Montbrun et al.[13] showed that, despite the enhanced rotarod performance, 3xTg-AD mice at 16 months of age display a reduction in walking distance and speed compared with the wildtype mice in a balance beam task, suggesting an age-related decline in motor performance. Orta-Salazar et al.[46] also found a reduction in locomotion distance and time in 11-month 3xTg-AD mice in an open field test. Overall, we did not observe any evidence of an enhanced motor phenotype in the 3xTg-AD mice. In fact, our results are more in favour of a reduced motor phenotype, starting from reduced whisker angles at 3 months, and then seeing changes in whisking capacity at 12.5 and 17 months, later also showing up as reduced whisker amplitude at 17 months. One issue in the analysis of motor phenotypes in the 3xTg-AD mice is the background strain used. Background strains can have a significant effect on behavioural phenotypes[47] and the 3xTg-AD mice are available from the JAX Labs on three different backgrounds: B6;129 (Stock No. 004807), 129S4 (Stock No. 0319881), and C57BL/6J (Stock No. 033930). Recent research[48] suggests that the motor phenotype of the 3xTg-AD mice on the C57Bl6 background differs from that of the mice on the B6129 background that we used.

We observed variation in whisker movements between wildtype mice of different ages. Specifically, pre-contact whisker amplitude was significantly higher in 17-month wildtype mice compared with 3-month wildtype mice, and pre-contact mean angular position was significantly higher in 3-month wildtype mice compared with older mice. Very young mice (10-13-days-old) also have smaller whisker amplitudes than weaned (21-days-old) mice.[34] Therefore, there might be a tendency for pre-contact whisker amplitude to increase with age in wildtype mice. Although studies of age-related changes in whisker movements are few, Garland et al.[23] show a visible amplitude increase in older wildtype mice when testing Q175, Hdh Q150 and Hdh Q250 mouse models of Parkinson's disease (all mice tested at 10, 20 and 90 weeks, Hdh Q150 and Hdh Q250 mice also tested at 55 weeks; amplitude increasing at every age). They also show decreasing mean angular position in wildtype mice when testing the R6/2 CAG250 mice (decreasing from 8 to 10 weeks and from 12 to 18 weeks). However, these age-related changes were not statistically evaluated in their work. Investigating the changes in whisker movements over an animal's lifecycle would be a useful addition to this work.

Data from 17-month-old mice analysed by Simanaviciute et al.[11] were in agreement with our data, as they found that 17-month-old female 3xTg-AD mice had lower whisker angular positions than wildtype mice. However, they also found that retraction speed was significantly lower in the 3xTg-AD mice. While retraction speed was

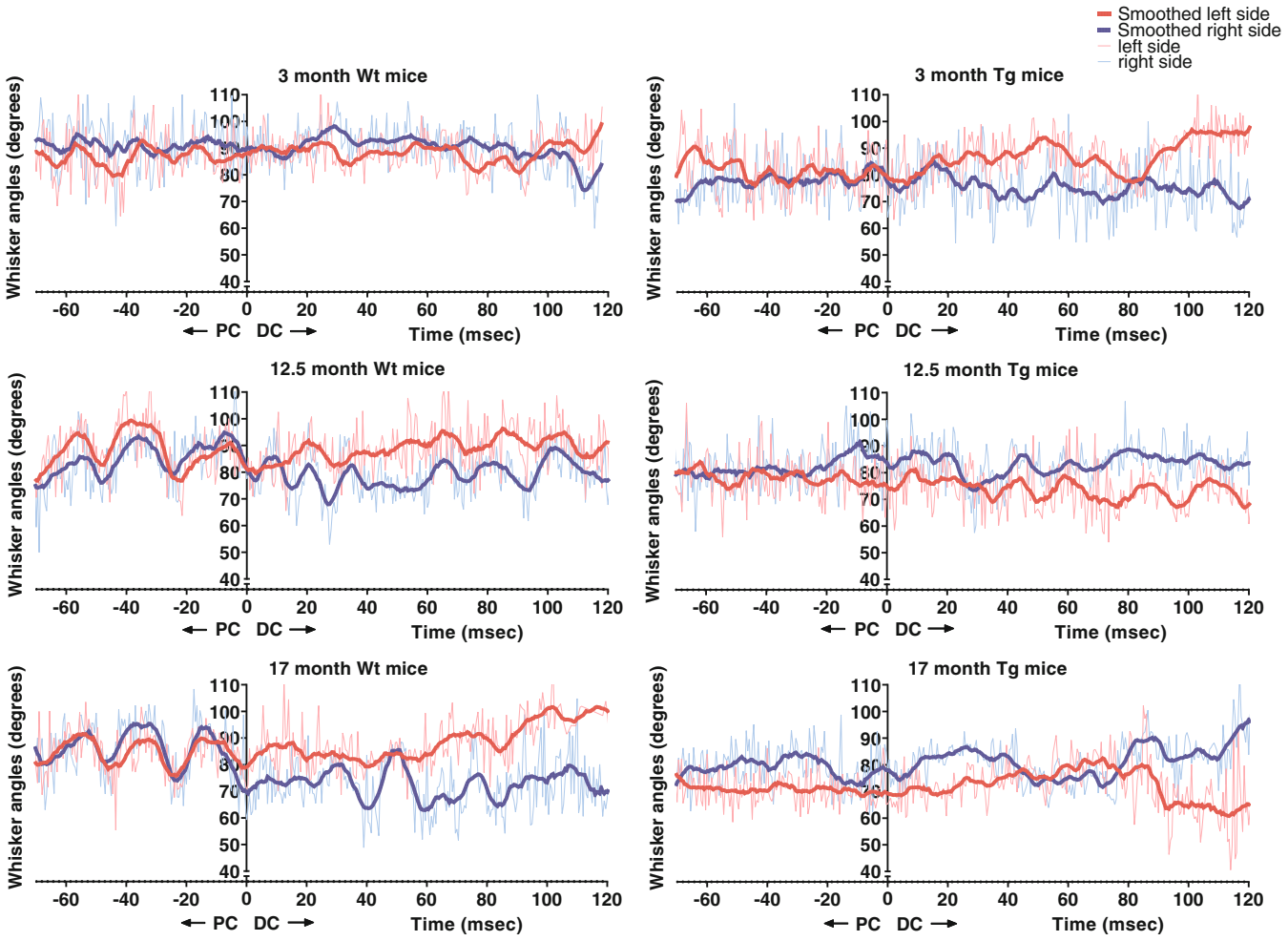

FIGURE 6 Example whisker angle traces of wildtype and 3xTg-AD mice at each age. Raw data points are shown in fine lines, and smoothed data (2nd order, 15 neighbours) are presented in thicker lines. Red colour traces are from the whiskers on the left side, and blue from the right side. 0 msec is the point of contact on the x-axis; therefore, left from the *Y*-axis is PC and right from the *Y*-axis is DC.

consistently lower in our 3xTg-AD mice compared with the wildtype mice (Supplementary Figure 1C), this difference was not significant in our analyses. Simanaviciute et al.[11] used per-clip measures for statistical analyses, whereas we use a stricter linear mixed effect model here. In statistical analyses, treating every trial as an independent data point can lead to pseudorepetition[49] and inflate the power of the statistical test. Therefore, per-trial, or, in this case, per-clip measures should not be used as independent data points, despite this often occurring in animal studies, especially where the sample size drops due to unforeseen experimental circumstances or data quality issues. In this case, we recommend a mixed-effect model that automatically determines degrees of freedom for the dataset instead of using standard parametric and non-parametric tests.[50,51]

4.2 | Contact-related movements

The 3xTg-AD and wildtype mice at all ages made robust object contact-related whisker movements, as indicated by a decrease in whisker speeds, spread, and increased amplitude and asymmetry following whisker contact (Figure 4B and C for amplitude and spread; Supplementary Figure 1 for all other parameters). Contact-related spread was affected in the 3xTg-AD mice, compared with the wildtype control mice. In the qualitative scoring, 12.5-month 3xTg-AD mice reduced whisker spread following contact less often than the controls. In the quantitative tracking, contact-related whisker spread was significantly higher in the 3xTg-AD mice than wildtypes at 17 months. 17-month 3xTg-AD mice also reduced their whisker spread following contact more than 12.5-month 3xTg-AD mice. It is unknown exactly what the sensory implications are of reducing whisker spread following contact, although it seems to play a role in increasing the number of whiskers contacting an object.[30] Why there is a difference in age in the spread reduction upon contact is not clear and demonstrates the need for more research in this field.

While some of these contact-related changes tend to be robust across animals,[11] some are still relatively variable and do not occur on every object contact. For example, 3-month-old wildtype mice show CIA significantly more often than other wildtype or 3xTg-AD mice

(Figure 3D), and HTA seems to be quite variable (Figure 3B). The reason for this is unknown, although it is likely due to variation in behaviour and motivation between individuals. Spread reduction, HTA and CIA have all been associated with orienting of the whiskers towards a region in space or an object, and hence with the animal's attention.[33] Contact-related whisker movement deficits observed in whisker spread and spread reduction could, therefore, imply an attentional deficit in 3xTg-AD mice. Attentional deficits have previously been documented in these mice in a visual task,[52] although in any sensory task it is challenging to separate attentional and sensory deficits.[52] Overall, our results suggest that contact-related sensory or attentional whisker movement deficits are likely to be present in 12.5 and 17-month-old 3xTg-AD mice.

We further compare our findings with those of other studies involving behavioural and cognitive tasks in Supplementary Table 5. Overall, in our study, and those of Stevens and Brown[14] and Fertan et al.[5,16,20] there is an early behavioural phenotype at 2–4 months old. This age group shows the most deficits in working memory and spatial learning,[5,14,16,20] despite being at the early stage of Alzheimer's disease. Our results show contact-related whisker movement differences at this age too – especially in contact-induced asymmetry scores and asymmetry, which may be associated with attentional or cognitive disturbances. We also describe an early motor phenotype, with pre-contact whisking amplitude significantly affected in these young mice. The 6-month group, which we did not test here, did not show any differences in the previous studies[14,16]; however, they observed some significant differences in working memory and spatial learning in the 12–13-month-old mice (Supplementary Table 5). We also observed differences in contact-related spread reduction and in pre-contact whisking scores at this age, perhaps indicating both motor and cognitive deficits. Surprisingly, deficits observed in 12–13-month-old mice were not maintained in older animals at 15 months in the studies by Stevens and Brown.[14] Indeed, previous studies do not show differences at later stages. We did not test 15-month-old animals; however, at 17 months, mice showed differences in both contact related and pre-contact measures, with whisking scores being maintained from the 12-13-month-old group. This suggests that in later stages of the disease, whisker movement measurements might be a better test to adopt than other, more standard behavioural tasks.

4.3 | Limitations

After the data selection process using our validated criteria, only three mice could be included in the 3-month 3xTg-AD group. We recognise that this sample size is low; however, we have kept the data with additional indication for a low sample size in figures and figure captions. We are also confident that the statistical method selected is appropriate to make the most of the uneven sample sizes. The difficulty of including more clips from this group might indicate that the 3-month 3xTg-AD mice behave differently from the other groups, since their clips did not often fit the selection criteria, while we were able to include a lot more clips from their control group. This could mean that we need to refine the data collection method to focus on collecting more clips from the young mice, given that in the previous studies from this laboratory as well as this study, the young female mice seem to be affected the most.

4.4 | Future recommendations

Following recommendations from Fertan et al.,[16] we observed the mice at different time points to examine age-related behavioural changes. However, since behavioural measures can be relatively variable, observing the same mice at each time point in a longitudinal study might be more beneficial than observing different groups of mice in a cross-sectional study. Nevertheless, it is rather difficult to conduct such a study, especially to 17 months, due to the increased mortality rates in older 3xTg-AD mice.[19] In addition, repeat testing of the same animal can impact behavioural tasks, as animals will habituate and learn tasks over time, which may affect their behaviour.[53] Indeed, we have previously shown that a mouse model of anxiety has different whisker movements to control mice[24]; therefore, an altered sensitivity to stress is likely to affect our results. The lack of automation of our set-up may also confound testing over different ages, while here we made sure that all data was collected over a period of just a few days, with all the equipment kept the same throughout.

As there are clear sex differences in 3xTg-AD mice[16,18] and whisker movements differ between sexes in other mouse models,[11,23,25] investigating whisker movements in male 3xTg-AD mice at different ages would be beneficial. It would also be interesting to investigate whether the amyloid quantity in the barrel cortex is related to whisking impairment in 3xTg-AD mice. We have previously shown that models of cortical development disorders have whisker movement deficits (in Robo3R3–5-CKO and RIM-DKOSert models), suggesting that cortical differences can affect whisker movements. However, our previous studies have also shown differences in whisker movements in non-neurodegenerative mouse models (MCAO model of stroke and heterozygous Reeler mice, Simanaviciute et al.[11]), which would suggest that whisking impairment is not specifically related to neurodegeneration and amyloid levels in the cortex, but likely caused by many changes in the brain.

In agreement with Simanaviciute et al.,[11] measuring whisker movements is a quick, robust and semi-automated way to capture motor, sensory and cognitive behaviours in rodents. While the qualitative scoring of whisking, spread reduction, CIA and HTA were valuable at assessing whisker behaviour, they require manual scoring and are relatively time-consuming to complete. We wanted to assess whether measuring spread automatically was a more sensitive method than manual scoring, and it has shown differences at more advanced disease stages than were found by manual scoring. Therefore, it might be worth developing ARTv2 to measure these qualitative scorings automatically. Developing quantitative data and better analytical methods will improve the robustness of repeated testing. Our findings differed from Simanaviciute et al.,[11] probably due to the difference in

statistical methods. We suggest using a linear mixed effect model for future analyses (package lme4 in R-studio, Bates et al.[37]) as we did here, which makes the most of smaller and uneven sample numbers, without assuming per-clip or per-trial independence. Small improvements in automation and analysis techniques will also help to develop whisker movements as a powerful behavioural measurement tool, with particular benefits in capturing behavioural deficits in mouse models that show complex or subtle phenotypes, such as in the 3xTg-AD mouse model. Indeed, the barrel cortex has been found to contain amyloid plaques in several mouse models of AD, including Tg19959 mice at 3 months,[54] APP transgenic mice Tg2576 at 17.5 months[55] and APP/PS1 mice at 19.5–21 months of age.[56] In order to understand the relationship between amyloid levels and whisker movement impairments, it would be beneficial to study whisking in these mouse models.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

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