Outdoor environments for people with dementia: an exploratory study using virtual reality

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ABSTRACT

Few studies have investigated how outdoor environments might disable people with dementia. The issue is rarely considered in planning and design guidelines and not at all in regulations, despite dementia being within the scope of disability discrimination legislation in the UK and other countries. This article reports a study that involved older people with mild to moderate dementias taking two walks, one in a real town centre and one in a virtual reality (VR) simulation. Adaptations were made to the VR simulation to test possible design improvements. Overall, the town centre posed relatively few problems for the 38 older people with dementia who participated, although more difficulty was evident with greater impairment. Some features of particular places were liked more than others, notably where they were separated from motor traffic. There were measurable benefits from using clear textual signs to support wayfinding and to identify objects and places in the environment. Diminished outdoor activity is likely to be experienced as a decrease in quality of life and may accelerate the progression of dementia. We conclude that older people with mild to moderate dementia should be encouraged to be active outdoors and that this can be facilitated by small environmental modifications. Some limitations of the VR technology used for the study are also reported.

KEY WORDS – dementia, environment, virtual reality, walking

Running head: Outdoor environments for people with dementia
Introduction

Dementia is a common disabling condition among older people, with prevalence growing in all ageing societies. Both of the main dementias, Alzheimer’s disease and vascular dementia, cause disorientation and difficulty with comprehension, and these can make navigating and understanding everyday environments challenging. Although severe dementia may eventually necessitate moving into a residential or nursing home, in the United Kingdom an estimated 80 per cent of people with dementia live in the community (Audit Commission 2000). Many remain active outdoors until this becomes impossible. Rarely, however, are outdoor environments considered by planners or designers in terms of the opportunities and barriers they present for a person with dementia (Blackman et al. 2003).

There is evidence that the more that older people walk and take exercise, the less their risk of dementia (Abbott et al. 2004; Larson et al. 2006; Scherder et al. 2005). There are several possible explanations for this association, including the effects of walking on cognitive activity and cardiovascular health. A study by Weuve et al. (2004) found that older women who are more physically active had better cognitive function across general cognition, memory, fluency and attention. Women with cognitive impairment who walked more also experienced less cognitive decline. Despite this evidence, little is known about how the outdoor environment may disable a person with mild to moderate dementia and therefore discourage walking and deny its potential benefits.

There is a body of work on dementia-friendly design but this concentrates on indoor care settings such as day centres and residential homes, although adjacent gardens have received attention. Much of this evidence is reported in a useful review by Zeisel et al. (2003). Paths with features of interest along the way have been found to decrease exit seeking from homes and improve the mood of residents. Gardens have been found to reduce attempts to leave the home, reduce aggression, improve sleep and engage family members.
with residents. Common spaces that are homely have been observed to reduce social withdrawal, and an ambience with meaningful and understandable sounds, sights and activities has been found to reduce agitation. Privacy has been linked with reduced aggression and agitation and better sleep.

These findings are inevitably biased towards the severer end of dementia when people are more likely to be in residential care. They promote a relatively recent concern with making the environment as easy as possible for people with dementia to understand and navigate. However, just as learned dependency can result from institutionalisation, there is also a possibility of over-compensating with design and planning adaptations so that the environment no longer offers stimulation or challenge (Lawton 1980, 1982, 1989, 1998; Scheidt and Windley 2003). If this extends to someone with dementia staying indoors with no or little outdoor activity it may be more difficult for them to retain cognitive capacities such as wayfinding that no longer receive stimulation.

Only one study of dementia and the outdoor environment has been published, based on observations from accompanying people with mild to moderate dementia on short walks around their neighbourhoods (Burton, Mitchell and Raman 2004). The authors recommend measures to assist with recognising and remembering streets, places and buildings by limiting changes in the physical environment to small-scale and incremental alterations, creating variety in urban forms such as street scenes, and ensuring that elements such as seating, telephone boxes and entrances to buildings are easily understood by making their function obvious and using traditional designs. They recommend retaining distinctive landmarks to assist with wayfinding and keeping signs simple, with obvious symbols and large, clear lettering. There are also features that they recommend should be avoided: changes in level that are not clearly marked, paving patterns with sharp colour contrasts, exposure to loud traffic, and areas of extreme contrasts between light and dark.
The major drawback with this study is that it was not possible to test whether environmental adaptations resulted in improvements for older people with dementia. This is clearly important given the implications of some of their recommendations and how restrictive they would be if required by regulations, such as traditional designs and incremental change. The recommendations are deduced from observing and interviewing participants but not comparing a ‘dementia-friendly’ environment with one lacking any specific adaptations for dementia. To overcome this it would be necessary to make the adaptations to evaluate them, raising practical and cost problems in real world environments.

In this article we report on a study that has tackled this issue by experimenting with the use of computer-generated environmental simulations that enable a person with dementia to take a virtual walk. This has the potential for participants to identify what helps or hinders them and then for changes to be made to the simulation that test these findings by the participant repeating the walk with adaptations added along its route. This is a new area of research where our aims are as much about evaluating the technology for this purpose as producing substantive findings relevant to planning and design practice. We report some limitations of the technology as well as results that indicate both an ability among our participants to remain competent in non-adapted environments and some specific adaptations likely to enhance these competencies.

**Methodology**

A computer-generated virtual environment (VE) can be relatively easily adapted by changing the computer model to incorporate design changes, enabling a participant to experience and test the adapted setting. The resulting person–environment interaction can be observed and performance of various tasks can be compared before and after adaptations are made. A useful feature of VEs is that a person can interact directly by using a joystick, moving through the VE as if really there, an experience called ‘presence’.
Prior to the main study reported in this article, a pilot project was carried out that successfully demonstrated with six older participants with dementia that they could navigate a VE, that they perceived elements in it as real, and that they could undertake tasks in the environment such as posting a letter and finding somewhere to sit down (Flynn et al. 2003). The first stage of the research was to plan a walk through the town centre of a Northern English town, Middlesbrough, close to the Virtual Reality Centre at the University of Teesside that was used for the VE exercises. The walk offered various environmental encounters such as crossing roads and finding different destinations. Three parts of the town centre were selected as appropriate: a route along a quiet side street to a fairly quiet shopping street and then along to a post office and across to a taxi rank; a route through a modern shopping precinct; and a route along a busy road with bus stops that ended at public toilets. These environments were then recreated in a detailed virtual reality (VR) model using a three-dimensional modelling and animation software package, with pavements, kerbs, signs, road markings, diverse street furniture, pedestrians and moving traffic. The model was transferred to a visualisation package run on a personal computer, and the images projected onto a large curved 6 x 2 metre screen, accompanied by ambient street sounds. Participants could view the environment by sitting in front of the screen and move themselves through it using a joystick.

Participants were recruited with their carers through local National Health Service (NHS) consultant psychiatrists of old age on the basis that they had a diagnosed dementia of the Alzheimer’s or vascular type at the mild to moderate stage and were mobile outdoors. A total of 38 people participated, aged between 71 and 84 years. Half were males and half females. All had Mini Mental-State Examination (MMSE) scores between 15 and 29 (Folstein, Folstein and McHugh 1975). Following initial agreement to participate during a visit to their consultant, the researcher working on the project made a home visit to the
person, showed them a short video of the VR cinema and invited him or her to sign a consent form together with their main carer. Subsequently, participants and their carers were interviewed separately at home to gather background data about their outdoor lives. They then took part a few days later in the walking exercises.

The VE simulated the real town centre and participants walked the routes through both the VE and in the real-world. This enabled their walks to be compared to investigate any differences between the real and virtual environments. The research was conducted in two phases: a first phase in which each participant undertook the real-world walk and the simulated VE walk, and a second phase when participants undertook the VE walk following adaptations. These adaptations were based on results from the first phase. Participants in phase two were either the same as in phase one if there had been no change in their condition, or were new participants matched with participants in phase one by age, gender, MMSE score and Activities of Daily Living (ADL) score based on the Bristol Activities of Daily Living Scale (Bucks et al. 1996).

The VE walks followed a set route with the research assistant sitting next to the participant. After an initial familiarisation exercise, participants were invited to proceed towards the first destination, a quiet side street. The walk continued with invitations to make their way to a destination or undertake a specific task. All the walks were recorded on video. Navigability was investigated by how well each participant proceeded to destinations and managed at junctions and other decision points. Legibility was evaluated by how well each participant recognised and located features in the environment, such as a taxi rank or a post office. Safety was assessed by observing each participant’s behaviour at points such as road-crossings and asking them about their own perception of safety. Comfort and well-being were assessed by asking participants about how they felt at different stages of the walks and their likes and dislikes regarding features of the environment.
The walks were conducted using an interview schedule administered in conversational fashion. The video of each session was then analysed by scoring each navigation, legibility, safety and comfort question or task. The use of quantitative measures was important if the project was to demonstrate any measurable change in how well participants managed the walks and tasks along the way. Scoring used Likert scales based on degrees of success or the number of prompts needed and was undertaken from the video records by two independent raters, the research assistant and a research student. Reliability between the raters was analysed using the intra-class correlation coefficient and all correlations were significant, on average exceeding 0.7 for each type of walk. Given this degree of reliability, only one set of scores was used in the analysis, those of the research assistant, who had also accompanied participants on the walks.

Most tasks were scored using a four-point scale where one is best (e.g. needed fewer prompts or acted safely) and four is worst (e.g. could not do the task or did not act safely). The median and semi-interquartile range were computed for each task for the real-world walk, the VR model of the real-world walk and the adapted VE walk. Comparisons were made between these measures to assess (a) differences between the real-world town centre and the VR model of it, evaluating the validity of the VR simulation, and (b) differences between the VR model of the real-world town centre, and the VR model of the town centre with environmental adaptations incorporated into it. The Wilcoxon matched-pairs signed-ranks test was used to compare the real-world walk and the walk in the virtual model of the real world. The Mann-Whitney U test was used to compare the real-world walk and its virtual model with the virtual model incorporating the environmental adaptations. These different tests were used to take into account that all participants who undertook the real-world walk also undertook the walk in the virtual model of the real world, while not all
participants who undertook the walk in the virtual model incorporating the adaptations had undertaken the earlier phase one walks.

Results

The walks incorporated a series of tasks. Table 1 shows the median scores and their semi-interquartile ranges (Q) for the tasks where there were significant differences between the real world town centre and its VR simulation, the real town centre and the VR adapted town centre, or the VR simulation of the real town centre and the VR adapted town centre. These comparisons therefore explore both the extent to which the real town centre was successfully simulated and the effects of adaptations on task performance.

Table 1 near here

The first task was to find Baker Street, a quiet side street just ahead of the starting point. In both the real-world walk and the VE walk participants found the street easily, with a median score of 1 and semi-interquartile range (Q) of 0. They used the street name sign, which was located on a wall at the entrance to the street. No change was made in phase two to what was a clear and easily visible sign. A further task of finding a particular address along the street, no. 17, was also completed successfully, with a median score of 1 in the real world and a Q of 0.5. The VR simulation presented more of a challenge because the visual resolution of the house numbers was not as clear as in the real street, giving a median score of 2 and a wider Q of 1.

Participants walked along Baker Street to emerge at a shopping street where they were invited to find somewhere to sit down. This involved locating some circular seating of modern design in stainless steel that was across the road. It was thought that this might be a difficult task given that the seating was not of a traditional design, but all participants found it easily and there was little difference in performance between the real world and VE (all median scores were 1, with a Q of 0 in the real world and 0.5 in the VE).
The next task was to find a post office, which was not visible from the seating and required participants to find their way via a landmark. In the real world and in the VE this was a church. It was generally used successfully for wayfinding in both the real world and the VE. Although the median score of 1 in the real world (Q = 0.3) was better than the median of 2 in the VE (Q = 0.5), the difference was not statistically significant (z = -1.51, p = 0.13). The landmark was changed in the adapted VR model to a prominent red post box and, although this led to a significant improvement compared to the unadapted VE, there was no significant change in scores compared with performance in the real world. It is likely that this change helped with perception of the projected image but with no real-world implications. In both the real-world and virtual walks, all participants proceeded to locate the post office with its traditional insignia without difficulty.

A short route from the post office to a taxi rank involved crossing a road with no curbs and only a series of bollards marking the road’s route. One participant commented that they thought the bollards meant no traffic was allowed along the road, while another believed that because there was no marked road-crossing, pedestrians were not allowed to cross the road. In both the real world and the unadapted VR model some participants had difficulty distinguishing between the pedestrian and motor traffic surfaces. The adapted VE in phase two incorporated a clear difference in the colour of these surfaces, resulting in a significant improvement in participants’ ability to distinguish between them in the adapted VE compared to the non-adapted VE (z = -2.12, p = 0.03) and in the adapted VE compared to the real world (z = -2.34, p = 0.02).

The next section of the walks involved entering a modern shopping precinct to find a bus station entrance from the precinct. On the way, participants were invited to find somewhere to sit down and had no difficulty locating the green metal seating. This was also the case when asked to deposit some litter, with all but one participant locating the green
litter bins. All participants successfully located the entrance to the bus station. This entrance was not obvious as a bus station except for a large sign with the words ‘bus station’ that most participants understood without difficulty.

Navigating within the precinct presented some problems. A *You are Here* map displayed both at the entrance and inside the precinct proved impossible for participants to use as a means of finding their way to the bus station. In the adapted VE these map displays were replaced with two directional landmarks, a fish sculpture and a ship, both pointing the way to the bus station (although with no word signs). Participants were told that these landmarks would lead them to the bus station, but they did not work and there was no significant improvement compared to the maps. The contrast with simple word signs was striking.

The next section of the walks started with participants being asked to locate modern glass and steel bus shelters along a busy road. The bus shelters generally proved easy to find and no adaptations were made for phase two. The bus numbers were displayed at a height on the bus stop signs and on a small notice board inside the shelters. Participants were asked to find the particular stop for ‘the number 65 bus’. Most did this successfully in the real world, with a median of 1 and Q of 0.5, but less successfully in the VE, where the median was 2 with a Q of 0.5, a statistically significant difference. This again appeared to be a resolution issue. A change was made for phase two by adding clearly displayed bus numbers at eye level on the outside of the shelters. There was a significant improvement between the unadapted and the adapted VEs (z = -3.97, p < 0.001). In effect, the clearer displaying of the bus numbers in the VE meant that participants performed as well in the VE as in the real world with finding the number 65 stop.

Participants were asked to locate a new-style steel-and-glass telephone box and most did so with little difficulty. Two types of wayfinding were then tested. In the real world and
the unadapted VR model, participants were invited to find the way to public toilets by using some telephone boxes as a landmark that cued them to turn right to the toilets. In phase two, the adapted VE included three signs that led the way to the toilets. Participants performed poorly using the landmark to remember where to go but much better using the signs. There was a significant difference between the real world and the adapted VE ($z = -3.02, p=0.003$) and between the unadapted VE and the adapted VE ($z = -3.17, p = 0.003$). The use of signs was tested further in phase two by including in the adapted VE three different types of directional sign on the way to the toilets: a picture symbol, a text sign (with the words ‘public toilets’) and a combined symbol and text sign. There was a significant difference between the symbol and text sign in favour of the text sign ($z = -2.12, p = 0.03, r = -0.34$) and between the symbol and combined symbol/text sign in favour of the symbol/text sign. There was no significant difference between the text sign and the combined symbol/text sign. When arriving at the toilets there was no difficulty in the real world or unadapted VE with recognising by the public toilets sign that they had arrived at their destination. The sign has black male and female graphical figures on a white background and is widely used in the UK.

**The attractiveness and safety of environments**

At various stages of the walk participants were asked about how attractive they found the environment and how safe. Safety behaviour was also observed and scored. The traffic-free shopping precinct was the best-liked environment, with comments such as ‘plenty of room to walk about’, ‘plenty of seats’, ‘nice paving patterns’ and ‘no traffic’. Its median score for attractiveness was 1 ($Q = 0.5$) in the real world and 1 ($Q = 0.1$) in the VE. The first shopping street encountered on the walk scored less well, with a median of 2 ($Q=0.5$) in the real world and VE. Although less liked than the shopping precinct, this score did not mean participants disliked this shopping street, with positive remarks such as ‘good variety of shops’, ‘not too many people about’ and ‘bright and wide’. The next street encountered towards the end of
the walk was busier but, although it again compared unfavourably with the shopping precinct, it received a similar score to the first shopping street. Wide and relatively litter-free pavements were commented upon favourably, as well as the modern bus shelters, which were liked as places to wait for a bus.

The least liked environment was the first side street, Baker Street, with participants making remarks such as ‘scruffy’, ‘too many cars’ and ‘too narrow’. The virtual model appeared to simulate this well, with no significant difference compared to the real-world street. Advised by the local authority planner on the project’s advisory group, the attractiveness of the street was improved in the adapted VR model by adding street furniture, small trees and traffic calming. This resulted in a significant improvement to how inviting participants perceived the street to be compared to the real world ($z = -2.32, p = 0.02$).

Dementia at the mild to moderate stages might be regarded as presenting particular risks when crossing roads. However, the VR technology had limitations regarding testing road-crossing behaviour. Despite the semi-immersive wide screen projection it was not possible to reproduce the extent of peripheral vision experienced in the real world. This resulted in road crossing behaviour that was often unsafe in the virtual environment, but all roads were generally crossed safely during the real-world walks. There was a close to significant difference in safety between crossing the first shopping street and a goods entrance encountered later on in the walk, with a higher safety rating in favour of the shopping street ($z = -1.90, p = 0.06, r = -0.33$). This seems likely to have been due to more traffic along the shopping street leading to participants behaving more safely.

Overall, we found that participants were very aware of the dangers of motor traffic, although this may mean that people with dementia are discouraged from using outdoor spaces with traffic. The popularity of the shopping precinct had much to do with the absence of motor traffic, while the busy shopping street elicited positive comments about how wide both
the pavements and the road were, attributes lacking in the widely disliked first side street. At the end of the walks, the participants were asked whether they would have liked to have walked the route on their own. The most common response for the real world, unadapted and adapted VE walks was a qualified ‘possibly’. When asked if they would like to walk the route with someone else, the answers for all the environments were generally positive. There was least variation for the adapted VE, with all participants answering that they would be confident to walk the route with someone else.

All tasks were analysed by the participants’ level of cognitive impairment, assessed using their MMSE score and Spearman’s rho correlation coefficient. In the real world, the lower the MMSE score, the more likely participants were to act unsafely in the first side street ($\rho = -0.70, p = 0.002$). This suggests that focusing on the task of finding a particular address distracted the more cognitively-impaired participants from the dangers of road traffic. A lower MMSE score was also associated with navigating less well out of the shopping precinct, liking the busy road less, and being less likely to identify the modern phone boxes as phone boxes.  

Discussion

The results suggest that a real town centre offers relatively few obstacles for people with mild to moderate dementia; their road crossing behaviour was generally safe and street furniture was recognised. Some improvements, however, made a measurable difference, particularly signage. Signs worked well for identifying a bus station, a post office and bus stops, and for finding the way to public toilets. The signs that appeared to work best were straightforward descriptive text signs in clear lettering. We conclude that clearly displayed signs using explicit words or numbers deliver a significant benefit for people with dementia, and indicate both the way to destinations and the function or purpose of buildings and other objects in the environment. This finding is consistent with other evidence that people with dementia are
better at understanding words than photographs, in particular when the subject is less familiar (Gross et al. 2004). The retention of semantic memory among many people with Alzheimer’s disease, despite the impairment of episodic memory, also points to the likely value of clear word signs that will often be understandable even though other abilities are significantly impaired. Maps, however, proved impossible to use as a navigation device.

Crossing roads safely was generally not a problem, although participants with a higher degree of cognitive impairment experienced more difficulty, including problems with surfaces shared between pedestrians and motor traffic unless very clearly demarcated. Participants tended to dislike motor traffic, which was reflected in how much they liked the pedestrianised shopping precinct compared to other settings, although wide pavements and roads appeared to ameliorate the effects of traffic. Contrary to Burton, Mitchell and Raman (2004), we found no significant problems with most participants’ ability to recognise the function and purpose of modern designs of seating, telephone boxes and bus shelters, although those with greater impairment again were more likely to have difficulty with the legibility of these objects. Adding landmarks as navigation aids was not successful, although landmarks in familiar environments may still be important (Sheenan, Burton and Mitchell 2006).

We did find that as impairment increased, the environment became more challenging for our participants, even with adaptations. It is difficult to generalise about a cut-off point at which adaptations become irrelevant, not least because people with severe dementia were excluded from our study. The changes we propose are quite modest but likely to have a benefit across a range of mild to moderate impairment. Whether they would actually encourage more outdoor walking would need further research, but this seems likely and could be promoted to raise awareness about the benefits of staying active outdoors.
Overall, the participants enjoyed their walks and encountered relatively few problems, although they preferred to be accompanied. During the interviews prior to the walks with the participants and their carers, there were many expressions of appreciation of both the social and aesthetic features of outdoor environments. Diminished outdoor activity was experienced as a decrease in quality of life. The most common reason for going out was shopping. Walking in a town centre and other outdoor environments should therefore be encouraged for people with dementia, and those at the mild to moderate stages appear still to be able to undertake many activities independently. Improving signage along the lines we recommend, and creating more spaces free or generously separated from traffic and with convenient seating, are likely to support this independence and also to enhance the experience of being outdoors when the support of a carer is needed.

There were technical limitations to the VR and projection technology, especially detailed resolution and the ability to reproduce the extent of peripheral vision participants had in the real world. Although the latter affected our ability to simulate road-crossing behaviour, safe road-crossing was not a problem in the real world, so this limitation does not affect our recommendations. Resolution problems with signage were more of an issue. While improving the visibility of the bus numbers clearly helped in the VE, the lack of an effect compared to the real world suggests that the VE effect is due to the poorer resolution of the VE projection compared to the real world. In addition, although we found that our participants were able to use a joystick to navigate the VEs, this presented more of a challenge than navigating the real-world environment because of the skill needed to co-ordinate joy stick movements with movement through the VE.

**Conclusions**

The United Nations Organisation identifies 45 countries that have passed disability-specific laws, but the institutional frameworks within which disability policies are established and
implemented vary (United Nations, Committee on a Comprehensive and Integral
International Convention on the Protection and Promotion of the Rights and Dignities of
Persons with Disabilities Organisation 2006). For example, Sweden incorporates disability
rights into other legislation, such as its Planning and Building Act which requires built
environments to be designed with ‘means enabling people with limited mobility or
orientation capacity to use the area’ (Sweden, National Board of Housing, Building and
Planning 2006: 13). Although Sweden is one of the few countries that explicitly recognises
orientation capacity as a disability, there is little guidance about how built environments
should be designed to take this into account, in Sweden and elsewhere.

We conclude that planners and designers should make more use of simple text signs
as aids for navigating and identifying the purpose of objects and places in the environment.
This explicit labelling of features in the outdoor environment would be an explicit dementia
adaptation likely both to benefit others and raise awareness of dementia. Because the number
of people with dementia is sizable and growing, even small improvements from a change like
this could represent a large global gain because a substantial number of individuals benefit.
These improvements could also complement and even enhance the benefits of drugs that help
with the symptomatic effects of dementia. Further benefits may extend to family carers by
helping to make trips outdoors a more shared and enjoyable experience. Yet it is important
not to exaggerate how much the outdoor environment should be adapted. Our participants
enjoyed their walks and some would clearly have liked to have got out more. We have
identified some environmental problems that should be addressed but there seems little
reason why our group of participants, with mild to moderate dementia, should regard a town
centre environment like Middlesbrough’s as on the whole unfriendly to their impairment.

NOTES
A subsequent grant application to support a major project was successful and also received National Health Service (NHS) research ethics approval (as did the pilot research). The study was guided by an advisory group comprising two consultant psychiatrists of old age, an architect, a representative of a company manufacturing street furniture, a local authority town planner, a local authority social worker and a carer.

The successive test statistics ($\rho$, $p$) were: -0.50, 0.04 for poorer navigation; -0.57, 0.02 for dislike of the road; and -0.61, 0.01 for poor identification of telephone boxes.
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References


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<table>
<thead>
<tr>
<th>Tasks</th>
<th>Real town centre</th>
<th>Virtual simulation</th>
<th>Virtual simulation with adaptations</th>
<th>Real compared to virtual</th>
<th>Virtual adapted compared to virtual unadapted</th>
<th>Real compared to virtual adapted</th>
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<td>2 (1.0)</td>
<td>2 (1.5)</td>
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<td>0.37*</td>
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<td>2 (0.5)</td>
<td>1 (0.0)</td>
<td>Ns</td>
<td>-0.43*</td>
<td>Ns</td>
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<td>2 (1.0)</td>
<td>1 (0.0)</td>
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<td>-0.70***</td>
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<tr>
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<td>2 (0.5)</td>
<td>1 (0.5)</td>
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<td>-0.64**</td>
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<tr>
<td>Using third toilet sign to remember where to go</td>
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<td>4 (1.5)</td>
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Notes: *p < 0.05; **p < 0.01; ***p < 0.001; Ns=not significant.