RESEARCH PAPER

Hippocampal dysfunction defines disease onset in Huntington’s disease

Faye Begeti, 1 Laetitia C Schwab, 1 Sarah L Mason, 1 Roger A Barker 1, 2

ABSTRACT

Background Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder characterised by a triad of motor, psychiatric and cognitive deficits with the latter classically attributed to disruption of frontostriatal networks. However, emerging evidence from animal models of HD suggests that some of the early cognitive deficits may have a hippocampal basis. The objective of this study was to link previous rodent findings in this area to clinical practice.

Methods In this study, 94 participants included patients with early HD, premanifest HD and age-matched controls underwent hippocampal-based cognitive assessments. These included a virtual reality version of the Morris water maze, a task involved participants having to swim through a virtual pool to find a submerged platform using a joystick, and the Cambridge Neuropsychological Test Automated Battery (CANTAB) paired associates learning task, a test also known to rely on hippocampal integrity.

Results Patients with early HD showed impaired performance in both the virtual Morris water maze and the CANTAB paired associates learning. Such deficits were also correlated with estimated years to diagnosis in premanifest participants.

Conclusions This study highlights the merit of using analogous tests in the laboratory and clinic and demonstrates that hippocampal impairments are an early feature of HD in patients as previously shown in rodent models of the disease. As such, they could be used not only to assist in the diagnosis of disease onset, but may also be useful as an outcome measure in future therapeutic trials.

INTRODUCTION

Huntington’s disease (HD) is an autosomal genetic disorder caused by a mutation in the huntingtin protein. Clinically, it presents with a combination of motor, cognitive and psychiatric features, which typically begins in the fourth to fifth decade of life. 1 Although the motor features of the disease form the basis of the clinical diagnosis, 2 it is now widely accepted that cognitive abnormalities occur early in the course of the disease, and in many cases predate the onset of motor features of HD. Furthermore, it is the cognitive and psychiatric difficulties experienced by patients, and not the movement disorder, that put the largest burden on HD families, and which are most predictive of the need for nursing home care. 3–6

The origin of cognitive abnormalities in HD have been largely, but not exclusively, attributed to disruption of the neural pathways connecting the frontal lobe and the striatum, a region of the basal ganglia highly affected in HD. 7–8 However, even in the early stages of HD, MRI and postmortem studies have shown that pathology is much more widespread with several other structures affected, including the hippocampus, 9–10 a region known to be important for learning, memory and spatial navigation. 11 A variety of hippocampal abnormalities have been reported in a number of HD mouse models, 12–18 including the early appearance of aggregates of the mutant huntingtin. 17–19 All of this would help explain why transgenic HD animals have impairments in a task called the Morris water maze (MWM), 17 where rodents have to learn and memorise the location of a hidden platform in a pool of water using visual cues. However, despite evidence of hippocampal deficits in transgenic animal models of HD, and cell loss in this region from human postmortem and neuroimaging studies, 16–22 hippocampal function has not been overtly studied in patients with HD.

In this study, we sought to investigate this using patients with HD and matched controls and two spatial memory tasks; a virtual human version of the MWM task and a computerised spatial memory task called the paired associates learning (PAL) task.

MATERIALS AND METHODS

Participant recruitment and screening

Ethical approval for this study was granted by the local Research Ethics Committee (reference number 09/H0308/2) and Addenbrooke’s Hospital R&D department. Informed consent was obtained from all participants in compliance with the Declaration of Helsinki.

Patients with HD in the early stages of the disease and premanifest gene carriers (premanifest HD, preHD) were identified from the HD clinic in the John van Geest Centre for Brain Repair, Cambridge, UK. Patients with manifest HD had received a diagnostic confidence rating scale of 4 by an accredited HD clinician and the Total Functional Capacity (TFC) score was used to identify patients in the early manifest stages of the disease (TFC score ≥ 10).

Age-matched and sex-matched control participants were recruited from the patients’ partners and the local community via advertisement. Inclusion criteria were (1) no family history of HD or a negative genetic test for HD; (2) Mini Mental State Examination (MMSE) ≥24 and (3) absence of any other known neurological or psychiatric disorder.


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Participants were assessed using the Addenbrooke’s Cognitive Examination—Revised (ACE-R), a test of global cognitive performance and a sensitive screening tool for dementia, and the National Adult Reading Test (NART), a measure of estimated premorbid verbal IQ to ensure that the groups were well matched.

Cognitive assessment

Participants underwent cognitive testing following a routine clinic visit or during a separate prearranged appointment. Owing to time issues and fatigue effects, some participants who chose to participate in this study following their routine clinic visit were only tested in one of the two tasks described below with a small proportion of participants completing both tasks.

Virtual human MWM

The MWM was designed using commercially available software from neuroinvestigations. This task is set in a virtual landscape, similar to a computer simulation game, and was developed from the classic MWM experiments. During the task, participants have to swim through a virtual reality swimming pool on the computer using a joystick in order to learn the location of a submerged hidden platform relative to external cues on the wall (see online supplementary figure S1A).

Participants initially habituate to the testing equipment by swimming towards a visible platform without navigational cues for four trials (see online supplementary figure S1B). Prior to the beginning of hidden platform training, participants are advised that the platform will be submerged under the surface of the water and so will no longer be visible and they have 60 s to find the platform by swimming through the pool (see online supplementary figure S1C). The computer will inform them, by a high-pitched sound, when they swim over the correct location and the message ‘platform found’ is displayed on the screen for 2 s, during which participants can turn around to orientate themselves before another trial is automatically initiated. If the platform is not found within 60 s, it becomes visible and participants have to swim towards the visible platform in order to initiate the next trial (see online supplementary figure S1D). The hidden platform training consists of five blocks of four trials, with each trial beginning from a different location of the pool (north, east, south and west) during which the platform location remains constant in the northeast quadrant.

At the end of the hidden platform training, there is a probe test which is a single trial lasting 60 s, indistinguishable from previous trials, but in this case there is no platform and no associated high-pitched sound. Recorded measures during the probe test include the percentage of time spent in the platform quadrant, initial heading error and total distance travelled.

Following the hidden platform training and probe test, there is a motor screening session to assess potentially confounding movement or motivational issues. During this screening session, participants are advised to swim towards a visible platform as quickly as they can for the next eight trials.

Paired associates learning

This is a touch-screen task of visuospatial memory and associative learning from the Cambridge Neuropsychological Test Automated Battery (CANTAB). During the encoding phase, boxes are opened one at a time in a random order from a circular display (see online supplementary figure S1E). One or more boxes contain a pattern which has been designed, so that a verbal label cannot be given to it. After all the boxes have been opened, in the recognition phase, each pattern is displayed one at a time and the subject must correctly identify the box that contained each specific pattern (see online supplementary figure S1F). The task gets progressively more difficult as the number of boxes containing a pattern at each stage increases (initially 1 pattern followed by 2, 3, 6 and 8 patterns). Following incorrect trials, all the boxes are reopened as before and the recognition phase is repeated; this continues until the location of all patterns are correctly identified or 10 incorrect trials are completed. Recorded measures include the number of errors made at each stage of the task.

Statistical analysis

Statistical analysis was performed using IBM SPSS software V20.0 and V21.0, with missing data excluded from the analysis. Disease burden score was calculated using the following formula: DBS=(CAG−35.5)×age−0.146×(CAG)−age. Repeated measures, one-way analysis of variance and Pearson χ² were used where appropriate and all significant results were further analysed using post hoc Bonferroni corrected t tests. Graphs were created using graphPad prism V5.0 and are presented as the mean±SEM unless otherwise stated. The level of statistical significance was set at 0.05 and all F and p values are reported to three significant figures.

RESULTS

Participant characteristics

A total of 94 participants were recruited to the study and were divided into early HD participants, preHD and age-matched controls. As not all participants completed both tests the demographics of the groups that were tested on PAL or the MWM are shown in table 1. The virtual MWM was performed by 21 healthy controls, 35 patients with preHD and 18 patients with manifest HD. The PAL was performed by 13 healthy controls, 34 patients with preHD and 18 patients with manifest HD.

All participants were matched for age, gender and estimated premorbid verbal IQ as assessed by the NART. Controls and preHD participants were also matched for performance on baseline cognitive tests like the ACE-R and MMSE. The control group was also matched to the HD group in terms of age and estimated premorbid verbal IQ, but due to the nature of the disease, the HD group had significantly lower performance on the baseline cognitive tests (although above the range for dementia) (ACE-R: t(58)=4.23, p<0.0001; MMSE: t(58)=3.11, p=0.003), a common finding in many HD studies.

As expected, due to onset of manifest disease, the HD group also had higher Unified Huntington’s disease rating scale (UHDRS) Total Motor Score (TMS) and lower Functional Assessment (FA) scores than the preHD participants with no significant differences in TFC (table 1).

Thirty per cent of preHD and 50% of HD participants were taking antidepressant medication and 0% of preHD and 50% of HD participants were taking dopamine antagonists at the time of participation. Owing to the different types and varying doses of medication in these two classes, it was not possible to add these as covariates in the analysis. However, the impact of medication on performance was assessed using a Student t test to directly compare those taking and those not taking either antidepressants or dopamine antagonists. All analysis produced non-significant results; therefore, we concluded that medication did not significantly influence performance in this study.

General neurology
Patients with early HD are impaired in the virtual MWM

The MWM consisted of 20 trials of ‘hidden platform training’, subdivided into five blocks of four trials each (starting at different positions), where participants attempt to find a hidden platform (for screenshots of task, see online supplementary figure S1). There was no significant difference in latency between groups during the initial block (F(2,73)=0.838, p=0.437). As participants did not know the location of the platform, the latency of all groups tested decreased throughout the blocks indicating learning of the platform location. However, the HD patient group showed impaired learning compared with the control and preHD groups with statistically significantly worse performance during blocks 3–5 (block 5: F(2,73)=5.56, p<0.006) (figure 1A).

At the end of ‘hidden platform training’, there was a probe test, a single trial where the platform was omitted but was indistinguishable from previous ‘hidden platform training’ trials. Whereas controls and premanifest patients spent the majority of their time searching for the platform in the correct quadrant (55.9%±5.80% and 53.6%±5.43% for controls and preHD, respectively), HD participants spend significantly less time searching in the correct quadrant (28.2%±5.49%) (F(2,73)=7.42, p=0.001) (figure 1B, C), in a pattern suggestive of a random search (75% time searching in each quadrant). In addition, performance in this task was significantly correlated with disease burden score (r=-0.446, n=45, p=0.002) (figure 1D).

Since a feature of HD is motor abnormalities and this task depends on being able to move a joystick, the movement aspects of this task were recorded. There was no difference in the path length travelled during the 60 s of the probe test (F(2,73)=0.143, p=0.867) (figure 2A) indicating that patients with HD are equally motivated to reach a visible platform in the patients with HD compared with preHD and controls (F(2,73)=7.36, p=0.001) (figure 2B). This is likely to reflect a combination of early motor dysfunction and subtle psychomotor slowing which are both well known in HD. Given that there is only a 3.1 s difference in latency to the visible platform between controls and patients with HD (and 2.3 s difference between preHD and HD), this would only account for a small proportion of the 15.3 s difference in latency observed during block 5 of hidden platform training. As such we are confident that this difference cannot be entirely explained as result of either a motor or psychomotor abnormalities. A similar difference has been observed in the swimming speed of transgenic mice in the corresponding rodent task and is thought to be too small to account for the difference in latencies to find the hidden platform.27

To confirm that the motor features of manifest HD are not confounding the results of this task, a number of other measures were also analysed such as path length and the route taken towards the hidden platform (smaller values indicate a more direct route) which was significantly higher in patients with HD (block 5: F(2,73)=11.1, p=0.009) (figure 2C). Additionally, heading error, which is based on the angle by which the initial direction taken differs from the location of the platform, was significantly higher in patients with HD than preHD and controls (block 5: F(2,73)=11.1, p<0.001) (figure 2D). Both of these measurements did not differ when patients with HD were moving towards the visible platform during motor screening indicating that these measurements provide an independent assessment of spatial navigation without being affected by the movement features of HD itself (path length: F(2,72)=0.899, p=0.412; heading error: F(2,73)=0.672, p=0.514) (figure 2E, F).

Patients with early HD have worse performance on a PAL task

We next investigated whether the same findings could be observed using the CANTAB PAL task, which has been found to be sensitive and specific for diagnosing Alzheimer’s disease (AD) and has recently been shown to activate the hippocampus on fMRI.28–30 There was no difference in performance during the three-pattern stage of this task (F(2,65)=5.89, p=0.122) but patients with HD made a greater number of errors at the six-pattern and eight-pattern stage (six patterns: F(2,65)=16.1, p<0.001; eight patterns: F(2,65)=8.37, p=0.001) which was also reflected in a significant number of overall errors (F(2,65)=13.0, p<0.001) (figure 3A, B) and indeed the number of errors made on this task significantly correlated with Disease burden score (DBS) (r=−0.469, n=34, p=0.005). There were no differences between controls and the overall preHD group in performance on this task.

MWM and PAL performance correlates with estimated years to diagnosis

Cognitive decline in HD is thought to occur prior to the onset of the motor features and in order to assess whether this is the

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**Table 1** Participant demographics according to cognitive task

<table>
<thead>
<tr>
<th></th>
<th>Virtual MWM</th>
<th>PAL</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>preHD</td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>35</td>
</tr>
<tr>
<td>Age</td>
<td>48.0 (±3.14)</td>
<td>46.1 (±1.60)</td>
</tr>
<tr>
<td>NART</td>
<td>120 (±0.865)</td>
<td>114 (±1.80)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.7 (±0.133)</td>
<td>29.2 (±0.214)</td>
</tr>
<tr>
<td>ACE-R</td>
<td>97.7 (±0.511)</td>
<td>94.1 (±0.02)</td>
</tr>
<tr>
<td>TMS</td>
<td>—</td>
<td>3.11 (±0.358)</td>
</tr>
<tr>
<td>FA</td>
<td>—</td>
<td>24.5 (±0.206)</td>
</tr>
<tr>
<td>TFC</td>
<td>—</td>
<td>12.6 (±0.214)</td>
</tr>
<tr>
<td>CAG</td>
<td>—</td>
<td>41.4 (±0.306)</td>
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</table>

*Indicates a significant result when compared with preHD participants.
†Indicates a significant result (p<0.05) as compared with controls. ACE-R, Addenbrooke’s Cognitive Examination-Revised; CAG, length of CAG repeat; FA, Functional Assessment; HD, Huntington’s disease; MMSE, Mini Mental State Examination; MWM, Morris water maze; NART, National Adult Reading Test; PAL, paired associates learning; preHD, premanifest Huntington’s disease; TFC, Total Functional Capacity; TMS, Total Motor Score.
case for hippocampal deficits, we analysed the premanifest cohort further. A number of measures in both tasks, in particular, reduced performance in the MWM probe test performance and increased total errors in PAL, significantly correlated with estimated years to diagnosis (MWM probe performance: \( r = -0.293, n = 30, p = 0.025 \); PAL total errors: \( r = -0.525, n = 23, p = 0.01 \)) (figure 4A,B). Further analysis, by dividing the PAL preHD group into early and close to estimated time of disease onset groups (using a median of 8.23 years to diagnosis), revealed that the close to onset group exhibited a significantly worse performance with almost three times as many errors in the six-pattern and eight-pattern stages of the task as well as total errors (total errors: \( t(21) = 2.880, p = 0.015 \)) (figure 4C, D).

**DISCUSSION**

In this study, we have used tests analogous to those previously employed in transgenic mouse models of HD to demonstrate that hippocampal-dependent impairments exist in patients ahead of them developing overt motor features of HD. This was so advanced in patients with early stage HD that they were unable to learn the location of the platform in the virtual MWM, an impairment which we showed was not due to the motor component of the task. Similar findings were obtained using the PAL task with performance in both this and the MWM task correlating to the estimated years to disease onset in patients with preHD.

**Hippocampal-dependent deficits contribute to the cognitive features of human HD**

In HD, the striatum is undoubtedly one of the earliest and greatest sites of neuronal loss.\(^9\)\(^10\) There are, however, changes at other sites including the hippocampus where postmortem and MRI studies have consistently shown that there is a smaller, albeit significant, 9% decrease in volume early in the disease.\(^9\)\(^10\) Atrophy of the hippocampus, as examined by MRI in the TRACK-HD study, begins in the premanifest stages of HD, with a more rapid decline as the disease becomes manifest which correlates with clinical measurements (eg, TMS and TFC).\(^10\) However, there have been no behavioural studies focusing solely on hippocampal performance in human HD, despite a well-accepted recognition that not all of the early cognitive deficits have a frontostriatal basis. This is in contrast to rodent studies where there is substantial evidence for early hippocampal deficits in the disease process\(^18\) including in HD transgenic mice on the MWM and other spatial navigation tasks.\(^17\)\(^31\) In this study, we translated these findings to patients by showing that manifest HD individuals are unable to learn the location of a hidden platform in a virtual reality MWM task and that there is a decline in performance as preHD participants start to develop motor features of the disease.

Since its creation in the 1980s, a number of studies have demonstrated a crucial role for the rodent hippocampus in the MWM.\(^32\)\(^33\) The neural substrate for the human version of this task is likely to be the same as that used in this study with similar virtual reality versions of this test, being shown to be affected following unilateral trans-sylvian selective amygdalohippocampectomy,\(^34\) anterior temporal lobectomy,\(^21\) unilateral hippocampal sclerosis as a result of temporal lobe epilepsy\(^35\) but not as a result of frontal lobe injury.\(^21\) Furthermore, better navigation performance on this task has been found to correlate with larger hippocampal volumes on MRI\(^16\) and posterior hippocampal \(\theta\) waves.\(^37\)

**Patients with HD are impaired in PAL**

HD participants were also tested on the CANTAB PAL task—a task that is sensitive and specific for early AD and hippocampal
Figure 2  Impairment in the Morris water maze task is not due to the motor features of Huntington’s disease (HD). (A) Patients with HD travelled an equal amount of distance during the probe test indicating that they were motivated to find the hidden platform. (B) The patients with HD had a significantly longer latency when navigating to the hidden platform. (C and D) Path length and heading error during hidden platform training is significantly higher in patients with HD than premanifest HD (preHD) and control participants. (E and F) Path length and heading error do not differ when patients with HD are moving towards the visible platform indicating that these measures are not affected by the motor features of HD. One asterisk indicates significance with p<0.05 whereas two asterisks indicate p<0.01.

Figure 3  Patients with Huntington’s disease (HD) show worse performance on the Cambridge Neuropsychological Test Automated Battery (CANTAB) paired associates learning (PAL). (A) HD participants made significantly more errors on the six-shape and eight-shape stages leading to a greater number of total errors overall compared with controls and premanifest HD (preHD) participants. There was no difference in performance at the three-shape stage. (B) Dot plot showing the distribution of total errors made by all participants with patients with HD making a significantly higher number of errors. One asterisk indicates significance with p<0.05 whereas two asterisks indicate p<0.01.
Figure 4  Deficits in the Morris water maze (MWM) and Cambridge Neuropsychological Test Automated Battery (CANTAB) paired associates learning (PAL) are present in patients with premanifest Huntington’s disease (preHD). (A) Significant correlation of total errors made on PAL and (B) performance in the MWM with estimated years to diagnosis. (C and D) PreHD participants close to disease onset make significantly more errors in PAL than those close to disease onset. One asterisk indicates significance with p<0.05 whereas two asterisks indicate p<0.01.

pathology. In this study, patients with HD made a greater number of errors at the six and eight stages of the task which led to a significantly greater total number of errors. Of particular significance is the fact that the number of errors in this task correlated with estimated time to disease onset, with participants closest to disease onset making three times as many errors as those farthest from it.

PAL has also been successfully translated into animal studies including non-human primates, rats and mice. All such studies have implicated the hippocampus as being essential in its execution. However, due to the extended training needed in rodents, it has yet to be successfully tested in HD transgenic mice, such as the R6/1 model, as these mice have major motivational impairments preventing them from completing the adequate number of trials needed for training.

Evidence from previous studies regarding hippocampal-dependent cognitive deficits in HD

Although no study has explicitly focused on the hippocampus in HD, some studies have examined two sorts of spatial navigation: allocentric navigation, where the location of an object is defined by the position of other objects and egocentric navigation, where the location of an object is determined relative to their own body. Davis et al found that both these memory tasks were impaired in HD and concluded that this suggests a role for the caudate nucleus in both egocentric and allocentric memory given its known early pathology in this disorder. The results of our study disagree with this and, coupled to the well-described early decrease in volume in the hippocampus in HD, our results suggest that the deficit in allocentric memory reflects the hippocampal pathology of HD. The recent study by Majerova et al in which it was found that there are deficits in both allocentric and egocentric navigational deficits in moderate-advanced disease but not early HD, can best be explained by differences in the tasks employed. Additionally, they recruited a large spread of patients with HD (TFC 3–13) whereas we minimised the variance in our study by limiting recruitment to participants in the premanifest or early stage of disease with a correspondingly high functional score TFC 10–13.

A number of previous studies have also found patients with HD to be impaired in pattern recognition memory (PRM), a cognitive test which is sensitive to amygdalohippocampectomy and temporal lobe lesions but not to lesions of the frontal lobe. In addition to the deficit in PRM, Lawrence et al also described in HD, deficits in delayed matching to sample and a significantly greater number of errors and trials in PAL. Together, this evidence argues in favour of early hippocampal involvement in the disease process at a behavioural level which would fit with that reported in the transgenic mouse models of HD.

In conclusion, this study highlights the value of using analogous tests in transgenic mouse models and human patients in order to detect the very earliest hippocampal abnormalities in HD, although bigger longitudinal studies with imaging are warranted to confirm this. This in turn will facilitate translation of laboratory findings into the clinic especially around agents that enhance cognition and/or modify disease progression given the ease with which identical tests can be used to measure efficacy in both preclinical and clinical drug trials.

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Contributors FB designed the research study, acquired and analysed the data and wrote the manuscript. LCS acquired data and critically revised the manuscript. SLM assisted with the Neuroinvestigations virtual software as well as all the patients and their families that were involved in this study. This study was supported by donations to the Huntington’s disease clinic in the John van Geest Centre for Brain Repair, and NIHR award of a Biomedical Research Centre to Addenbrooke’s Hospital and the University of Cambridge, a Medical Research Studentship and James Baird Fund awarded to FB.

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