Whisker exploration behaviours in the 5xFAD mouse are affected by sex and retinal degeneration

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Active whisking in mice and rats is one of the fastest behaviours known in mammals and is used to guide complex behaviours such as exploration and navigation. During object contact, whisker movements are actively controlled and undergo robust changes in timing, speed and position. This study quantifies whisker movements in 6- to 7-month old male and female 5xFAD mice, and their C57/SJL F1 wild-type (WT) controls. As well as genotype, we examined sex differences and the effects of retinal degeneration (rd). Mice were filmed using a high-speed video camera at 500 frames per second (fps), under infrared light while behaving freely in three tasks: object exploration, sequential object exploration and tunnel running. Measures of whisker position, amplitude, speed and asymmetry were extracted and analysed for each task. The 5xFAD mice had significantly altered whisker angular positions, amplitude and asymmetry during object contacts and female 5xFAD mice with rd had lower mean angular positions during object contact. There were no significant effects of genotype on sequential object exploration or on tunnel running but differences due to sex and rd were found in both tasks, with female mice making larger and faster whisker movements overall, and mice with rd making larger and faster whisker movements during object contact. There were sex differences in whisker movements during sequential object exploration and females with rd had higher whisker retraction speeds in tunnel running. These data show that measuring whisker movements can quantify genotype and sex differences and the effects of rd during exploratory behaviour in these mice.

KEYWORDS

Alzheimer Disease, exploratory behaviour, mice, motor control, rodent models, sex differences, transgenic, vibrissae, whisker movements

1 | INTRODUCTION

Alzheimer Disease (AD) is an age-related progressive neurodegenerative disorder and is the most frequent form of dementia in elderly persons.[1–4] Mouse models are key to understanding the progression of AD and for developing new AD treatments.[5–8] Many of these mouse models develop Aβ-plaque pathology and cognitive dysfunction, but much less is known about their development of age-related sensorimotor deficits as occurs in AD.[9] The majority of AD mouse models take over 6-months to develop phenotypic symptoms, but the transgenic 5xFAD mouse model exhibits amyloid plaques before 2 months of age[10] and recapitulates many pathological changes observed in AD, including cognitive impairment,[10,11] neuronal cell loss[12] and reduced cerebral glucose uptake.[13]

Behaviourally, the 5xFAD mice have memory impairments, showed by a lack of recognition for novel objects at 6 to 7 months of age[14] and spatial memory deficits.[15,16] They also have motor impairments after 9 months of age,[17–19] age-related deafness[20] and social impairments[11] from 12 months of age. The 5xFAD mice also show a reduction of inhibitory interneurons in Layer IV of the whisker barrel cortex, which leads to changes in vibrissae-related behaviour that include a lack of whisker barbering in the home cage and an avoidance of enclosed spaces, which dissipates when the whiskers are trimmed.[11] Although Jawhar et al[12] found reduced anxiety in 5xFAD

mice in the elevated plus maze (EPM), the results of Flanigan et al[11] suggest that these results are not due to reduced anxiety but to the mice avoiding the closed arms due to over-sensitive vibrissae.[11] In addition, some 5xFAD and wild-type (WT) mice have retinal degeneration (rd) and are completely blind[21,22] as a result of the rd gene (Pde6b^{RD1}) in the SJL/J background strain.[21] These mice, therefore, have a loss of vision and rely more on whisker touch to guide exploration navigation, and other behaviours.

Laboratory rats and mice use their whiskers as their primary sense for exploring their surroundings and employ them in navigation, object exploration and social interactions.[23,24] Whiskers are regularly studied as a model system for investigating fundamental principles of sensory processing.[25–27] During exploration, the whiskers move forwards and backwards (termed protractions and retractions) in a motion called *whisking*, which can occur at rates of up to 25 Hz in mice.[28] Detailed quantitative behavioural analyses have showed that rodents alter the timing, spacing and positioning of their whiskers to maximize sensory information.[28–31] For example, when contacting an object, they reliably and robustly: (a) reduce the retraction speed of the whisk so that their whiskers spend more time in contact with the surface of the object[30]; (b) reduce the amplitude of whisker movements to increase contact duration[29,30]; (c) increase the frequency of whisking to maximize the amount of sampling against the surface[32] and (d) reduce whisker angular positions to prevent whiskers being forced forward into the surface and enable light touches.[30,31] Following unilateral contacts, whisker asymmetry increases, as the side contralateral to the contact is positioned more forward to increase whisker contact, and the side ipsilateral to the contact is positioned more backwards to enable light touches against the surface.[28,30,31] Focussing of the whisker field towards objects during contacts is thought to indicate the spatial attention of the rodent.[33,34]

As the whisker movements of rodents are precisely controlled, and high-speed video footage can be measured using custom-made tracking software, whisking has been proposed as a quantitative behavioural measure of motor control and exploration abilities.[27,35] Indeed, deficits in whisker movements have been seen in mouse models of motor disorders, such as amyotrophic lateral sclerosis[36] and Huntington disease,[37] as well as in models of anxiety.[35] Because whisker movements are linked to attention and exploration, whisking behaviour might be a suitable model for testing cognitive functions in Alzheimer's model mice. In particular, we might expect to see whisker position and movement differences between 5xFAD and WT mice in sequential object exploration and tunnel running tasks, as 5xFAD mice have behavioural deficits in similar tasks.[14]

This study will, for the first time, assess the differences in whisker movements during exploratory behaviour between 5xFAD mice and their WT controls. Three behavioural tasks: object exploration (a well-defined task in whisker exploration), sequential object exploration (which is similar to the novel object task that is often used in AD mouse models) and tunnel running (as used by Fragkouli et al[14] to indicate sensitive whiskers in 5xFAD mice) will be used to determine if whisker movements differ in 5xFAD and WT mice at 6 to 7 months of age. As well as genotype differences, we will examine sex differences and the effect of rd on whisker movements in 5xFAD and WT mice in these three tasks.

2 | METHODS

2.1 | Animals

Fifty-nine 6- to 7-month old male and female 5xFAD mice were included in this study. All mice were the offspring of male hemizygous C57BL/6J × SJL/J F2 5xFAD (B6SJL-Tg (APPSwFILon, PSEN1*M146L*L286V) 6799Vas/Mmjax) and female WT C57BL/ 6J × SJL/J F1 mice obtained from Jackson Laboratories (Bar Harbour, Maine) and bred in our laboratory. The 5xFAD mice have five mutations; three on the APP gene, the Swedish (K670N/M671L), Florida (I716V) and London (V717I) mutations, and two mutations to the PSEN1 gene (PS1), which encodes presenilin-1PS1 (M146L and L286V).[10] As a result of the rd gene (Pde6b^{RD1}) in the SJL/J background strain, some mice have rd and are blind.[21] This type of rd is early onset and severe[21]; indeed, due to early degeneration of the retina there is a complete loss of rods by 35 days of age.[22] At 14 weeks of age, mice with the same RD1 mutation perform only at chance levels on a visual detection task, indicating that they were functionally blind.[38] There may be some variation of symptoms during development due to interactions with other genes,[39] however, by 6 months of age we would expect the mice to be completely blind, with no rods present. All mice were sexed and genotyped for the APP and PS1 transgenes, and the phosphodiesterase-6b retinal degeneration-1 (Pde6b^{RD1}) allele by Dr Chris Sinal (Pharmacology Department, Dalhousie University) from tissue samples obtained from ear punches at weaning. In our colony, around 50% of pups born had the 5xFAD gene and 25% had the Pde6b^{RD1} gene.

The mice were weaned at 21 days of age, separated into groups of 2 to 4 same-sex littermates and housed in 30 × 18 × 12 cm polycarbonate cages with wire tops and ad lib access to food (Purina Rodent laboratory chow #5001). The colony room was maintained at 22°C ± 2°C with a 12:12 hour reversed light:dark cycle (lights off at 9:45 AM). All behavioural testing was completed during the dark (active) portion of the light:dark cycle. 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.

2.2 | Apparatus and tracking

Mice were taken to the testing room, removed individually from their home cage and placed in a transparent, Perspex, rectangular arena (30 × 50 × 15 cm), which was lit from below by a bright, infrared light box (PHLOX LEDW-BL-400/200-SLLUB-Q-1R-24V). Mice were tested in three tasks that were designed to investigate whisker exploratory behaviours, including *object exploration*, *sequential object exploration* and *tunnel running* (Figure 1). Each mouse was introduced to the task arena, and clips were collected immediately during the initial exploration phase. The mouse spent only 10 to 12 minutes in each task arena, which was enough time to collect the allocated number of video clips for each task for each mouse as described below. In the object exploration task, a Pyrex bottle-stop was placed in the centre of the arena and a video clip was taken every time the mouse approached and investigated the object. Approximately 15 video clips

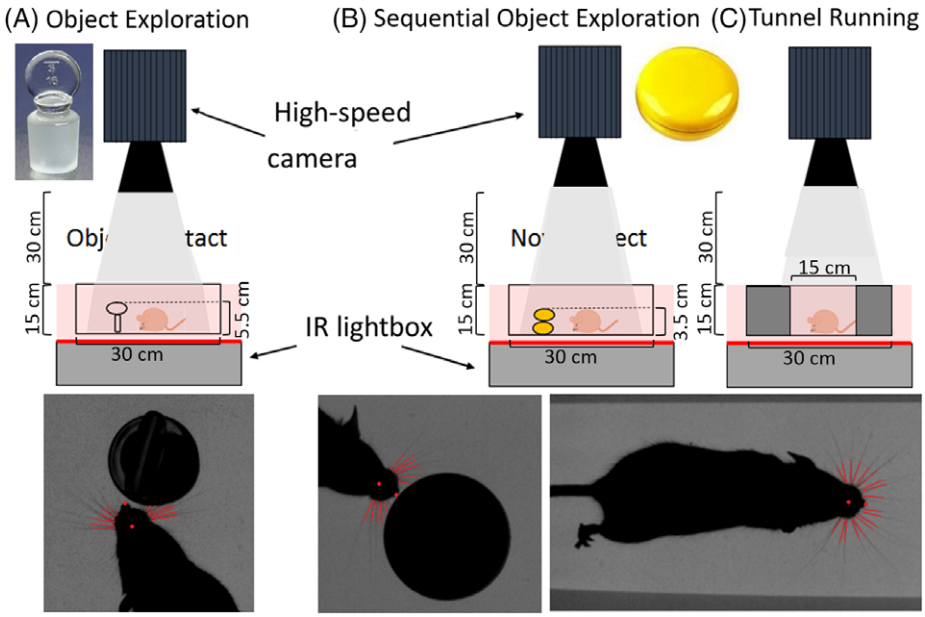

FIGURE 1 Schematic of the whisker behaviour tasks and tracking. The object exploration task (A) records exploration of a glass bottle stopper; the sequential object exploration task (B) records exploration of the bottle stopper and a novel small yo-yo object (photographs of both are inset). The tunnel running task (C) involves the mouse travelling continually down an enclosed corridor. Behaviours were recorded in semi-darkness in all tasks using an infrared lightbox and a high-speed video camera, with points on the nose, head and whiskers (in red) tracked in all suitable videos

were collected from each mouse. The bottle-stop object (5.5 cm tall) was semi-transparent, with a brushed textured bottom (1.5 cm tall with a 2.3 cm diameter), the top was domed with a smooth, transparent, circle on top (3 cm tall with a 2.5 cm circle diameter), as can be seen in Figure 1A. In the sequential object exploration task, the same arena was used as for object exploration, with four clips collected when the mouse explored the original object (bottle-stop). This was then replaced with a second (novel) object, a mini yo-yo, and another five video clips were collected. The yo-yo object was 3.5 cm tall with a 3.5 cm diameter; it was yellow, with smooth, gloss plastic, as shown in Figure 1B. This is a modification of the usual novel object task, where the familiar and novel object are both included in the same trial, because the high-speed camera can only image the whiskers during object contact if it is directly above the object. If two objects were present, they would obscure the whiskers, as a vertical view cannot be achieved on both objects simultaneously. In the tunnel running task, black Perspex dividers were placed in the arena to make an enclosed tunnel of 40 long × 15 wide × 15 high cm. Mice were filmed running up and down the tunnel and approximately eight clips were collected per mouse to represent one run along the tunnel. The sample size for each group is presented in the figure captions corresponding to each task (Figures 3, 5 and 7). All mice were filmed in the object exploration task first, then 1 to 2 days later in the tunnel running task, and then 3 to 4 days later in the sequential object exploration task.

In all tasks, filming was conducted under infrared light so the mouse would be filmed in perceived darkness. Mice were filmed from above using a digital high-speed video camera (Phantom Miro ex2) recording at 500 fps with a shutter-speed of 1 millisecond and a resolution of 640 × 480 pixels, suspended 30 cm above the top of the arena (Figure 1). Multiple 700-millisecond video clips were collected opportunistically (by manual trigger) when the animal moved in the camera's field of view. This was in accordance with other mouse whisker and locomotion studies,[35–37,40] as the camera was unable to store continuous footage, due to the large file size of high resolution, high-speed videos. Clips were selected with respect to a set of exclusion criteria (see individual sections for details), and in each clip, the snout and whiskers of the mice were tracked using the BIOTACT Whisker Tracking Tool.[41] This tracker semi-automatically locates the orientation and position of the snout, and the angular position (relative to the midline of the head) of each identified whisker field. Tracking was validated by manually inspecting the tracking annotations overlaid on to the video frames (Figure 1).

The movement of the entire whisker field was determined, frame-by-frame from the unsmoothed mean of all the tracked whisker angular positions for each side of the snout, to determine the naïve mean angle (nma),[28] and the following variables were calculated from the whisker angular position data. *Mean angular position* was calculated per-clip, and averaged for the left and right whisker sides. *Asymmetry* was calculated as the mean difference between the left and right mean angular positions. The mean angular position was removed from the whisking angle time series and the root mean square (RMS) value was computed to give the RMS whisking *amplitude*. These time series were approximately sinusoidal, so the "peak-to-peak whisking amplitude" was estimated by multiplying the RMS whisking amplitude by $2\sqrt{2}$.[42] This estimate of amplitude is reasonably robust to accommodate departures from a purely sinusoidal pattern. Mean angular *retraction* and *protraction speeds* were calculated as the average velocity of all the backward (negative) and forward (positive) whisker movements, respectively. Angular position, amplitude, retraction and protraction velocities were calculated individually for each whisker side, and then averaged over the left and right sides to give one value of each per clip. Raw data from each task can be referred to in an excel document in Supporting Information Appendix S1. The time taken to explore each object (first and second) was recorded, however, this was limited

to the length of the video clip (700 milliseconds), so could only include very short explorations. It did not differ between mice, over time or between the original and second object, so this data was not included in any analyses. *Whisking frequency* was also calculated using Fourier transforms of the whisker angular position traces, however, it did not differ significantly in any of the tests and was not included in any analyses. During behavioural testing, the genotype, gender and rd were recorded for each mouse, however, all clip selection, tracking and analysis was performed blinded. Although not directly measured here, we did not notice any stress or anxiety-like behaviours in any of the mice. Once introduced to the arena, they all explored away from the walls, at the centre of the arena, within the first minute, and performed a grooming bout at around 5 minutes into the testing session. There was no excessive defecation or urination by any of the mice.

2.3 | Object exploration

All 59 mice were used in this task (genotype: 30 5xFAD, 29 WT; sex: 30 male, 29 female; RD: 29 without, 30 with; exact mouse numbers for each group are shown in Figure 3), and a total of 662 video clips were collected from mice exploring the bottle-stop object. These clips were manually reviewed and selected for tracking using an automated tracker if: (a) the mouse was clearly in frame; (b) both sides of the face were visible; (c) the head was level with the floor (no extreme pitch or yaw); (d) there was a period prior to contact (pre-contact) of at least 50 frames and (e) a period during contact, where the nose-tip was clearly visible for at least 50 frames. All clips were then trimmed to include only periods of correct tracking, or removed from the analysis entirely. This left 387 clips for analysis, with 5 to 11 clips per mouse. Based on these criteria, around 44% of all clips were included for further analysis (genotype: 39% 5xFAD, 48% WT; sex: 48% male, 39% female; RD: 51% without, 36% with) (Appendix S1). Female 5xFAD mice with rd were the most affected (χ^2 = 16.0017, df = 11, P = 0.003). The inclusion criteria were relatively equal among all groups of mice, and the differences occurred in the automated whisker tracking stage, which we could not control.

A mixed-model multivariate analysis of variance (MANOVA) was conducted on all the whisking data with pre-contact and contact as the within factors, genotype (5xFAD, WT), sex (male, female) and rd (present, absent) as the between factors. Partial-eta squared (η^2p) was used for effect sizes (0.2 = small, 0.5 = medium, 0.8 = large). Individual MANOVAs using the same format were then conducted on just the male mouse data, and then the female mouse data, due to the large effects of sex multivariate ANOVAs were conducted to examine the effects of genotype and rd on the individual whisker variables. Analyses were also conducted to determine if there were differences in these whisker variables over subsequent contacts; however, no differences were observed in any of the mice so all contact data was combined for analysis.

2.4 | Sequential object exploration

From the original 59 mice, 27 were randomly selected for this task (exact numbers for each group are shown in Figure 5), and a total of 282 video clips were collected from mice exploring the first object (bottle-stop) and then the second object (yo-yo). The clips were manually reviewed and selected for automatic tracking if they met the five criterion listed above for object exploration, which resulted in 108 clips for analysis, with 5 to 8 clips per mouse. Around 39% of all clips were included for further analysis (genotype: 39% 5xFAD, 41% WT; sex: 43% male, 37% female; RD: 54% without, 43% with) based on these criteria, groups did not differ in the number of clips excluded (χ^2 = 0.9968, df = 11, P = 0.910) (Appendix S1). A mixed-model MANOVA was conducted on all the contacting whisking data with the object type (original [first]: bottle-stop, and second: yo-yo) as the within factor, genotype (5xFAD, WT), sex (male and female), and rd (present, absent) as the between factors, using η^2p for effect sizes. Individual MANOVAs were then conducted on the male mouse data, and then the female mouse data due to the large effect of gender; multivariate ANOVAs examined the effect of genotype and rd on the individual whisker variables, Bonferroni corrections were not needed here and throughout (as per Bock[43]).

2.5 | Tunnel running

The same 27 mice from the sequential object exploration task were also used for this task (exact numbers for each group are shown in Figure 7), and a total of 227 clips were collected from mice in the tunnel running task. Clips were manually reviewed and selected for automatic tracking as described above, with the added criterion that the mouse travelled, without pausing, from one end of the tunnel to the other, resulting in 126 clips, with 4 to 6 per mouse. Around 58% of all clips were included for further analysis (genotype: 62% 5xFAD, 55% WT; sex: 58% male, 59% female; RD: 63% without, 54% with) based on these criteria, which did not differ between groups (χ^2 = 0.8792, df = 11, P = 0.9275) (Appendix S1). A multivariate ANOVA was conducted on all of the whisking data with genotype (5xFAD, WT), sex (male, female) and rd (present, absent) as the between factors. Individual multivariate ANOVAs were then conducted on male and female mouse data separately for genotype and rd effects.

3 | RESULTS

3.1 | Object exploration

During contact with the object, the whiskers were positioned less forward and more asymmetrically (Compare Figures 2a-d with Figures 2e-f), compared with whisker positions prior to contact. Overall, there were significant effects of genotype (MANOVA: $F_{6,373}$ = 2.275, P = 0.036, η^2p = 0.035), sex (MANOVA: $F_{6,373}$ = 10.172, P < 0.001, η^2p = 0.141) and rd (MANOVA: $F_{6,373}$ = 3.194, P = 0.005, η^2p = 0.049) on the whisker variables during contact with the object in this test. All mice made robust changes in whisker protraction and retraction speeds, angular positions, asymmetry and amplitude following contact with the object (Figure 3). All of the mice changed their whisker movements following contact with the object (MANOVA: $F_{6,375}$ = 156.074, P < 0.001), with protraction speed (ANOVA: $F_{1,380}$ = 414.615, P < 0.001, Figure 3a), retraction speed (ANOVA: $F_{1,380}$ = 268.661, P < 0.001, Figure 3b) and amplitude

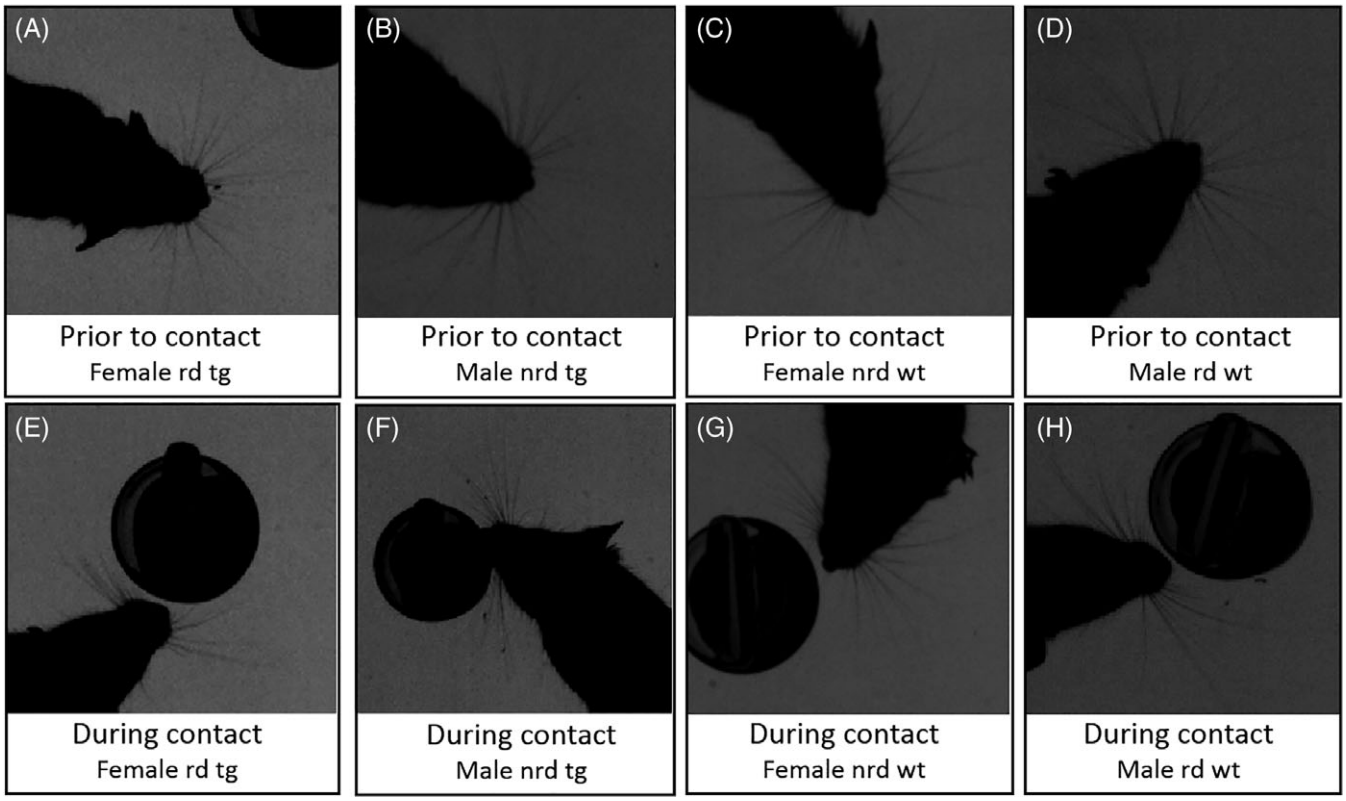

FIGURE 2 Screenshots of whisker positions prior to and during object contact. All screenshots were taken at maximum whisker protraction. Prior to contact, the whiskers were more forward and symmetrical (A-D), than during contact (E-H). NRD, no retinal degeneration, tg = 5xFAD transgenic

(ANOVA: $F_{1,380}$ = 747.503, P < 0.001, Figure 3e) all increasing following a contact. Mean angular position decreased following contact with the object (ANOVA: $F_{1,380}$ = 188.300, P < 0.001, Figure 3c). Whisker asymmetry increased following a contact (ANOVA: $F_{1,380}$ = 689.015, P < 0.001, Figure 3d) as can be seen by comparing Figure 2a-d to Figure 2e-h.

As the difference between the sexes had such a large effect (η^2p > 0.8), with females having much larger and significant differences between pre-contact and contact values for protraction speed, retraction speed, mean angular position and asymmetry, and smaller differences in amplitude between pre-contact and during contact (Figure 3), data were then analysed separately for males and females. Male mice all showed robust changes in whisker variables following object contact and there was a significant genotype effect (MANOVA: $F_{6,209}$ = 2.841, P = 0.011) but no significant effect of rd on the whisker variables in male mice (MANOVA: $F_{6,209}$ = 1.093, P = 0.368), but. Figure 3a shows that male 5xFAD mice had higher protraction speeds than WT males (ANOVA: $F_{1,214}$ = 4.176, P = 0.042) and Figure 3d shows that asymmetry was also higher in the 5xFAD than the WT males (ANOVA: $F_{1,214}$ = 15.306, P < 0.001). Indeed, the 5xFAD mice without rd had the highest levels of whisker asymmetry, especially following object contact (ANOVA: $F_{1,214}$ = 3.971, P = 0.048), which can be seen clearly by comparing Figure 2f with Figure 2e,g,h.

Female mice also showed robust changes in whisker variables following object contact, with significant differences due to genotype (MANOVA: $F_{6,159}$ = 3.473, P = 0.003) and rd (MANOVA: $F_{6,159}$ = 5.734, P < 0.001). Female 5xFAD mice had significantly lower mean angular position values than WT females (ANOVA: $F_{1,164}$ = 6.677, P = 0.011, Figure 3c). Females with rd had higher values of mean angular position than females without rd before object contact, but had lower mean angular position scores following contact (ANOVA: $F_{1,164}$ = 17.468, P < 0.001; Figure 3c), as can be seen in Figure 2a,c,e,f. Female 5xFAD mice with rd had the lowest mean angular position scores following a contact (ANOVA: $F_{1,164}$ = 5.782, P = 0.017; Figure 3c). Females with rd also had higher values of asymmetry (ANOVA: $F_{1,164}$ = 9.162, P = 0.003; Figure 3d) and amplitude than females without rd, following object contact (ANOVA: $F_{1,164}$ = 13.309, P < 0.001; Figure 3e).

3.2 | Sequential object exploration

All mice showed significant changes in whisker movements on the second object, compared with the original object, with all mice having consistently smaller amplitudes on the second object. Overall, there were no significant effects of genotype (MANOVA: $F_{5,40}$ = 2.056, P = 0.091, η^2p = 0.204), or rd (MANOVA: $F_{5,40}$ = 1.719, P = 0.152, η^2p = 0.177) on the whisker variables exploring the original and second object. However, there was a large and significant sex difference (MANOVA: $F_{5,40}$ = 4.442, P = 0.003, η^2p = 0.357), so data was then analysed separately for males and females.

Figure 4 shows whisker traces of a male 5xFAD mouse without rd, which illustrates the decrease in whisker amplitude and asymmetry when investigating the second object compared with the original object, which was observed in all mice, irrespective of genotype, sex

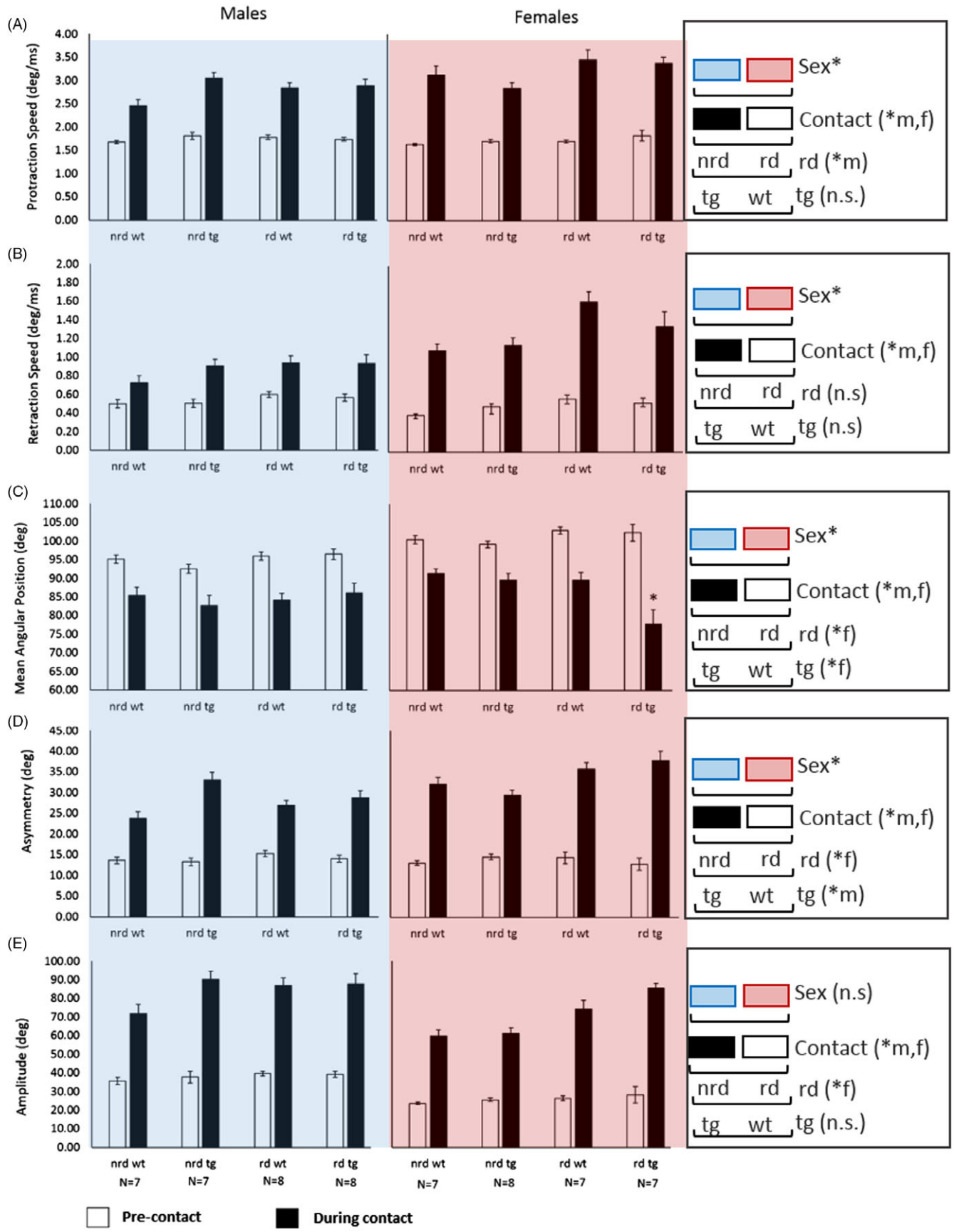

FIGURE 3 Summary of whisker movements during the pre-contact and object contact periods in male (left) and female (right) mice. Mean (±SEM) protraction speed (A) and retraction speed (B) both increased during object contact. Angular position (C) decreased during contact, while asymmetry (D) and amplitude (E) both increased during object contact. Female 5xFAD mice with retinal degeneration had lower angular positions during contact than other mice. *$P < 0.05$, with the sex of the interaction (m = male and f = female) indicated in the key. NRD, no retinal degeneration, tg = 5xFAD transgenic. All whisker variables measured showed significant changes on object contact (indicated by the white and black bars). The column on the right summarizes the results of the ANOVAs for differences due to sex, object contact, retinal degeneration and genotype

and rd. Both male (ANOVA: $F_{1,26}$ = 6.976, P = 0.014; Figure 5d) and female mice (ANOVA: $F_{1,19}$ = 19.612, P < 0.001; Figure 5d) showed significant decreases in whisker amplitude while exploring the second object. Male mice also showed a decrease in asymmetry during exploration of the second object (ANOVA: $F_{1,26}$ = 9.289, P = 0.005; Figure 5c) and male mice with rd had greater asymmetry when investigating the original object, and lower asymmetry with the second object than males without rd (ANOVA: $F_{1,26}$ = 4.253, P = 0.049; Figure 5c).

Female mice showed a reduction in protraction speed (ANOVA: $F_{1,19}$ = 4.916, P = 0.039; Figure 5a) and retraction speed (ANOVA: $F_{1,19}$ = 30.379, P < 0.001; Figure 5b) during second object exploration, compared with exploration of the original object. Female 5xFAD mice had greater retraction speeds with the original object, and lower retraction speeds with the second object, than WT females (ANOVA: $F_{1,19}$ = 7.958, P = 0.013; Figure 5b). Females with rd had greater retraction speeds with the original object and lower retraction speeds with the second object, than females without rd (ANOVA: $F_{1,19}$ = 5.926, P = 0.025; Figure 5b) Indeed, 5xFAD female mice with rd had the highest retraction speeds on the original object (P = 0.032; Figure 4d) and the lowest in the second object.

3.3 | Tunnel running

Overall, there was no significant effect of genotype (MANOVA: $F_{5,114}$ = 1.633, P = 0.157, η^2p = 0.067), rd (MANOVA: $F_{5,114}$ = 1.512, P = 0.192, η^2p = 0.062) or sex (MANOVA: $F_{5,114}$ = 2.298, P = 0.050, η^2p = 0.092) on the whisker variables during the tunnel running task. The data was split by sex to further investigate genotype and rd effects during tunnel running. Examples of whisker traces from female WT mice show that those with rd had higher amplitudes and retraction speeds than those without rd (Figure 6). Female mice showed no significant genotype differences on these measures (P > 0.05), but females with rd showed significantly higher retraction speeds (ANOVA: $F_{1,64}$ = 5.041, P = 0.028; Figure 7a) and amplitudes than females without rd (ANOVA: $F_{1,64}$ = 4.401, P = 0.040; Figure 7b). Male mice showed no significant effects of genotype or rd on their whisker retraction speed or amplitude (all P > 0.05, Figure 7a,b), and traces looked very similar to those in Figure 6c.

4 | DISCUSSION

All mice made robust changes in whisker movements and positions when they contacted an object. The 5xFAD mice differed from WT mice in whisker movements during object exploration, and mice with rd differed from sighted mice in whisker positions and movements in the object exploration, sequential object exploration and tunnel running tasks. The sex of the mice had the largest effects on whisker movements, with male and female mice having large and significant difference in all three of the experimental tasks.

All mice robustly altered their whisker movements during object exploration by increasing protraction and retraction speeds, asymmetry and amplitude and decreasing their angular positions (Figure 3). An increase in contact-induced asymmetry is associated with orienting to an object and maximizing whisker contacts.[31,34] The decrease in angular position is common during exploration; as a mouse orients towards an object, the amount the whiskers are moved forward is reduced to enable light touches, with the whiskers not striking too hard onto the surface.[30,31] The robustness of these findings across all mice supports the use of measures of whisker movements to quantify object exploration in rodents. Exploration has been associated with lower whisker retraction speeds[30,36] and amplitudes,[29,30] so these increases in whisker retraction speed and amplitude contradict results from previous studies. However, they do indicate an increase in the scanning area of the whiskers. Perhaps these variables are more task-specific or variable than asymmetry and angular position.

We did not observe any significant differences between 5xFAD and WT mice in whisker movements or other general behaviour in the tunnel running task. Flanigan et al[11] found that 5xFAD mice avoided a

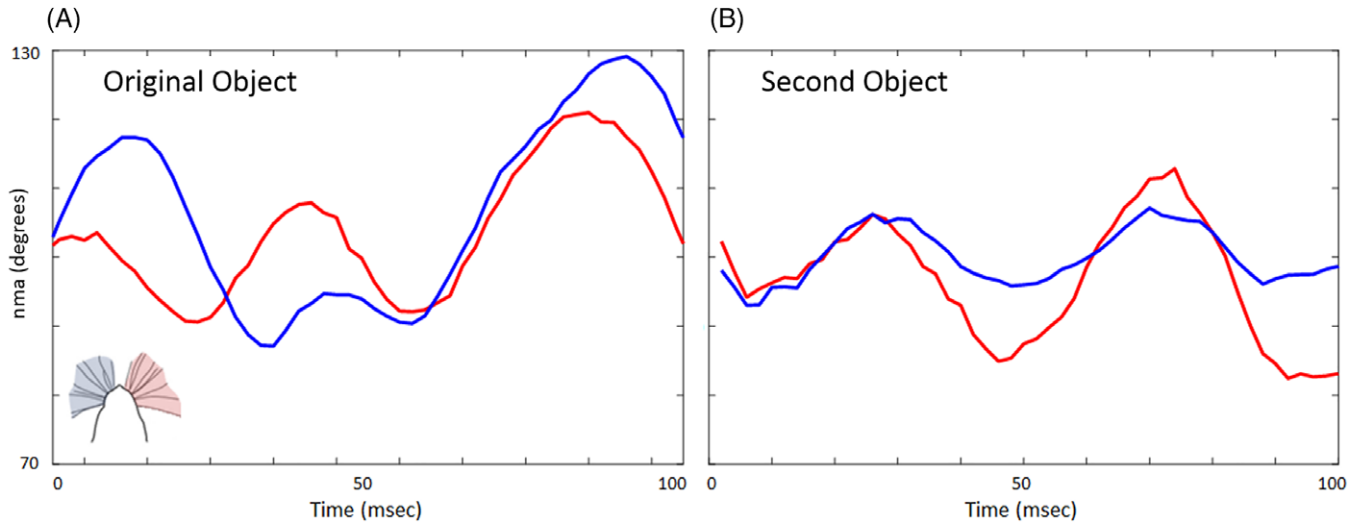

FIGURE 4 Examples of whisker traces from the sequential object exploration task. Whisker measurements of a male 5xFAD mouse without retinal degeneration exploring the original object (A); and the second object (B). Whisker amplitude and asymmetry were both larger when investigating the original than the second object. Left whiskers are shown with blue lines, and right whiskers with red lines. nma is the naïve mean angle, the average angular positions of all the whiskers on that side of the face

closed arm tunnel; however, when their whiskers were removed they no longer avoided the tunnel, suggesting that there might be an increased sensitivity, or aversive overstimulation, of their vibrissae. Although we did not observe any evidence of this in our studies, it may account for the reduced angular position values during contact in the 5xFAD female mice during the object exploration task (Figure 2c). Reduced angular positions would ensure lighter touches and lower force whisker contacts.[31] Although no previous study has measured whisker movements in 5xFAD mice, aspects of their exploratory behaviour have been examined. In an open field test, 12 month-old 5xFAD mice spent more time away from the walls of the arena than WT mice, indicating a reduction in their anxiety.[12] In our object exploration test, the object was in the centre of an open field and we did not notice the 5xFAD mice approaching it faster, or spending more

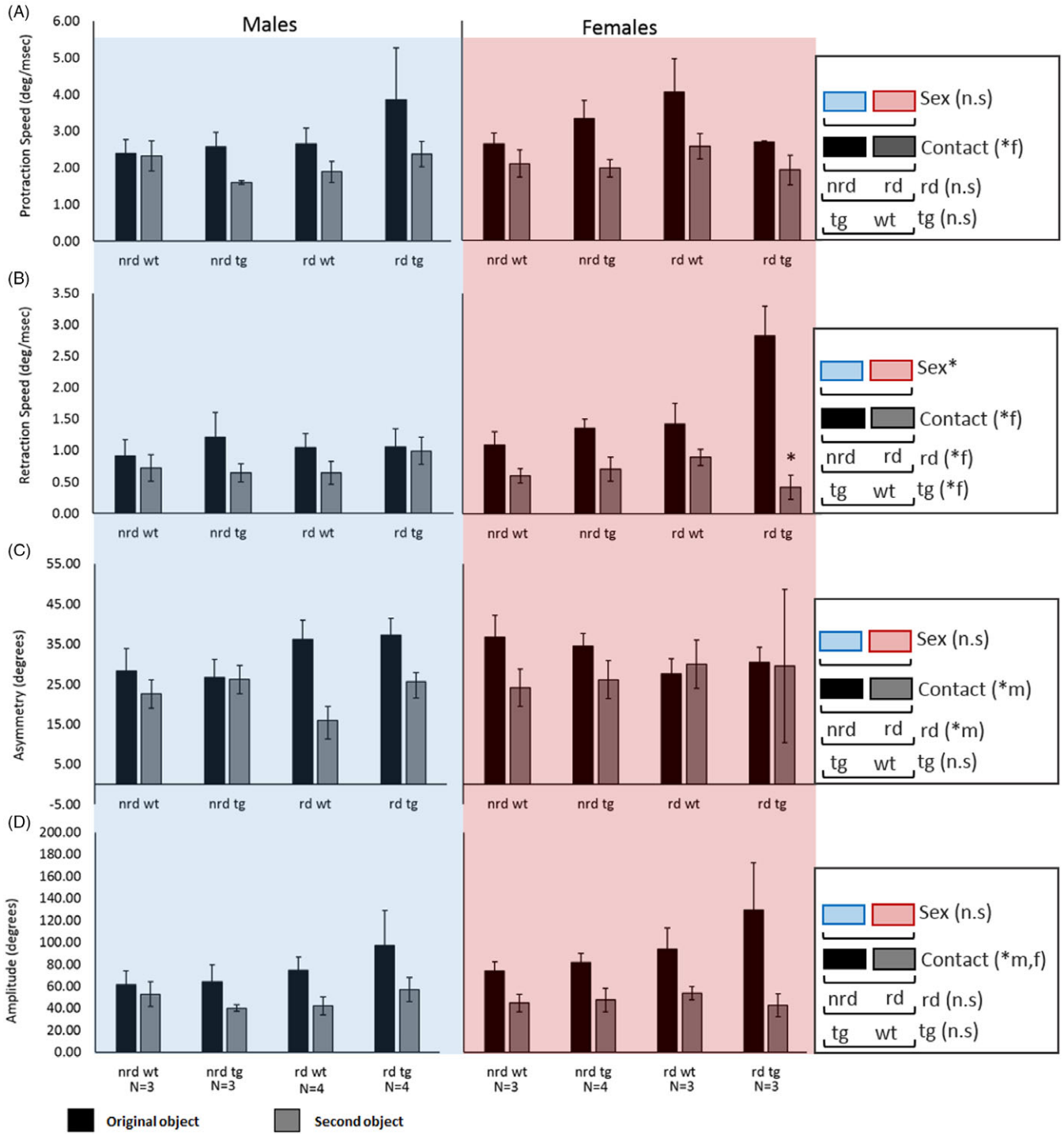

FIGURE 5 Summary of the whisker movements when exploring the original object and the second object by male (left) and female (right) mice. Mean (±SEM) whisker protraction speed (A); retraction speed (B); asymmetry (C) and amplitude (D). Female 5xFAD mice with retinal degeneration had the highest retraction speed on the original object, and lowest retraction speed on the second object, with the sex of the interaction (m = male and f = female) indicated in the key NRD, no retinal degeneration; tg, 5xFAD transgenic. The column on the right indicates whether there are significant differences due to sex, during contact on the original and the second object (*$P < 0.05$)

time in the centre of the arena than WT mice, perhaps because our mice were younger. The 6- to 7-month-old 5xFAD mice have been reported to spend less time exploring novel objects[14]; however, we did not observe a genotype difference in the time exploring the original or second (novel) object. In fact, the reduction in whisker retraction speed in the 5xFAD female mice with rd during exploration of the second object is more representative of an increase in object exploration duration. Indeed, reducing retraction speeds is thought to increase the time the whiskers spend in contact with an object,[30] increasing the exploratory time overall.

In many instances, the 5xFAD mice showed an overall increase in object exploration using their whiskers. However, these changes differed between tasks and sexes. In the object exploration task, female 5xFAD mice had lower angular position values (Figure 2c; Figure 3c) and male 5xFAD mice had increased asymmetry during object contact (Figure 2b; Figure 3d). In the sequential object task, female 5xFAD mice with rd had the lowest retraction speeds (Figure 5b). These behaviours are all associated with an increase in controlled and focussed exploration of an object.[30,33] No motor phenotype has previously been documented in 5xFAD mice at 6 to 7 months of age,[17–19] therefore, these differences in exploratory whisker movements may be due to cognitive or emotional (ie, anxiety) differences between the transgenic and WT mice. However, although not directly measured here, we did not notice any stress or anxiety-like behaviours in any of the mice. Therefore, if both motor deficits and anxiety-like behaviour are unlikely in the 5xFAD mice, changes in whisker behaviour may be due to alterations in sensory processing. It has been suggested that 5xFAD mice might have hypersensitive whiskers due to a reduction of inhibition in the barrel cortex,[11] with the barrel cortex producing large amplitude neural responses with a broad spatial spread following a whisker deflection.[44] It may be that whisker signals are not processed efficiently in the cortex, therefore, their exploratory movements increase to gain more sensory information; or that they are over-sensitive, so decrease whisker angular positions in order to reduce the force applied to the whisker from an object contact. Further investigations into whisker object contacts and forces in 5xFAD mice might help to tease apart these hypotheses (ie, Campagner et al[45]).

There were significant effects of rd on how the mice moved their whiskers; however, these changes were not consistent between different tasks and showed sex differences. Female mice with rd showed an increase in asymmetry and decrease in angular position in the object exploration task, and male mice with rd showed an increase in asymmetry in the sequential object exploration task (Figure 5), consistent with an increase in exploration. However, mice with rd also showed an increase in amplitude, in females in the object exploration task (Figure 3) and tunnel running task (Figure 7), and males in the

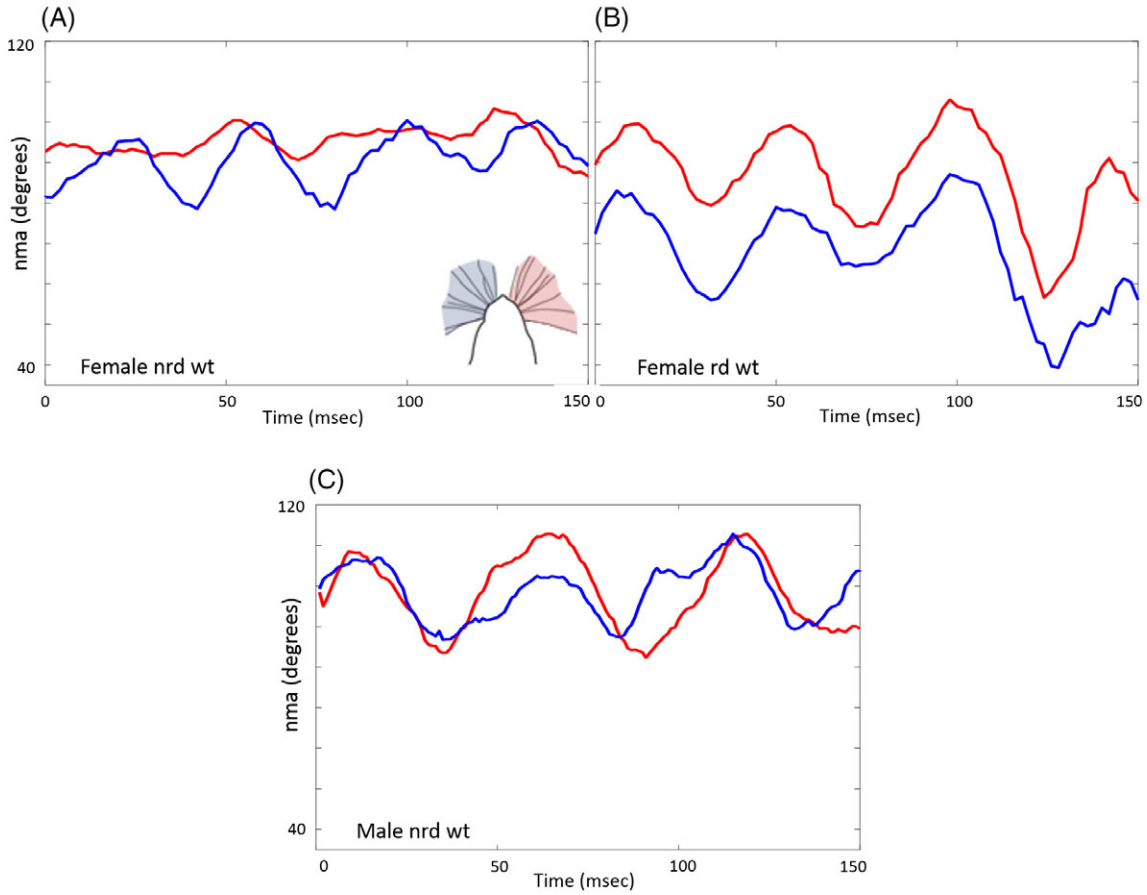

FIGURE 6 Example of whisker traces of three wild-type mice during the tunnel running task. Wild-type females with retinal degeneration (B) had larger whisker amplitudes than WT females with no retinal degeneration (A). They also had faster retraction speeds, indicated by the steep slopes of the retractions. Wild-type male mice had relatively small whisker amplitudes (C), similar to (B), with no differences between those with and without retinal degeneration. Left whiskers are shown with blue lines, and right whiskers with red lines. nma is the naïve mean angle, the average angular positions of all the whiskers on that side of the face

sequential object exploration task (Figure 5). Arkley et al[33] found that rats with retinal dystrophy, when introduced to an unfamiliar environment, also had larger amplitudes, compared with sighted animals. It might be that without sight, blind rodents will increase the area that they are scanning over (increasing amplitude) to prevent collisions. There were also many instances where the mice with rd did not show an increase in exploratory behaviour, for instance, female mice with rd showed an increase in retraction speed in the sequential object exploration task and male mice with rd showed an increase in protraction speed during the object exploration task. It is likely, therefore, that the interaction of vision and whisker touch might be quite complex. New strains of 5xFAD mice do not carry the rd allele Pde6b^{RD1}, however, the amyloid phenotype is less robust in these animals (jax.org 2017). We know that mice with rd are impaired in visuospatial learning tasks, but not in non-visual tasks,[46] so tactile sensory information from the whiskers may help compensate for lack of visual information.

Both sexes showed robust changes in all whisker position and movement variables following contact in the object exploration task, and in amplitude in the sequential object exploration task but there were no sex differences in each task. Female 5xFAD mice have a higher density of plaques than males at a similar age,[17] but it is not clear from our data whether 5xFAD female mice are more affected in their whisker movements than the males (compare Figures 3, 5, 7). However, the sex differences observed in this study indicate that the 5xFAD female mice used their whiskers more than males to investigate objects in both the object exploration and the sequential object exploration tasks (Figures 3 and 5). No studies have yet explored sex differences in whisker movement in different strains of mice or rats and these needs to be completed before whisker measurements become a standard behavioural test for exploration and object recognition. There are significant sex differences in mouse models, in general,[47,48] and in AD model mice specifically, in terms of disease pathology,[14,17] lifespan and healthspan[49,50] and behaviour.[14]

4.1 | Conclusions

Our study showed significant differences in genotype, sex and rd in whisker movements in 5xFAD and WT mice. The largest differences were found between male and female mice across the three tasks tested. Whisker movements also differed between mice with and without rd in all three tasks, and the object exploration and sequential object exploration tasks showed genotype differences in whisker movements. Therefore, the effects of sex and rd might interact with or confound aspects of mouse whisking behaviour more than the effects of the genetic mutations in the 5xFAD mice. Characterizing sex differences in whisker movements in other mouse models of AD and in non-transgenic mice and rats would be important in understanding some of the differences that we observed. All mice showed robust and reliable changes in measures of whisker movements from pre-contact to contact on the object exploration task (Figure 3). Therefore, this is likely to be a reliable test for future studies measuring object exploration. The sequential object exploration task also showed promising results. While there were strong effects of sex in some of the whisker variables, the reduction of whisker amplitude in

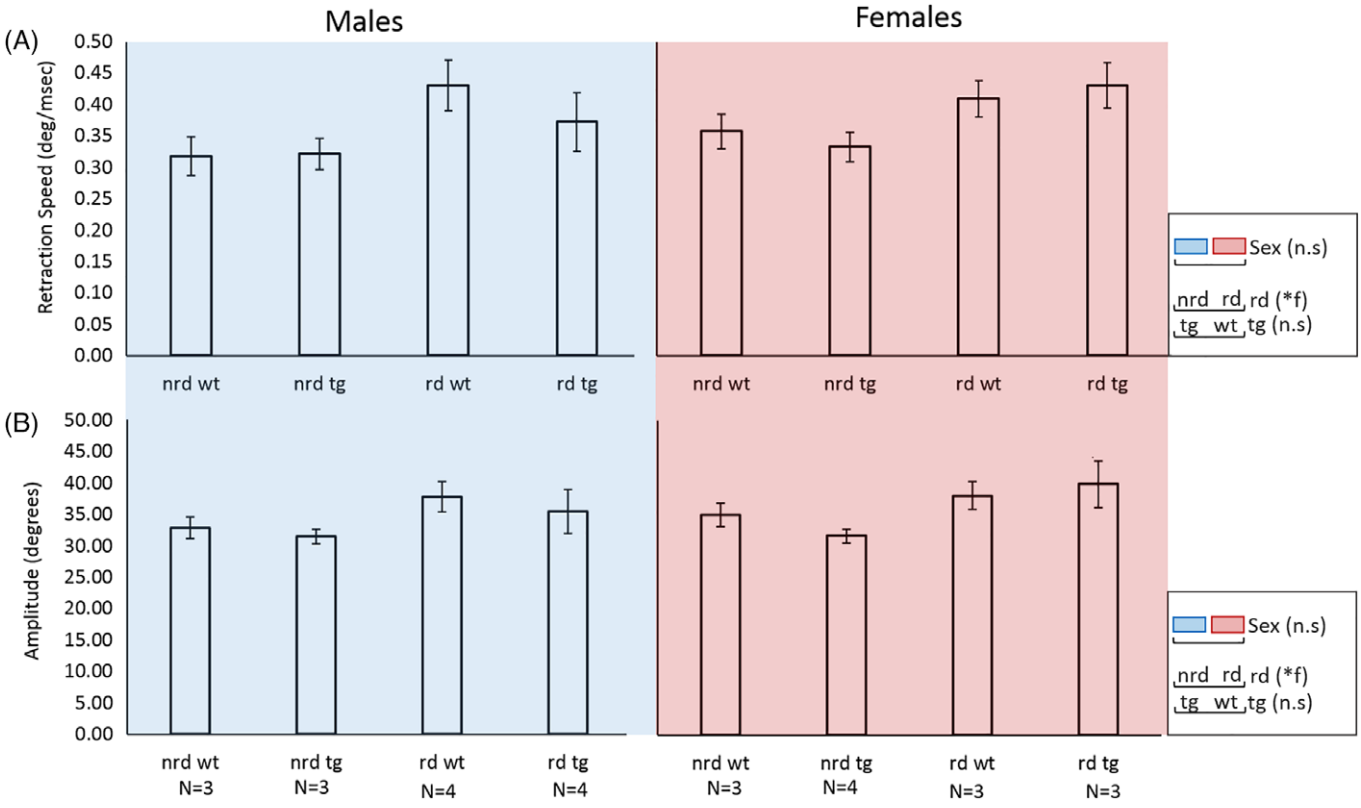

FIGURE 7 Summary of whisker movements of male (left) and female (right) mice during tunnel running. Mean (±SEM) whisker retraction speed (A); and amplitude (B). *$P < 0.05$, with the sex of the interaction (m = male and f = female) indicated in the key: NRD, no retinal degeneration; tg: Tg = 5xFAD transgenic

all mice encountering the second (novel) object indicates a focussing of attention[33,34] and suggests an increase in exploration of the new object,[29,30] as might be expected in habitution-dishabituation experiments.[51–54] Usually, novel object tasks simply measure the duration of time spent exploring the new object, whereas measuring whisker movements quantifies the tactile aspects of this behaviour. Therefore, measuring whisker movements during object exploration tasks may give new insights into rodent behaviours, and their phenotypic changes in mouse models of disease. Finally, the careful analysis of whisking behaviour is essential for understanding the neural basis of tactile sensation and the ability of mice to "see" with their whiskers.[55–57] Understanding the neural basis of whisking behaviour and its changes with genotype, sex, age and disease state may lead to new insights into the neural basis of behaviour.[58,59]

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