Evidence for motor learning in Parkinson’s disease: Acquisition, automaticity and retention of cued gait performance after training with external rhythmical cues

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**ABSTRACT**

People with Parkinson’s disease (PD) have difficulty learning new motor skills. Evidence suggests external stimuli (cues) may enhance learning; however, this may be specific to cued rather than non-cued performance. We aimed to test effects of cued training on motor learning in PD. We defined motor learning as acquisition (single task), automaticity (dual task) and retention of single- and dual-task performance (follow-up). 153 subjects with PD received 3 weeks cued gait training as part of a randomised trial (the RESCUE trial). We measured changes in cued gait performance with three external rhythmical cues (ERC) (auditory, visual and somatosensory) during single and dual tasks after training and 6 weeks follow-up. Gait was tested without cues to compare specificity of learning (transfer). Subjects were ‘on’ medication and were cued at preferred step frequency during assessment. Accelerometers recorded gait and walking speed, step length and step frequency were determined from raw data. Data were analysed with SAS using linear regression models. Walking speed and step length significantly increased with all cues after training during both single- and dual-task gait and these effects were retained. Training effects were not specific to cued gait and were observed in dual-task step length, and walking speed however was more limited in single-task non-cued gait. These results support the use of ERC to enhance motor learning in PD as defined by increased acquisition, automaticity and retention. They also highlight the potential for sustained improvement in walking and complex task performance.

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1. Introduction

Research has highlighted a role for the basal ganglia in learning, raising questions regarding the capacity for people with Parkinson’s disease (PD) to acquire and retain motor skills (Doyon, 2008). Motor skills and sequences of movement are acquired through a process of implicit learning, becoming automatic with practice and performed with little conscious awareness as opposed to explicit learning which refers to acquisition of factual knowledge. Automatic movements have functional benefits, allowing dual- and multiple-task performance, and are impaired in PD (Bond and Morris, 2000; O’Shea et al., 2002; Rochester et al., 2004). The main method of managing the symptoms of Parkinson’s disease (PD) is through levodopa therapy providing highly effective symptomatic treatment. However, after a variable period of time, motor complications develop that cannot be adequately controlled with medication. These complications include unpredictable and extended ‘off’ periods, dyskinesias, poor gait and balance which contribute to reduced mobility and increased risk of falls. Rehabilitation strategies that result in sustained change in function in PD may therefore offer an important contribution to patient management. Knowledge of motor learning is therefore critical for the optimal design of rehabilitation interventions (Abbruzzese et al., 2009).

Motor skill acquisition, automaticity and retention are considered hallmarks of motor learning. Learning a motor skill has two phases in which the skill is acquired over a short time scale (a few hours) and then consolidated (retained) into long-term memory with practice over several weeks (Karni et al., 1995). During the early stage attention is required and with repeated practice this is reduced such that performance can occur automatically with relatively little attention. Whilst a recent meta-analysis concluded that implicit sequence learning was impaired in PD (Siegent et al., 2006) others have reported that it was possible but attenuated compared to controls (Wu and Hallet, 2008; Wilkinson and Jananshanhi, 2007; Mentis et al., 2003; Carbon and Eidelberg, 2006). In PD the fast stage of learning where skills are acquired may be preserved; however, transfer of skills into later stages of learning with increased automaticity and retention of skill is less efficient compared to age matched controls (Doyon et al., 1998; Wu and Hallet, 2008; Wilkinson and Jananshanhi, 2007; Muslimovic et al., 2007).

Motor learning studies demonstrate that there is capacity for people with PD to learn a variety of motor tasks ranging from upper limb movement to functional tasks (Smiley-Oyen et al., 2006; Jessop et al., 2006; Mak and Hui-Chan, 2008). A common feature of these studies is the use of augmented feedback such as auditory pacing cues or visual cues for training. External information in the form of visual or auditory stimuli improves motor performance and motor learning in normal subjects (Heremans et al., 2009; Semjen et al., 2000). Augmented visual feedback also facilitated motor learning in a novel upper limb task in PD (Verschueren et al., 1997). Interestingly in this study, the effects were not retained in the absence of sensory information, highlighting the possibility that learning in PD is context specific with external feedback being incorporated into the motor program (Verschueren et al., 1997). The study by Verschueren et al. (1997) also highlights an important point in relation to methods of evaluating training as some studies test the effect of training with the cue and others test without the cue and therefore measure transfer of skill. This is an important distinction as cue specific learning (described by Verschueren et al., 1997) may be missed.

External cues have received much interest as a rehabilitation tool to improve function such as gait in PD (for review, see Rubinstein et al., 2002; Lim et al., 2005; Nieuwboer et al., 2008). External cues have been defined as temporal (e.g., auditory tones to step in time to) or spatial (lines on the ground to step on or over) stimuli associated with the ongoing facilitation of movement (Nieuwboer et al., 2007). In PD, where practice was given in a single training session external cues improved walking speed and step length (Morris et al., 1996; Rochester et al., 2007; Baker et al., 2007; Hausdorff et al., 2007). Dual-task walking speed and step length also improve suggesting that gait had become more automatic (Rochester et al., 2005, 2007; Baker et al., 2007). Furthermore a one off-training session with external cues had a short-term carryover effect to non-cued gait (up to 2 h) (Morris et al., 1996; Rochester et al., 2005, 2007; Baker et al., 2007, 2008; Hausdorff et al., 2007), which may indicate the early stages of learning or heightened attention. These studies measure cued gait and underscore the potential for cueing training as a motor learning paradigm. However they also raise the question as to whether a longer period of training may facilitate the later phase of motor learning in PD to enhance automaticity and retention.

Clinical studies have shown that externally cued practice over more extended periods (3–6 weeks) show significant benefits of training with a range of different external cues on gait, balance and transfers (Thaut et al., 1996; Nieuwboer et al., 2007; Mak and Hui-Chan, 2008; Sidaway et al., 2006). Cued training has also been shown to be more effective than other interventions such as non-cued exercise (Mak et al., 2007; Marchese et al., 2000). Evidence for retention is inconclusive however with some studies reporting retention of between 2 and 4 weeks (Mak and Hui-Chan, 2008; Sidaway et al., 2006) whilst others of longer duration (6 weeks) do not (Nieuwboer et al., 2007). In all of these studies, training effects were measured without the external cue; therefore, they primarily concentrated on generalisation to a range of functional skills and motor symptoms. It may be that learning in PD is cue specific as reported by Verschueren et al. (1997) and the effect of training on cued performance is therefore not clear to date.

As stated above, we have shown that gait training with external rhythmical cues (ERC) (the RESCUE trial) (Nieuwboer et al., 2007) improved a variety of functional tests relating to a broad range of mobility outcomes. These effects were not retained at 6 weeks; however, the method of assessment could be insensitive to learning effects specific to cued performance. We were therefore also interested in the specificity of training with ERC on cued gait performance, which we evaluated using a specially designed protocol that measured cued single- and dual-task gait performance (Rochester et al., 2007). We used three different ERC modalities (visual, auditory and somatosensory) and found ERC (especially auditory) immediately enhanced dual-task gait performance before training and had
a short-term carryover to non-cued gait but these effects were not retained when measured 3 weeks later (Rochester et al., 2007). Here we extend our findings to report the effect of training with ERC on cued gait performance during single- and dual-task gait and retention of performance. These are new data and have not been previously reported. In this study we defined motor learning as acquisition, automaticity and retention of skill. We hypothesized that an extended period of training with ERCs would further enhance cued gait performance during single (acquisition) and dual tasks (automaticity) and would be retained. We also tested training effects during non-cued single- and dual-task gait to see learning was specific to cued trials only.

2. Results

Demographic subject data are shown in Table 1. There was no difference between the characteristics of the early and late groups. Most patients had mild to moderate disease severity as 46.4% of patients (n=71) were in Hoehn and Yahr stage II, 41.8% (n=64) in stage III and 11.8% (n=18) in stage IV. During the 3-week training period, 102 (67%) trained with the auditory cue, 51 (33%) with the somatosensory cue and no one selected the visual cue. During assessment however cued gait was tested with all ERC types (see Fig. 1).

Walking speed, step length and cadence were estimated from raw data obtained with accelerometers as shown in Fig. 1. These data were collected during single- and dual-task walking. Descriptive data (mean and SD) for all gait variables are shown in Table 2. We estimated the effect of cueing training using multiple linear regression models which accounted for repeated measures inherent in the study design (Fig. 2). During single and dual tasks the training effect for each ERC (visual, auditory and somatosensory) was estimated using the first three assessments (T1, T2, T3). Statistical models corrected for differences between early and late groups at baseline, time effects (T1–T2 late group) and carryover effects (T2–T3 early group) (Fig. 2 for study design). The same procedure was used for each variable (speed, step length and cadence). The change scores reported in Table 3 are therefore the estimates of training effects on cued performance with baseline, time and carryover effects controlled for (Table 3). Data from early and late groups are pooled for descriptive purposes before, after and at follow-up. Missing data were due to equipment failure or recording difficulties which occurred in a random fashion and the characteristics of those patients were not different from those included in the analysis. Motor learning was defined as acquisition, automaticity and retention and the results are presented according to this definition.

2.1. Acquisition (single task)

Following cueing training single-task walking speed significantly increased with each ERC and this was largely achieved by a significant increase in step length. There was a significant increase with all ERC modalities (visual, auditory, somatosensory) despite the fact that no one chose to train with the visual cue. There was no significant difference in training effect between cue types. Unsurprisingly cadence did not change significantly following training due to the fact that gait was cued at preferred rate and effectively controlled this parameter. There was limited transfer to non-cued gait in the single task with significant improvement in step length; however, walking speed did not change significantly and cadence decreased.

2.2. Automaticity (dual task)

We used gait performance under dual-task conditions as a measure of automaticity and change in attentional resources with increased proficiency in performance (Doyon, 2008; Jueptner et al., 1997; Wu and Hallett, 2008). Firstly, we identified the attentional demands of the dual task by comparing single- and dual-task non-cued gait using pooled data from early and late groups at T1. Walking speed (pre p<.0001; post p<.0001) and step length (pre p<.0001; post p<.0001) were significantly reduced during the dual-task compared to single-task gait; however, cadence remained unchanged. The baseline characteristics of the early and late groups are shown in Table 2, and again differences between groups at baseline were adjusted for in the analysis. The training effects on cued gait during dual tasks are shown in

## Table 1 – Comparison between early and late cueing training groups for demographic and clinical characteristics (n=153). Data are described as median and interquartile range (Q1–Q3) values except for age and freezers/non-freezers where the total number of each is given. Significance level is p=0.05.

<table>
<thead>
<tr>
<th>Demography</th>
<th>Early n=76</th>
<th>Late n=77</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female *</td>
<td>48/28</td>
<td>40/37</td>
<td>0.16</td>
</tr>
<tr>
<td>Age</td>
<td>67.5 (61.5–72)</td>
<td>69 (62.5–73)</td>
<td>0.7</td>
</tr>
<tr>
<td>PD characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease duration</td>
<td>7 (4–11)</td>
<td>8 (4–12)</td>
<td>0.59</td>
</tr>
<tr>
<td>H&amp;Y (on)</td>
<td>2.5 (2.5–3)</td>
<td>3 (2.5–3)</td>
<td>0.56</td>
</tr>
<tr>
<td>H&amp;Y II/III/IV *</td>
<td>39/29/8</td>
<td>32/35/10</td>
<td>0.48</td>
</tr>
<tr>
<td>Freezers/N-freezers *</td>
<td>31/45</td>
<td>32/45</td>
<td>0.92</td>
</tr>
</tbody>
</table>

| Clinical data       |            |           |         |
| UPDRS-total (on)    | 54 (46–65.5) | 56 (49–63) | 0.62    |
| UPDRS I (on)        | 4 (2–5)    | 3 (2–4)  | 0.1     |
| UPDRS II (on)       | 16 (12–19.5) | 16 (12–20) | 0.67    |
| UPDRS III (on)      | 31 (25–37) | 34 (28–41) | 0.32    |
| UPDRS IV (on)       | 3 (1–5)  | 2 (1–5) | 0.34    |
| Levodopa (mg)       | 500 (300–700) | 350 (200–550) | 0.07    |
| MMSE                | 28.5 (27–30) | 29 (27–30) | 0.99    |
| Brixton             | 4 (2–6)   | 4 (2–6)  | 0.45    |
| HADS anxiety        | 6.5 (4–10) | 6 (4–10) | 0.97    |
| HADS depression     | 7.5 (5–10) | 6 (4.5–9) | 0.45    |

H&Y=Hoehn and Yahr stages during on; UPDRS=Unified Parkinson’s Disease Rating Scale; PD=Parkinson’s disease; MMSE=Mini Mental State Examination; HADS=Hospital Anxiety and Depression Scale.
*Expressed as number of patients and p values based on Chi-squared test.
Table 3 as change scores for each gait variable. Similar to single-task gait, dual-task cued walking speed and step length significantly increased with all ERC modalities after training, whilst again cadence remained unchanged. There were also no significant differences in training effect between cue types. In contrast to single tasks however, there was a greater transfer of training to the non-cued dual-task trials where a significant improvement in walking speed and step length was observed.

2.3. Retention (single and dual)

We measured retention of cued gait performance as an indicator of consolidation into long-term memory (Doyon, 2008). Retention was determined by comparing the change between T3 and T4 for early and late groups combined. The pooled descriptive data for the early and late group and change scores after 6 weeks are shown in Table 3. For all cued...
trials and all gait variables the effects of training were retained during both single and dual tasks with some evidence for a slight increase as shown by positive values. These changes were very small however and difficult to interpret. In addition, retention was also observed for all gait variables during non-cued trials for both single and dual tasks.

3. Discussion

The key findings in this study were that gait training with ERC increased single- and dual-task cued walking speed and step length and these effects were retained at follow-up. These changes were consistent with our definition of motor learning supporting our hypothesis that cued training would increase motor learning. Practice also transferred to non-cued gait, generalised across cue types and was retained. These data are robust given the study design and novel and add to knowledge regarding motor learning and its application to rehabilitation in PD.

We saw significant improvements in walking speed and step length with all ERC during the single task, which we termed acquisition of skill. Prior to training however, the effect of ERC during a single task was limited with either no change or a small increase in walking speed or step length compared to non-cued gait (Rochester et al., 2007). It is interesting that significant improvements with training were seen with all ERC despite individuals electing to train with their preferred cue type only (67% auditory and 33% somatosensory). These data suggest that cued gait performance not only became more proficient following training but also transferred to all cue types providing additional support for motor learning. The fact that cadence did not change significantly in response to ERC is not surprising given that this temporal aspect of gait was constrained by the cueing frequency used. No other studies have reported cued gait performance after a period of training.

We found that cued dual-task walking speed and step length also significantly increased with all ERC after training. The dual-task was intentionally demanding (observed by significant reductions in walking speed and step length compared to single-task gait) and therefore improved dual-task performance supports increased automaticity. We also found that cues had an immediate and significant effect on dual-task walking speed and step length before training (Rochester et al., 2007). It seems therefore that practice increased proficiency and generalized to all cue types as seen in the single task. Significant improvements in dual-task gait performance in PD following training have also been
reported by others (Canning et al., 2008) indicating improved automaticity of movement, this study was however un-cued. Studies in PD in the upper limb demonstrate that automaticity can be achieved but with more difficulty and requires greater activation of neural networks (Wu and Hallett, 2005). Activity was increased in networks involving the cerebellum, premotor area, parietal cortex, precuneus and dorsolateral prefrontal cortex (DLPFC) compared to normal subjects (Wu and Hallett, 2005). Networks involved in the control of attention may also contribute. Increased activity in the DLPFC and anterior cingulate is associated with attentional control of movement when learning a new task (Jueptner et al., 1996; Johansson-Berg and Matthews, 2002) and disappears when the task is performed automatically (Jueptner et al., 1996). When finger movements are externally paced by a metronome the DLPFC is only activated when the rhythm is modified and subjects are fully conscious of their movements (Stephan et al., 2002). These networks may therefore compensate for basal ganglia dysfunction and contribute to motor learning in PD.

We demonstrated retention after 6 weeks, which is longer than other studies of functional motor learning which range from 15 min to 4 weeks (Chuma et al., 2006; Mak et al., 2007; Smiley-Oyen et al., 2006; Jessop et al., 2006). However, whilst we report a relatively long retention period, studies over longer time scales (1 year) in PD have shown that retention is impaired (Doyon et al., 1998). Increased cued single-task gait speed and step length may be related to a relatively fast phase of learning (explicit learning) (Karni et al., 1995), however, retention of single- and dual-task cued gait performance supports consolidation of training into long-term memory.

We found only limited transfer to non-cued single-task gait with no significant change in walking speed although step length increased. These results agree in part with the findings of Verschueren et al., (1997), who found no transfer of effect to non-cued trials. However, we were surprised to find that training did transfer to non-cued dual-task walking speed and step length. Farley and Koshland (2005) also found generalization of amplitude training of limb movements to speed improvements in non-cued in PD. Requirements for motor learning (Schmidt and Wrisberg, 2000) advocate varied practice which is functionally relevant and we adhered to these principles, which may explain transfer of skill to non-cued conditions and retention. Participants were required to synchronize their gait with the cue and to practise this in a variety of different tasks and environments. Whether this is related to the type of training is uncertain. Transfer of training was also observed across different cue modalities as seen by increased gait speed and step length in visual cued trials despite the fact that this cue type was not selected for practice by any participant. These results present the possibility that motor representation of rhythm may become stored in cerebellar circuits providing a role for the cerebellum in how movements are performed in relation to their timing (Rannan and Passingham, 2001). Whether different neural networks contribute to augmented learning with rhythmic cues cannot be answered in this study as we did not have a control group where gait without cues were practised. This question still needs to be addressed in future work.

There are some limitations to this study which should be addressed. Because of the study design (crossover) placebo effects were not controlled for in this study. Also we did not correct for multiple comparisons in the analysis which amounted to eight comparisons for each gait outcome. The results should therefore be interpreted cautiously. It is also important to note however that the statistical model used in the analysis provided a conservative estimate of the training effects as it corrected for time and carryover effects inherent in the study design, estimating training independently which will partly ameliorate the above limitations. The findings of this study cannot be generalized to people with PD who have significant cognitive decline and other co-morbidities and future studies should focus on these effects. It is highly likely that subtle cognitive deficits and cognitive decline associated with PD will impair motor learning. The impact of different stages of the disease and disease severity was also not a focus of this study and these factors are likely to impact on learning ability.

Training increased cued gait performance for both single and dual tasks and these effects were retained. This is the first report of learning in cued gait and also on automaticity and the findings are therefore novel. These results together with large subject numbers and randomised controlled trial design provide robust evidence regarding the capacity of people with PD for motor learning. The possibility to increase automatic performance of dual tasks has functional implications for people with PD with potential impact on independence and safety. The use of external cues to augment gait performance may therefore be considered an effective motor learning paradigm for people with PD.

4. Experimental procedure

4.1. Subjects

153 participants with idiopathic PD were recruited from three centres around Northumbria University, Newcastle upon Tyne (UNN), Katholieke Universiteit of Leuven (UNL) and the Department of Neurology at Vrije Universiteit Medical Centre of Amsterdam (UNA). The study was approved by the ethics committee of each participating centre. All participants gave informed written consent to the study. Eligibility criteria were: a diagnosis of idiopathic PD, defined following the UK Brain Bank Criteria; stable medication usage; Hoehn and Yahr stage II, III or IV; at least one score >2 for one or both limbs for either the tremor, rigidity or the bradykinesia items of the UPDRS; able to walk independently; age 18–80 years; no severe cognitive impairments (Mini Mental State Examination (MMSE) >24; no other severe neurological, cardiopulmonary or orthopaedic disorders; absence of drug-related fluctuations making testing difficult, and no participation in a physiotherapy program two months prior to commencing the trial. Participants were excluded if they had undergone functional neurosurgery.

4.2. Experimental protocol measuring the effect of ERC training (Fig. 1)

The effect of ERC training on cued gait performance with ERC (visual, auditory and somatosensory) was investigated using a...
test of functional gait (Rochester et al., 2007) which incorporated a single and a dual task. Participants started from standing and were instructed to walk 6 m to pick up a tray with two cups on it (filled to a standard level), turn around through 180°, carry the tray back to the start position and stop. Participants were asked to walk at their preferred speed and concentrate equally on all elements of the test. The test was divided into two conditions: walking without tray (single task) and walking with the tray (dual-task). Seven trials of the test were carried out: non-cued; two cued trials with each cue type (visual, auditory, somatosensory). Trails were measured without cues to determine transfer of cued training and specificity of learning. A prototype cueing device worn on a belt around the waist provided three rhythmical cueing modalities: (1) auditory (a beep delivered through an earpiece); (2) visual (light flashes delivered through a Light Emitting Diode attached to a pair of glasses); and (3) somatosensory (pulsed vibrations delivered by a miniature cylinder worn under a wristband). The order of cue modalities was randomised by selecting cards; however, baseline gait was always tested before cued trials. Participants were asked to synchronise each step with the rhythm of the cue set at preferred stepping frequency determined during a 6 m walk test conducted at preferred walking speed. Gait was measured using a Vitaport Activity Monitor® (VAM) (TEMEC Instruments Inc.) which is a valid and reliable tool for measuring gait in PD (Lord et al., 2008). The activity monitor measured movement with five accelerometers, one attached to each leg and three placed on the lower third of the sternum. Data were sampled at a frequency of 25 Hz and stored on a removable memory card for later analysis. Data were analysed using a specifically designed software program (Vitagraph) (TEMEC Instruments Inc.). Movement was not restricted in any way by either the monitor or the cueing device. Measurements were taken approximately 1 h after medication to ensure participants were in the ‘on’ phase of their medication. Walking speed, mean step length and cadence were estimated from analysis of the raw data collected by the VAM and validated in a previous study (Lord et al., 2008) (Fig. 1C). The test was divided into two conditions (described above) for data analysis (Fig. 1C): (1) walk only-single task; (2) walk + carry tray = dual task. Four trials were determined for each condition (single and dual): non-cued walking, visual, auditory and somatosensory cued walking, the cued trials being the mean value of each pair of cued trials. The test of functional gait was carried out in parallel with a single-blind, randomised clinical trial with a crossover design (the RESCUE trial) (Fig. 2) (Nieuwboer et al., 2007). The experimental protocol was performed by a blinded assessor in the persons home immediately before randomisation (T1) at 3 (T2), 6 (T3) and 12 (T4) weeks (Fig. 1) in the early and late groups.

4.3. ERC training

Participants were randomised to receive 3 weeks of cueing training either immediately (early) or after a 3-week delay (late) and both groups were followed-up for 6 weeks after cues were withdrawn (Nieuwboer et al., 2007) (Fig. 2). A prototype cueing device (described above) was used to deliver the cues during training. This was worn on a belt around the waist and provided three rhythmical cueing modalities: (1) auditory; (2) visual; and (3) somatosensory. Cueing was rhythmical, addressing temporal control of gait and aimed to improve step length and walking speed. Participants used ERC to augment gait performance during nine sessions which lasted for 30 min each. Participants tried all cue modalities in the first week and then selected their preferred modality to train with. To assess the effect of cued training however, participants were assessed with all three cues to evaluate generalisation from one cue type to another. A trained therapist facilitated the use of ERC during walking using the prototype cueing device, and this was carried out in the participants home. Gait was augmented by asking participants to step in time to the rhythmical cues which were applied during single-, dual- and multi-task walking. Cueing therapy consisted of the following components: gait initiation and termination, heel strike and push-off, sideways and backwards stepping, walking while dual tasking, and walking over various surfaces and long distances. Complex tasks were used to reduce available explicit resources and encourage generalisation and automatisability. Evidence based cueing guidelines were used that specified cueing parameters and instructions (available on CD-ROM at http://www.rescueproject.org). Cues were delivered at preferred frequency according to the environment in which the participant was practising. Medical treatment continued unchanged throughout the study.

4.4. Data analysis

Data were analysed separately in one centre (Newcastle) by a tester who was not involved in data collection and was blinded to cue order for the trials. The effect of training on cued gait performance was analysed as described below. To measure acquisition and automaticity cued gait performance with each ERC was compared to gait performance after training during single (acquisition) and dual tasks (automaticity). In addition non-cued gait was also measured to allow transfer from cued to non-cued trial to be examined. The effects of cueing training on gait variables during single and dual tasks were estimated using the first three assessments (T1, T2, T3). Multiple linear regression models were used to account for repeated measures. The linear models were fitted for each gait variable (walking speed, step length and cadence) with PROC MIXED in SAS (V8.2). In this model the effects of cueing training were corrected for differences of variables at baseline, time effects and carryover effects. Retention was assessed by comparing the change between T2 and T4 using a model with two factors (time and group) fitted to the outcomes of tests 1–4 for early and late groups combined. Results are reported as beta estimates and standard errors (SE). Bonferroni corrections were not applied. Two-tailed analysis was performed on all tests with a significance level of p=0.05.

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