Walking and wheelchair navigation in patients with left visual neglect

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Abstract

Patients with neglect veer to one side when walking or driving a wheelchair, however there is a contradiction in the literature about the direction of this deviation. The study investigated the navigational trajectory of a sample of neglect patients of mixed mobility status in an ecological setting. Fifteen patients with left sided neglect after right hemisphere stroke were recorded walking or driving a powered wheelchair along a stretch of corridor. Their position in the corridor and the number of collisions was recorded. The results showed that patients’ path was dependent on their mobility status: wheelchair patients with neglect consistently deviated to the left of the centre of the corridor and walking patients with neglect consistently deviated to the right. A further two ambulant patients with neglect were recorded both walking and using the wheelchair to determine whether the differences were task or patient dependent. These two patients also exhibited leftward deviation when driving the wheelchair, but a rightward deviation when walking. These results suggest that the direction of the deviation is task dependent. Further work will be required to identify what features of the two modes of navigation lead to this disassociation.
**Introduction**

Neglect, or lack of attention to the affected side is a common consequence of stroke that greatly hinders independence in many activities of daily living including mobility (Paolucci, Grasso, Antonucci, Bragoni, Troisi, Morreli, D. et al., 2001). Patients with neglect veer to one side and bump into furniture and doorframes when driving wheelchairs. When walking they position themselves too close to walls and the edges of pavements and ramps and like wheelchair users sometimes collide with doorframes and furniture (Towle & Lincoln, 1991; Azouvi, Marchal, Samuel, Morin, Renard, Louis-Dreyfus et al., 1996; and personal observations). These behaviours lead to the need for close supervision when patients are moving in their environment. In fact for safety reasons patients with neglect are often not supplied with wheelchairs (see Dawson & Thornton, 2003). Given that independent mobility is important for daily living, research into the mobility status of neglect patients is surprisingly limited and a better understanding of the effect of unilateral inattention on navigation is needed to guide clinical practice; the development of new therapeutic interventions and develop theory in this area.

Existing evidence tends to agree that neglect patients show a larger deviation in their walking trajectory than control patients (e.g. Robertson, Tegner, Goodrich, & Wilson, 1994; Berti, Smania, Rabuffetti, Ferrarin, Spinazzola, D’Amico et al., 2002; Huitema, Brouwer, Hof, Dekker, Mulder & Postema, 2006). However, the research is contradictory with the direction of the apparent deviation being debated. Some studies indicate that neglect patients consistently veer to the ipsilesional side (e.g. Robertson et al., 1994; Berti et al., 2002) while others report a deviation to both sides (e.g. Tromp et al., 1995). Furthermore there appears to be a possible distinction between the direction of deviation for patients with mild versus severe neglect (Tromp, Dinkla, & Mulder, 1995) and for patients with good versus poor walking abilities (Huitema et al., 2006). Healthy subjects demonstrate a veering trajectory when deprived of visual feedback with approximately 50% showing a consistent deviation to one side versus 50% who deviate randomly to both sides (Boyadjian, Marin & Danion, 1999). These contradictions between neglect patients may simply reflect a general
heterogeneity between people. However, several different explanations in terms of the task environment, task difficulty and instructions have also been proposed to account for the above contradictions (Huitema et al., 2006).

Not only is there debate about the direction of deviation, but a deviation to the ipsilesional side is also at odds with reports that neglect patients collide on their contralesional side (Azouvi et al., 1996). Robertson et al. (1994) proposed a model to account for this contradiction that predicts both left and right-sided collisions in patients with neglect following right hemisphere stroke. They suggested that a veering trajectory to the right is due to an over-reliance on right-sided visual cues that reduces attention to obstacles on the left. Their theory places the problem of collisions as secondary to that of a veering trajectory.

The literature on wheelchair navigation in patients with neglect concentrates on collisions rather than trajectory and confirms the clinical impression that right hemisphere patients with neglect tend to bump into or brush obstacles on their left side (Webster, Cottam, Gouvier, & Blanton, 1988; Webster, Rapport, Godlewska, & Abadee 1994; Webster, Roades, Morrill, Rapport, Abadee, Sowa, et al., 1995). It is a common assumption that these collisions are a consequence of reduced attention to obstacles on the left.

Studies investigating wheelchair navigation in patients with neglect have tended to use contrived trails laid out in a large open space, (e.g. Webster et al., 1988; 1994; 1995; Dawson & Thornton, 2003; Quiang, Sonoda, Suzuki, Okamoto & Saitoh, 2005; Punt, Kitadono, Hulleman, Humphreys & Riddoch 2007), while some walking studies have sought to understand navigation by asking patients to aim for a single target or position in a doorway (e.g. Berti et al., 2002; Huitema et al., 2006). Matching body position to a defined target may require different attentional processes to voluntary orienting of self in space (Corbetta, Kincade, Ollinger, McAvoy & Shulman, 2000).

The purpose of this study was to investigate the natural trajectory of right hemisphere stroke patients with neglect in an ecologically valid environment. We based our investigation in a corridor of a hospital and compared the direction of deviation and frequency of collisions of a sample of neglect patients with mixed mobility status; wheelchair and walking.
Experiment 1

Methods

Participants

Inclusion criteria for participation were the presence of left neglect defined by scoring below the normal cut-off on the star cancellation test or line bisection test of the Behavioural Inattention Test (BIT; Wilson, Cockburn, & Halligan, 1987). Participants needed to be medically well, be able to understand and follow instructions, be able to walk independently (with or without a walking aid) or alternatively be physically able to drive a powered wheelchair. Since this was a novel task, potential wheelchair-driving participants were given practice in a different and wider corridor in the hospital to ensure they would be able to drive the chair for the study. Patients with dementia or severe anxiety were excluded. The Occupational Therapists decided these by clinical judgement.

Fourteen right hemisphere stroke patients with left unilateral neglect were recruited. They were recruited as part of a larger intervention trial, which was operating across two hospital sites. These fourteen were included in this smaller investigation based on their location at the hospital in which the navigation task was set up, or their ability to be transported there after discharge from the alternative site. The age of participants ranged from 55 to 91 (median 76 years) and the sample consisted of seven males and seven females. All participants were first time neglect patients (although not necessarily first time stroke patients). The diagnosis of lesion location was made with a CT or MRI scan.

All patients were entered into the study at a minimum of twenty days post-stroke, with a mean length of 39.7 days (SD 19.8). Nine of the participants undertook the study in a wheelchair and five walked (four using a walking stick). The comfortable walking speed of the walking participants was slow and ranged from a 0.4 m/s to 0.8 m/s, (median 0.6 m/s)

Clinical Assessments

The severity of neglect was assessed using the conventional subtests from the BIT (Wilson et al., 1987), the Catherine Bergego Scale (CBS; Azouvi, Olivier, de Montety,

The CBS is a unidimensional assessment of self-care and mobility neglect behaviours and is more sensitive to neglect than pencil and paper tasks (Azouvi, Bartolome, Beis, Perennou, Pradat-Diehl, & Rousseaux, 2006).

Neglect behaviours can fall along a spectrum from predominantly perceptual neglect to predominantly motor neglect (Bisiach, Ricci, Lualdi & Colombo, 1998; Harvey, Krämer-McCaffery, Dow, Murphy & Gilchrist, 2002). The Milner Landmark test was included as it estimates the degree of motor bias, which may influence the path taken in navigation. The short form Landmark test comprised twelve 20 cm lines, presented to the participant on separate A4 sheets. Each line had a ‘landmark’ bisecting it at or close to its mid length. Six of the lines had landmarks that were exactly midway. The participant was asked to point to the end of the line that appeared closer to the landmark. The responses for the six lines with midway landmarks were recorded. An index along the perceptual-motor spectrum was determined by subtracting the number of rightward responses from the leftward responses and dividing by the total number of tests (i.e. Freq left responses - frequency right responses / 6). Only one participant (N12, a wheelchair patient) was assessed as having a bias towards the motor neglect end of the spectrum.

The clinical assessments were carried out by a research Occupational Therapist. Table 1 summarises this information for each patient.

(Table 1 about here)

**Stroke Participants without neglect**

Nine stroke patients without unilateral neglect were included in the study as control participants. Their participation was important in confirming that collisions were not due to the novelty of driving a powered wheelchair. These participants were a convenience sample taken from current patients at the Stroke Rehabilitation Unit and were matched as far as possible to the participants with neglect in age and mobility status. Inclusion criteria remained the same as above, but of course it was also necessary that control participants did not present
with unilateral neglect. This was checked using the Star Cancellation and Line Bisection tests from the BIT (Wilson et al., 1987). The control sample consisted of five males and four females with an age range of 58 to 85 and a median age of 75 years. Four participants walked (one with the aid of a walking frame) and five used an electric wheelchair. Seven of the control group had left hemisphere strokes, one had evidence of damage to both hemispheres and one had a mid-brain lesion. Table 2 summarises the characteristics for each control participant.

(Table 2 about here)

**Procedure**

All the procedures were performed in accordance with a protocol approved by Local NHS Research Ethics Committee.

(Figure 1 about here)

A quiet stretch of corridor in the hospital was used to record mobility (see figure 1). It is on average 1.32 metres wide and approximately 11 metres long with a handrail running along each wall. There are doors distributed on both sides and two windows, one at the side and the other at an end. Figure 1 includes a scale diagram including the positioning of doors and window. On each handrail black duct tape was placed at seven approximately equal intervals along a 6.6m stretch. These provided markers from which to measure the patients’ position along their journey. Padding was wrapped around the ends of the handrails to prevent the participants injuring themselves in the event of a collision.

The participant’s path along the corridor was recorded on a JVC digital camcorder, operated by remote control. The video camera was placed on a windowsill at one end of the corridor on a specially constructed stand. The stand was a triangular base plate with three adjustable feet below and mountings for the camera, a circular spirit level and a laser pointer on its topside. Placing the camera with its base plate feet on marked points on the windowsill and then aligning a laser pointer to a mark on a facing wall at the end of the route ensured consistent positioning of the camera. The spirit level ensured that the base was flat. The camera zoom was adjusted so that all seven markers were captured on screen.
The powered wheelchairs used were Apollo 16 and Apollo 18 types, with seat widths 40.6cm and 45.7cm respectively. Outside width measurements including control box were 54cm and 60cm. The wheelchair used was selected according to the size of the participant. The joystick control was always attached to the right arm of the chair for the neglect patients, so that they could easily drive the chair using the non-paretic hand. For control patients the control was set to the left side, due to poor hand function on the right side. The speed of the wheelchair was pre-set to the slowest setting in all cases.

Two pieces of adhesive tape were stuck onto the front and back of the participant to make a ‘+’ shape over the participant’s midline for taking measurements from the video recording. The colour of the duct tape, black or white, was selected to be distinguishable from the participant’s clothing. For wheelchair participants the back target was placed onto the back of the chair. The target was set horizontally at 80cm from the ground and corresponded with the bottom of the handrail and vertically in accordance with the patient’s midline. For walking patients the vertical setting was once again their midline but the horizontal line was set at 100cm, which corresponded to the top of the handrail. The discrepancy between walking and wheelchair target placement was based on the height difference between the two sets of participants when doing the task.

The participant was asked to navigate (either drive the wheelchair or walk) along the corridor and back ten times. Chairs were available at each end for walkers to sit down if necessary. The participant was told that if help or a rest was required at any point he or she could ask for it. Wheelchair participants were warned that it was not uncommon to collide with objects and that if this happened they should not be discouraged.

The participant began at the far end of the corridor from the camera’s point-of-view. For each run up or down the corridor, the participant started around a corner so that position in the corridor was self-determined. Each run was complete when the participant had navigated around the final corner. On the up trip (towards the camera) the run was completed with a right-hand turn. On the down trip (away from the camera) the run was completed with a left-hand turn (see figure 1).
Although the participant navigated the corridor alone a researcher or therapist was positioned out of sight around the corner at either end of the corridor to help them turn around. Participants in the wheelchair who collided were frequently unable to free themselves to continue; in these cases the researcher or therapist intervened and guided the chair to face in the right direction again. So as not to distract or influence the participant’s path, the therapist at the end of the corridor from which the patient was driving away, watched to judge if help was necessary.

**Measurements and Video Analysis**

Distance from the participant’s midline to the wall on his/her left and number of collisions were assessed from the video recordings using SiliconCOACH Pro Software. A scaled line was drawn between the duct-tape markers on either side of the corridor. The video was then forwarded or rewound as necessary until the point when this line was aligned with the marker on the front or back of the patient (depending on the participants’ direction in the corridor). The distance (cm) from the centre of the participant to the wall at his or her left was recorded at each of the seven markers along the corridor. These measurements were averaged and then transformed to find the mean deviation from the centre of the corridor for each trip with negative values represented deviation to the left of centre and positive values to the right of the centre.

There were three cases in which data was incomplete. Two of the participants with neglect (N11, N12) were unable to complete the full ten trials required due to mechanical problems with the electric wheelchair. In another case (N10) there was a fault with the video camera and measurements could only be taken from four of the markers. A criterion of obtaining at least 28 measurements for each direction in the corridor was set for a participant’s data to be included; for example, at least four runs either way with seven markers.

In order to measure the number of collisions the corridor was divided into zones. A zone constituted the space between the markers or the region at either end of the corridor beyond the final marker. The video was played in slow motion in order to determine how
many times the patient collided in each zone. The side of collision, i.e. the left or right wall, from the participant’s perspective, was also recorded to give separate frequencies of left-sided and right-sided collisions.

The mean number of collisions for each direction was calculated by summing the total number of collisions on all individual runs (in the appropriate direction) and dividing by the total number of runs. This compensated for the patients who had not completed the full number of trials. For videos missing some of the markers collisions could only be recorded in a limited number of zones. To compensate for this the following formula was applied to calculate the total number of collisions for individual trips:

\[ \text{No.of collisions} = T_z \left( \frac{c}{z} \right) \]

\( T_z \) = total number of zones there should have been
\( c \) = number of collisions recorded in limited zones
\( z \) = number of zones on the video

The number of collisions was also calculated for control participants. This was to ensure that neglect participants’ collisions were not due to the novelty of the mobility task, particularly for wheelchair users.

**Statistical Analysis**

To analyse the trajectory position in the corridor a repeated measures ANOVA with one within-subjects factor (direction in the corridor: up versus down) and two between-subjects factors (mobility status: wheelchair versus walking; patient status: neglect versus control) was conducted with mean position as the dependent variable. Post hoc analysis was carried out using t-tests.

To analyse the collisions a repeated measures ANOVA was used. There were two within-subject factors (side of collision: right versus left and direction in corridor: up versus down) and one between-subject factor (mobility status: wheelchair versus walking). The control participants never collided with the wall and were therefore excluded from the analysis. For post hoc analysis using the Wilcoxon non-parametric test an index of collision
side was produced by subtracting the mean frequency of right-sided collisions from the mean frequency of the left (i.e. Left – Right collisions).

Results

(Table 3 about here)

Direction of deviation

Table 3 shows the mean deviation measurements for participants when travelling along the corridor in each direction. Irrespective of the direction of travel all of the neglect participants driving a wheelchair deviated to their left while those with neglect who walked deviated to their right. On average control wheelchair participants deviated to the right of centre, especially in the down direction while walking control participants deviated very slightly to the left of centre. Although the size of the effect in some individual patients was small the direction of the deviation was surprisingly consistent with all neglect patients showing the same pattern as the group means (see Figure 2A). ANOVA showed the interaction between patient group and mobility task was significant (F(1,19) = 30.048, p <0.01). Post hoc t-tests of the neglect patients’ position in the corridor confirmed that the wheelchair group were significantly to the left of the middle of the corridor (t=−6.78, p<0.001) while the walking group were significantly to the right of the middle of the corridor (t=7.08, p=0.002). An independent samples t-test comparing the paths of wheelchair and walking control participants showed no significant difference (t=1.4, p=0.206).

(Figure 2 about here)

The deviation in control participants was affected by the direction of their journey in the corridor: their mean deviation was greater when going down the corridor than coming up it. While, the direction*patient status interaction effect was significant (F(1,19) = 8.103, p =.01), ANOVA results showed that main effects for direction and patient status were not significant (F(1,19) = .037, p =.850 and F(1,19) = 1.391, p = .253 respectively).

(Table 4 about here)
Collisions

Table 4 shows the mean number of collisions for participants with neglect on their journeys up and down the corridor. Participants with neglect who used a wheelchair had significantly more frequent collisions that those walking (F(1,12) = 10.403, \( p = .007 \)). In keeping with their different navigational paths along the corridor wheelchair participants collided more often with the left hand wall while those walking collided more often with the wall on their right (see figure 2B). This interaction is statistically significant; F(1,12) = 13.478, \( p = .003 \). Non-parametric comparison of the ‘bumpside’ index (i.e. frequency of left–right collisions) confirmed that the wheelchair drivers had a significantly greater tendency for left sided collisions; Wilcoxon W=15.0, \( p=0.003 \).

There was no effect of direction on the frequency of collisions for either wheelchair or walking participants.

Experiment 2

Experiment 1 suggests that the trajectory deviation for neglect patients depends on whether they are walking or in a wheelchair. There are two possible explanations for this result. First, it may be that the task demands of the two types of navigation are so different that deviation reverses or second it may be that these two neglect patient groups are different in some other way, for example they may have distinct lesion characteristics. To test this we were able to recruit two further patients with neglect who were able to walk. They were screened for neglect with the conventional tests from the BIT. Details of these two patients; Na and Nb are given in table 1. These two participants were recorded both walking and driving a wheelchair in the corridor using the same procedure as in Experiment 1. The order of tasks was counterbalanced with patient Na using the wheelchair first. The significance of deviations from the centre of the corridor (zero) for walking and wheelchair tasks for each participant was tested with one-sample t-tests. Independent samples t tests were used to test
for differences between mean deviations in walking and wheelchair conditions for each participant.

**Results experiment 2**

Mean deviations in the wheelchair and walking conditions for the two participants who performed both tasks are given in table 5. Both patients deviated to the right of centre when walking (Na: $t(4) = 18.2, p<0.001$; Nb: $t(5) = 3.95, p=0.01$) and to the left of centre when driving the wheelchair (Na: $t(7) = -14.7, p<0.001$; Nb: $t(5) = -8.3, p<0.001$). The difference between conditions was also statistically significant in each case (Na: $t(11) = 21.69, p=0.000$; Nb: $t(10) = 8.33, p<0.000$).

(Table 5 about here)

**Discussion**

The aim of this study was to investigate, within an ecological setting, the direction of navigational deviation in right hemisphere stroke patients with neglect. A central pathway would allow maximum clearance of the walls and doorways on either side in the narrow corridor used. However, the neglect patients exhibited a path that was off centre and the direction of this deviation was determined by the patients’ mobility status; wheelchair patients with neglect deviated to the left of centre while walking patients with neglect deviated to the right. A secondary aim was to evaluate the side of collisions in wheelchair and walking patients with neglect. Collisions in participants who were driving the wheelchair were much more frequent than in walking participants. The predominance of left sided collisions when driving the chair was related to the deviation in path taken.

These results add support to a growing body of evidence reporting an abnormal trajectory as a distinct issue for patients with neglect (e.g. Robertson *et al.*, 1994; Berti *et al.*, 2002; Huitema *et al.*, 2006). This study has distinguished between mobility types, wheelchair driving and walking, and by doing so a very clear distinction has emerged.
The dissociations in the direction of deviation according to task in experiment one could arguably be interpreted on the basis of differences in the lesion site. A more anterior and medial lesion is more likely to lead to mobility problems and so these patients are more likely to be in the wheelchair group in Experiment 1. In addition, a larger lesion is more likely to lead to mobility problems but could also result in hemispheric differences that may also affect the trajectory. For example patients with hemiparkinsonism preferentially turn toward the more affected hemisphere (Bracha, Shults, Glick & Kleinman, 1987). However, the results of Experiment 2 showed that deviation from the centre of the corridor within individual participants with neglect differed when walking and when driving a wheelchair. Thus, based on the results of these two participants, differences in path appear to be task specific and not the result of individual differences. Further investigation of patients who can perform both tasks would be useful to confirm this task dependence.

A potential confound between tasks may have been in differences between the therapist’s warnings to the neglect patients. To prevent them from being demoralised those who were driving the wheelchair were warned that it was not uncommon to collide with objects and not to worry if they did bump; the walking patients were not given this warning. Collisions were more prevalent in the wheelchair drivers and it is possible that the warning led to less care being taken.

Although this study has shown robust task dependent differences between the navigational path of patients with neglect, there remains the possibility that we are seeing behaviour that relates to the hemisphere damaged by stroke rather than to neglect. Goal-directed pointing movements, in patients with right hemisphere stroke, have shown no effect of spatial inattention (Himmelbach & Karnath 2003). However these upper limb tasks are performed purely in peripersonal reaching space. There may be different requirements of attention in navigation which involves attention to far space and different motor effectors. Our control group of non-neglect patients mostly had left hemisphere strokes and so were not suited to addressing the importance of hemisphere in walking and steering a wheelchair. This would be an area to address in a further study.
The striking dissociation in the neglect patients’ navigation when walking and when driving a wheelchair has important implications for clinicians evaluating the safety risks involved in discharging patients with cognitive impairments from hospital (National Clinical Guidelines for Stroke, 2004). Most therapists are aware of the potential for left sided collisions when patients with neglect are driving wheelchairs, however the tendency for walking patients to veer or position themselves to the right is not well recognised. This information has implications for patients’ safety on pavements, railway platforms and in busy pedestrian situations, such as supermarkets. Knowledge of direction of veering is also important for remediation. With consistent encouragement some patients may change their path. However an understanding of the mechanisms operating or failing in patients’ navigation is likely to yield other strategies for improving safety in mobility.

The current study is unable to provide a explanation of the mechanisms that underpin the striking and consistent differences in path taken, however there are several possible explanations which would warrant further investigation. There are fundamental differences between walking and driving a wheelchair and so the attentional mechanisms deployed in both tasks may well be different. For example the domain of space that is attended to may vary according the task, or the strength of cueing from motoric, proprioceptive or optic flow afforded by the mobility task may influence the heading (e.g. Robertson & North 1992). Biases in coding a central heading, rather like line bisection errors, might explain the ipsilesional deviation in walking patients (Robertson et al., 1994; Berti et al. 2002) and there may be attention capacity effects in the mobility tasks that require attention to more than one component. Huitema et al., 2006 argued that patients who have difficulty walking might not be able to attend to their heading in addition to their gait. The wheelchair drivers with neglect in our study appeared to find the task more difficult than the walkers with neglect. Further work is needed to tease apart possible mechanisms.

Huitema et al (2006) reported contralesional veering in neglect patients who walk fast. They reported a strong and significant correlation between walking speed and deviation in a sample of six neglect patients - with slow patients veering to the ipsilesional side and fast
walking patients veering to the contralesional side. In agreement with the slow walkers in the Huitema et al. study our sample of slow walking patients with neglect deviating ipsilaterally. Further studies should explore in more detail the interaction between the mobility speed and the walking vs. wheelchair navigation.

While the current set of results cannot distinguish between possible mechanisms underlying impaired navigation, the differences observed between the trajectory of wheelchair patients and walkers provides an interesting basis in which to ground future research. It certainly seems that the mobility task is a key to understanding navigation in neglect patients, for planning rehabilitation and for informing the development of therapeutic interventions.
References


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**Legends**

**Table 1.**
Summary of information for neglect patients

Key: R = right, EWch = electric wheelchair, Wlk = walking, Wlk* = walking with a stick, CBS score 0 = no neglect, BIT high score indicates less neglect, cut-off score of 129 indicating normal performance. Landmark index perceptual neglect (+1) to motor neglect (-1)

Table 2.
Control patients’ characteristics

Key: EWch = electric wheelchair, Wlk = walking, Wlk** = walking with a walking frame

Table 3.
Position in the corridor for patients with neglect and control participants
Position is coded in relation to the patients, with positive values being to the participant’s right side and zeros being the centre of the corridor.

Table 4.
Frequency of left and right-sided collisions in neglect patients on their journeys up and down the corridor.

Table 5.
Mean deviations, with 95% confidence intervals, when walking and driving a wheelchair for participants who performed both mobility tasks.

Figure 1.
Photographs and diagram of the corridor including the positioning of doors and window
On the up trip (towards the camera) the run was completed with a right-hand turn. On the down trip (away from the camera) the run was completed with a left-hand turn.
Figure 2.

A. Interaction of mobility task and group. Negative values represent deviation to the left of centre and positive values to the right of the centre. Neglect participants driving a wheelchair deviated to the left of the centre of the corridor, while control wheelchair participants deviated to the right of centre. Neglect participants who walked deviated to the right while walking control participants deviated very slightly to the left of centre. The dotted lines indicate the corridor walls. Error bars indicate standard error of the mean.

B. Interaction effect of mobility task and side of collision for participants with neglect

Participants driving wheelchairs collided more often with the left hand wall while those walking collided more often with the wall on their right. Error bars indicate standard error of the mean.