Progressive imbalance in the interaction between spatial and procedural memory systems in the R6/2 mouse model of Huntington's disease

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ABSTRACT

When Huntington's disease (HD) patients are tested on cognitive tasks that involve both striatal and hippocampal memory systems, a decline in their striatal function is compensated for by an increase in hippocampal activity that allows these patients to achieve an optimal performance [Voermans, N. C., Petersson, K. M., Daudley, L., Weber, B., van Spaendonck, K. P., Kremer, H. P. H., et al. (2004). Interaction between the human hippocampus and the caudate nucleus during route recognition. *Neuron, 43*, 427–435]. Our recent study suggests that there is also an imbalance between hippocampal and striatal memory systems in R6/2 mice, a widely used animal model of HD [Ciamei, A., & Morton, A. J. (2008). Rigidity in social and emotional memory in the R6/2 mouse model of Huntington's disease. *Neurobiology of Learning and Memory, 89*, 533–544]. However, interactions between multiple memory systems have never been studied directly in HD mice. Here, we used a water maze task to examine striatal and hippocampal systems directly. R6/2 mice were trained to swim from a fixed starting point to a cued platform. During the probe test, the apparatus was rotated by 180°, and mice had to choose between a hidden platform located where the cued platform had been during training (place learning), and a cued platform that was now located in the opposite quadrant (cue learning). Probe trial results showed that in 8 week old R6/2 mice the escape response was driven mainly by a cue-based strategy (striatal), whereas by 12 weeks of age, a higher proportion of mice adopted a place-based strategy (hippocampal) to escape from the maze. We conclude that following striatal decline in R6/2 mice between 8 and 12 weeks of age, hippocampal functions emerge to drive the escape response of R6/2 mice.

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1. Introduction

HD is a progressive neurodegenerative disorder caused by an expanded CAG repeat in the HD gene. Symptoms are associated with profound neuronal loss in the striatum and cortex (The Huntington's Disease Collaborative Research Group, 1993) and include motor abnormalities, cognitive decline and psychiatric disturbance (Bates, Harper, & Jones, 2002; Snowden et al., 2003; Sprengelmeyer, Schroeder, Young, & Epplen, 2006). Cognitive impairment observed early in HD patients is consistent with a dysfunction in striatum and cortex, the brain structures predominantly affected by neuronal loss. When tested in tasks of visual discrimination, HD patients respond normally to reinforced associations of visual stimuli paired on the basis of a specific dimension (e.g. the shape). However, when the reinforcement is shifted on a different dimension (e.g. the colour), HD patients persevere in responding to the first dimensional association reinforced (Lawrence, Sahakian, Rogers, Hodges, & Robbins, 1999; Lawrence et al., 1996; Watkins et al., 2000). This deficit, also known as cognitive inflexibility or rigidity,

involves networks within the fronto-striatal loop that are disrupted in HD patients. Then, with progression of the disease, other brain regions begin to degenerate, adding their malfunction to that of fronto-striatal networks and eventually generating the complexity of behavioural alterations observed in HD patients, where cognitive inflexibility may coexist with depression, social withdrawal and other psychiatric symptoms (Bates et al., 2002).

A recent study showed that the early decrease in striatal functionality can be compensated for by other memory systems. For example, HD patients tested in a route recognition task could reach a performance level similar to that reached by healthy controls. However, while in control subjects route recognition was achieved through similar levels of activation of both the hippocampus and the striatum, in HD patients a decrease in striatal activation was compensated for by an increase in hippocampal activation, that allowed HD subjects to achieve a performance level similar to that of healthy controls (Voermans et al., 2004).

Both hippocampus and striatum acquire information during tasks of spatial navigation such as the one described by Voermans et al. (2004), with different systems storing information relating to different aspects of the task learned (McDonald, Hong, & Devan, 2004). The hippocampus is known to be mainly involved in the

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formation of detailed cognitive 'maps' of the context in which learning occurs (place learning), while the striatum is engaged by repetitive stimulus–response (S–R) association (response learning). However, hippocampal and striatal memory systems do not act as independent entities, despite the absence of direct anatomical connections between the two regions. Rather they can interact, either cooperatively or competitively, thus 'managing' the behavioural response required in complex tasks (Kim & Baxter, 2001; Packard & McGaugh, 1996; Poldrack & Packard, 2003). Indeed, a main feature of competitive interactions is that lesioning or damaging of either one of the two systems can lead to an increase in the influence of the other on behaviour (Chang & Gold, 2003; Middei, Geracitano, Caprioli, Mercuri, & Ammassari-Teule, 2004; Packard & McGaugh, 1996; Poldrack & Packard, 2003; Schroeder, Wingard, & Packard, 2002).

In a recent paper we discussed the results of behavioural experiments suggesting that in the R6/2 mouse model of HD, a perturbation of balance between the hippocampal and striatal memory systems might occur following striatal decline (Ciamei & Morton, 2008). The R6/2 line of transgenic mice is widely used to study the pathological mechanisms of HD because these mice show a progressive deterioration of locomotor and cognitive abilities that models many of the symptoms shown by HD patients (Carter et al., 1999; Lione et al., 1999; Murphy et al., 2000). R6/2 mice express the N-terminal fraction of the human *Hd* gene containing a highly expanded CAG repeat (Mangiarini et al., 1996). In R6/2 mice, abnormalities in hippocampal synaptic plasticity are present as early as 3 weeks of age (Gibson, Reim, Brose, Morton, & Jones, 2005), and cognitive deficits are measurable by 4 weeks of age (Lione et al., 1999). The onset and progression of neurological dysfunction in R6/2 mice correlates with a neuropathological feature of HD and of other polyglutamine repeat diseases, namely the formation of polyglutamine aggregates in intranuclear and extranuclear inclusions (Davies et al., 1997; Morton, Lagan, Skepper, & Dunnett, 2000; Morton et al., 2009; Stack et al., 2005). In R6/2 mice, inclusions have been observed immediately after birth (Stack et al., 2005) and their number and size has been shown to increase during the first 3 months of life (Morton et al., 2000; Stack et al., 2005). In the hippocampus, inclusions appear first in the CA1 region, and then in neurons of the CA3 field and the dentate gyrus (Morton et al., 2000). Similarly, at early stages, the distribution of inclusions in the striatum is compartimentalized (Morton et al., 2000). Interestingly, although no differences were observed in the distribution of inclusions between the medial and lateral aspects of both the dorsal and ventral striatum (Morton et al., 2000), Stack et al. (2005) reported early signs of neuronal atrophy in the dorsomedial striatum of R6/2 mice, supporting the idea that also in the striatum, progression of the disease might distinguish between different subregions.

The cognitive deficit of R6/2 mice has been mainly studied using classical behavioural paradigms known to engage learning mechanisms based in either the hippocampal or striatal memory systems (Lione et al., 1999; Morton, Skillings, Bussey, & Saksida, 2006; Murphy et al., 2000; Pallier et al., 2007). Results obtained from testing R6/2 mice in tasks of spatial navigation, such as the Morris water maze task, have shown deficits in spatial learning that can be detected as early as the fourth week of age. When tested in a two-choice swim tank task, R6/2 mice show no deficits until the sixth (reversal learning) or the tenth (acquisition) week of age (Lione et al., 1999; Morton et al., 2006), suggesting that deficits in hippocampal-based learning skills appear earlier than that of striatal-based learning skills. Moreover, these findings seem to confirm that the dorsomedial aspect of the striatum, which is known to be important for the flexibility required by reversal learning (Palencia & Ragozzino, 2004) is likely to be the first striatal subregion to become dysfunctional in R6/2 mice, since deficits in

reversal learning precedes acquisition deficits in two-choice discrimination tasks (Lione et al., 1999; Morton et al., 2006). However, our recent findings suggest that the deficits displayed by R6/2 mice in tasks known to rely either on the hippocampus or the striatum do not fully describe the cognitive phenotype of this line of transgenic mice (Ciamei & Morton, 2008). For example, it is noteworthy that, at ages in which both systems appear to already be dysfunctional, R6/2 mice do not show an impairment in a one-trial inhibitory avoidance task that is known to engage both the hippocampal and striatal memory system (Cammarota, Bevilaqua, Köhler, Medina, & Izquierdo, 2005). Moreover, the hippocampal deficit inferred by the impaired performance of R6/2 mice tested in a water maze task does not seem to affect other forms of memory known to be dependent on structures within the medial temporal lobe, such as social memories formed in a social transmission of food preference task (Alvarez, Wendelken, & Eichenbaum, 2002; Ciamei & Morton, 2008; Eichenbaum, 2000). In this study, we tested R6/2 mice in a water maze competition paradigm designed to measure the occurrence of hippocampal and striatal learning in an experimental situation in which both spatial and procedural strategies can be used to perform the escape response (Martel et al., 2007).

2. Methods

2.1. Animals

Mice were taken from a colony established at the University of Cambridge bred on a CBA × C57BL/6 F1 background as described previously (Morton et al., 2005). Mice were housed in single sex, mixed genotype groups of 10 mice. All testing was performed on male mice. WT littermates were used as controls for R6/2 mice. CAG repeat length for R6/2 mice was 280 ± 2 (mean ± SEM, $n = 70$). Genotyping and repeat length measurement were performed by Laragen, Los Angeles, USA. All studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Mice were housed within a 12 h light/dark cycle (lights on at 7:30 AM and off at 7:30 PM) in a temperature-controlled (19–21 °C) and humidity-controlled (55 ± 10%) environment. Dry food, mash, and water were available *ad libitum* as described previously (Carter, Hunt, & Morton, 2000). All experiments were carried out during the light phase of the cycle.

In the present study, we used R6/2 mice carrying a repeat length of 280 CAG. R6/2 mice with a number of CAG repeats between 250 and 300 normally die by 40 weeks of age (Morton et al., 2009). Although in our mice overt locomotor symptoms are not evident at 12 weeks of age, signs of disease progression (decrease in body weight, eating and drinking abnormalities) can already be detected at this age (Wood et al., 2008).

2.2. Water maze competition test

The water maze consisted of a circular water tank, made from white polypropylene (diameter, 200 cm; height, 60 cm). It was filled to a depth of 40 cm with water (23 °C) and rendered opaque by the addition of a small amount of nontoxic white paint. Four positions around the edge of the tank were arbitrarily designated north (N), south (S), east (E), and west (W); this provided four alternative start positions and defined the division of the tank into four quadrants: NE, SE, SW, and NW. A video camera was fixed 1.6 m above the centre of the swim tank, and connected to a HVS tracking system (HVS Image 2020, Hampton, UK). The N and E sides of the pool were in proximity with the walls of the room (1 m from each cardinal point), while a poster board was placed behind the SW quadrant at the same distance, in order to hide the experimenter and to provide a richer environment for this side of the maze. Extra-maze visual cues were provided by printouts of