Cognitive models of neurological disorders: the role of perceptual integration, decision-making and multiple memory systems

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Table of contents

List of abbreviations

alpha synuclein - ASN

aMCI - amnestic mild cognitive impairment

ANOVA – analysis of variance

BDI - Beck Depression Inventory

BNT - Boston Naming Test

BS - basal ganglia

CNS - central nervous system

EDSS - Expanded Disability Status Scale

FSS - Fatigue Severity Scale

IGT - Iowa Gambling Test

MMSE - Mini-Mental State Examination

MS - multiple sclerosis

MTL - medial temporal lobe

PD - Parkinson's disease

RAVLT - Rey Auditory Verbal Learning Test

SF - semantic fluency

S-R learning- stimulus-response learning

VEP - visual evoked potential

VFQ-25- 25-item National Eye Institute Visual Function Questionnaire

WCST - Wisconsin Card Sorting Test

Papers related to the thesis

I. Nagy H, Bencsik K, Rajda C, Benedek K, Beniczky S, Kéri S, Vécsei L. The effects of reward and punishment contingencies on decision-making in multiple sclerosis. *Journal of the International Neuropsychological Society* 2006; 12: 559-562. IF: 2.367, Cited: 8

II. Nagy H, Kéri S, Myers CE, Benedek G, Shohamy D, Gluck MA. Cognitive sequence learning in Parkinson's disease and amnestic mild cognitive impairment: dissociation between sequential and non-sequential learning of associations. *Neuropsychologia* 2007; 45: 1386-1392. IF: 3.630, Cited: 4

III. Nagy H, Bencsik K, Rajda C, Benedek K, Janáky M, Beniczky S, Kéri S, Vécsei L. Lateral interactions and speed of information processing in highly functioning multiple sclerosis patients. *Cognitive and Behavioral Neurology* 2007; 20: 107-112. IF: 2.614, Cited: 1

IV. Kéri S, **Nagy H**, Myers CE, Benedek G, Shohamy D, Gluck MA. Risk and protective haplotypes of the alpha-synuclein gene associated with Parkinson's disease differentially affect cognitive sequence learning. *Genes, Brain & Behavior* 2008; 7: 31-36. IF: 3.890, Cited: 0

Summary

Characteristics clinical signs and symptoms of neurological disorders, such as multiple sclerosis (MS) and Parkinson's disease (PD), indicate the impairment of basic sensory and motor processes. However, recent evidence raised the possibility that fine-scale perceptual integration, decision-making, and multiple memory processes are also affected. In this series of studies, we investigated perceptual integration, contingency-dependent decision-making, stimulus-response (S-R) learning of sequences, and context representation in patients with MS, PD, and amnestic mild cognitive impairment (aMCI). We also assessed the effect of genetic traits related to PD on cognition in healthy volunteers.

Our hypotheses were as follows:

1. Highly functioning MS patients without visual complaints show subtle abnormalities in perceptual integration, which are mediated by lateral connections in the primary visual cortex.
2. Affective problems in MS can be modeled using a test of decision-making. For such purposes, we used the Iowa Gambling Test (IGT), which is sensitive to the lesions of the ventromedial prefrontal cortex.
3. In a combined S-R sequence learning and context representation test, PD patients with basal ganglia dysfunctions show impairments in the S-R learning phase, whereas aMCI patients with medial temporal lobe pathology show context representation dysfunctions.
4. The S-R learning deficit is related to risk variants of the alpha synuclein (ASN) gene, a significant risk factor of PD.

Study I. Sensory integration in MS

Visual impairment is a common feature of MS. The aim of this study was to investigate lateral interactions in the visual cortex of highly functioning MS patients and to compare that with basic visual and neuropsychological functions. Twenty-two young, visually unimpaired MS patients with minimal symptoms (Expanded Disability Status Scale <2) and 30 healthy controls subjects participated in the study. Lateral interactions were investigated with the flanker task, during which participants were asked to detect the orientation of a low-contrast Gabor patch (vertical or horizontal), flanked with two collinear or orthogonal Gabor

patches. Stimulus exposure time was 40-, 60-, 80-, and 100-ms. Digit span forward/backward, digit symbol, verbal fluency, and California Verbal Learning Test procedures were used for background neuropsychological assessment. Results revealed that MS patients showed intact visual contrast sensitivity and neuropsychological functions, whereas orientation detection in the orthogonal condition was significantly impaired. At 40-ms exposure time, collinear flankers facilitated the orientation detection performance of the patients resulting in normal performance. In conclusion, the detection of briefly presented, low-contrast visual stimuli was selectively impaired in multiple sclerosis. Lateral interactions between target and flankers robustly facilitated target detection in the patient group.

Study II. Decision-making cognition in MS

Many patients with MS show cognitive and emotional disorders. The purpose of this study is to evaluate the role of contingency learning in decision-making in young, non-depressed, highly functioning patients with MS (n=21) and in matched healthy controls (n=30). Executive functions, attention, short-term memory, speed of information processing, and selection and retrieval of linguistic material were also investigated. Contingency learning based on the cumulative effect of reward and punishment was assessed using the IGT. In the classic ABCD version, advantageous decks are characterized by immediate small reward but even smaller future punishment. In the EFGH version, advantageous decks are characterized by immediate large punishment but even larger future reward. Results revealed that patients with MS showed significant dysfunctions in both versions of the test. Performances on neuropsychological tests sensitive to dorsolateral prefrontal functions did not predict and did not correlate with the IGT scores. These data suggest that patients with MS show impaired performances on tasks designed to assess decision-making in a situation requiring the evaluation of long-term outcomes regardless of gain or loss, and that this deficit is not a pure consequence of executive dysfunctions.

Study III. Multiple memory systems in PD and aMCI

Dopaminergic mechanisms in the basal ganglia are important in the learning of sequential associations. To test the specificity of this hypothesis, we assessed never-medicated patients with PD and MCI using a chaining task. In the training phase, each link in a sequence of stimuli leading to reward is trained step-by-step using feedback after each decision, until the complete sequence is learned. In the probe phase, the context of S-R associations must be

used (the position of the associations in the sequence). We found that patients with PD showed impaired learning during the training phase, but their performance was spared in the probe phase. In contrast, patients with aMCI with medial temporal lobe dysfunctions showed intact learning during the training phase, but their performance was impaired in the probe phase. These results indicate that when dopaminergic mechanisms in the basal ganglia are dysfunctional, series of S-R associations are less efficiently acquired, but their sequential manner is maintained. In contrast, medial temporal lobe dysfunctions may result in a non-sequential learning of associations, which may indicate a loss of contextual information.

Study IV. The genetic polymorphism of the ASN gene affects sequence learning

ASN is a key factor in the regulation of dopaminergic transmission and is related to PD. In this study, we investigated the effects of risk and protective haplotypes of the ASN gene associated with PD on cognitive sequence learning in 204 healthy volunteers. We found that the 3'-block risk haplotypes were associated with less effective S-R learning of sequences and with superior context representation. In contrast, participants with protective haplotypes exhibited better S-R learning and worse context representation, which suggest that these functions are inversely affected by risk and protective haplotypes. The Rep1 promoter polymorphism did not influence cognitive sequence learning. Because S-R reward learning may be mediated by the basal ganglia and context learning may be related to the medial temporal lobe, our data raise the possibility that dopaminergic signals regulated by ASN inversely affect these memory systems.

Introduction

Cognitive neurology

It is well established that lesions of the central nervous system results in characteristic dysfunction in fundamental domains of cognition. During the last two decades, traditional clinical neuropsychology was revolutionized by cognitive neuroscience, leading to the emergence of a new discipline, called *cognitive neurology* (Cappa, 2001). The term cognition refers to higher-level mental processes such as thinking, perceiving, imagining, speaking, acting, and planning. Cognitive neuroscience integrates the methods and theoretical framework of several disciplines, with a special relevance to cognitive psychology and neuroscience, and uses experimental paradigms from functional neuroimaging, electrophysiology, cognitive genomics, and behavioral genetics. Clinical studies of patients with cognitive deficits constitute a central aspect of cognitive neuroscience. The definitive clinical signs and symptoms of neurological disorders, such as MS and PD, indicate the impairment of sensory and motor processes. However, there is emerging evidence that subtle alterations in perceptual integration, decision-making, and multiple memory processes are also affected in these disorders, which may shed light on hidden neuronal mechanisms.

Visual sensory integration in MS

MS is a common inflammatory disease of the central nervous system with genetic, environmental, and autoimmune causes, which eventually result in the loss of myelin, axons, and cortical atrophy (Peterson and Trapp, 2005). Based on recent evidence, the characteristic physical consequences of the disease (sensory loss, ataxia, weakness and clumsiness of the limbs, urinary dysfunctions) seem to be associated with cognitive and emotional disorders and together significantly contribute to the psychosocial consequences of MS (quality of life, work activity, and social functions) (Wishart and Sharpe, 1997; Chiaravalloti and DeLuca, 2008). Cognitive impairment is quite common in MS, especially in the domains of learning/memory, processing speed, and working memory. These impairments may be detected even during the first demyelinating attack and the level and pattern of the deficit depend on the subtype, stage, and severity of the disease (Zakzanis, 2000; Bobholz and Rao, 2003). Recent evidence suggests that brain atrophy accounts for more variance than lesion burden, with a particular reference to thalamic atrophy, which could explain the diversity of cognitive impairments as a critical structure for the coordination of cortico-subcortical

pathways (Benedict et al., 2004). Although visual deficits have obvious clinical implications, even MS patients with intact visual acuity and contrast sensitivity may demonstrate deficits, including slowed automatic visual information processing (Vleugels et al., 2001; Lycke et al., 2001; Pula and Reder, 2009). Given that visual perceptual deficits can be independent from other cognitive deficits, they may be caused by a focal dysfunction within the visual system. Such focal and subtle abnormalities can be investigated by testing the integrative function of early-stage vision, that is, to assemble local information across the visual field to a global representation of spatially extended objects (Kovács and Julesz, 1994). The impairment of such integration can be due to micro-scale disconnections within early visual areas.

The *flanker task* is a new psychophysical method, which is suitable for the assessment of lateral connections in the primary visual cortex. During this task, participants are asked to detect the orientation of a low-contrast target patch (vertical or horizontal), flanked with two collinear or orthogonal patches. If the target and the flankers are collinear, detection is significantly enhanced compared with the scenario when they are orthogonal (Polat and Sagi, 1994). The physiological bases of this simple phenomenon have been extensively investigated, and the data support that lateral interactions in the primary visual area (V1) play an essential role in flanker facilitation. For example, Kapadia et al. (1995) measured contrast thresholds in humans in parallel with single cell recordings from monkey V1. They found that contrast threshold for a target bar was 40% improved by a lateral flanker. Recordings from monkey V1 revealed that neurons showed increased activity for a lateral flanker. Therefore, human behavioral data and electrophysiological responses of V1 neurons followed the same rule.

In summary, the flanker task targets two aspects of visual information processing. First, using briefly presented stimuli, the speed of information processing can be assessed. Second, the test explores the effect of flankers on target detection, which provides information about the functional integrity of lateral connections in early visual areas.

Decision-making and MS

In addition to the physical and cognitive impairments, many patients display emotional problems, including depression, euphoria, pathological laughing and crying, altered personality, and psychosis. Cognitive and emotional disorders seem to be associated (Feinstein, 2004). It has been suggested that the prefrontal cortex plays a critical role in

emotional disorders in MS via its connections to subcortical structures (e.g. amygdala) regulating mood and affect (Passamonti et al., 2009).

Affective problems in MS can be modeled using tests of decision-making processes. The IGT, which is sensitive to the lesions of the ventromedial prefrontal cortex, provides a unique opportunity to investigate special aspects of decision-making problems: hypersensitivity to reward, insensitivity to punishment, and "myopia for the future" when decisions are guided by immediate prospects instead of long-term outcomes of decisions (Bechara et al., 2000).

In the IGT, participants are asked to select cards from four decks in order to win as much money as possible. The classic (ABCD) version of the task investigates the possibility that decision-making abnormality is based on hypersensitivity to reward, that is, when large immediate gain outweighs even larger future loss. In contrast, the modified (EFGH) version of the task investigates the possibility that decision-making problems are due to the failure of high reward to outweigh immediate punishment. In this version, advantageous decks are characterized by high immediate loss but even higher future gain. If decision-making problems are due to insensitivity to long-term outcomes, patients will show impairments in both versions of the IGT (Bechara et al., 2000).

Yechiam et al. (2005) described a cognitive model of the IGT, which takes into consideration the attention paid by patients with different neurological and psychiatric disorders to gain, loss, and recent outcome instead of long-term consequences of decisions. Patients with lesions of the ventromedial prefrontal cortex pay excessive attention to recent outcomes regardless of loss and gain. Patients with Parkinson‘s disease and Asperger‘s syndrome are less influenced by gain, whereas patients with cocaine dependence and Huntington's disease pay excessive attention to both gain and recent outcomes.

Kleeberg et al. (2004) demonstrated impaired learning in the ABCD task in patients with MS, which was not associated with executive dysfunctions. Slower learning in the IGT was associated with impaired emotional reactivity, as revealed by abnormal anticipatory skin conductance responses. Given the negative consequences of impaired decision-making on daily life, Kleeberg et al. (2004) suggested that this factor might be associated with altered quality of life in MS.

Associative and context learning in PD and aMCI

Traditionally, explicit and implicit memory systems in the brain are defined as distinct functional and structural domains (Squire et al., 2004; Yin and Knowlton, 2006). However, recent evidence suggests that these memory systems interact in a cooperative and competitive manner (Poldrack and Rodriguez, 2004). It is thought that explicit learning is dependent on medial temporal lobe and diencephalic brain structures, whereas habit learning and implicit skill learning are closely associated with the neostriatum. Following this anatomical distinction, PD accompanied by striatal dysfunctions is characterized by impaired skill and habit learning, whereas in Alzheimer's disease and aMCI, medial temporal lobe functions and explicit memory are more disrupted (Salmon and Filoteo, 2007). The most frequently used experimental task to examine implicit learning in PD has been the serial reaction time task, which includes the learning of motor sequences (Siegert et al., 2006). However, cognitive sequence learning with increasing complexity of S-R associations has not been investigated in details. Basic research suggest that the medial temporal lobe is not necessary for slow feedback-based S-R learning, but it is definitely important when stimuli are presented in a novel context (Eichenbaum et al., 1989). Patients with aMCI, who are at a high risk to develop Alzheimer's disease, display relatively spared general cognitive abilities and daily functioning, but their explicit memory is impaired, presumably due to the pathology of the medial temporal lobe (Petersen et al., 1999).

In PD, Shohamy et al. (2005) demonstrated that dopaminergic mechanisms in the striatum are involved in the learning of sequential ("chaining") S-R associations, in which each link in a sequence of stimuli leading to reward is trained step-by-step using feedback after each decision, until the complete sequence is learned. In PD, cellular death in the substantia nigra pars compacta leads to the depletion of dopamine in the striatum In addition to the motor symptoms, dopaminergic loss in the striatum results in a variety of cognitive dysfunctions, with a special reference to habit and skill learning, which is based on trial-by-error choices, feedback, and reward. Frank et al. (2004) proposed that in unmedicated PD patients the low level of dopamine in the striatum is not sufficient for reward during positive feedback, whereas in PD patients receiving L-DOPA substitution, dopamine "overshoots" disrupt learning about the absence of reward during negative feedback. In this respect, the chaining task is informative because patients with PD tested off their normal dopaminergic medication perform more poorly on this task than patients with PD who receive L-DOPA

substitution (Shohamy et al., 2005), which suggests that L-DOPA ameliorates sequential association learning deficits.

Cognitive genetics is a new discipline, which focuses on the relationship between cognitive functions and genetic variations (single nucleotid polymorphisms, haplotypes, and copy number variations) (Reichenberg et al., 2009). Based on the assumptions outlined above on the relationship between sequence learning and dopamine, we hypothesized that the genetic variation of the ASN gene may be related to this type of learning. ASN, which is a key component of Lewy bodies, is predominantly localized in the presynaptic terminal of the neurons as a molecular chaperone in the SNARE complex, which regulates neurotransmitter release, vesicle recycling, synaptic plasticity, and neuronal survival (Chandra et al., 2005). ASN is especially prevalent in dopaminergic neurons and influences the release of the transmitter. It may be directly related to the regulation of the reward prediction function of dopamine, given that the decreased expression of the ASN gene results in the sensitization of the reward system and leads to significantly altered operant behavior (Oksman et al., 2006).

Aims and hypotheses

Our hypotheses were as follows:

1. Highly functioning MS patients without visual complaints show subtle abnormalities in perceptual integration, which is mediated by lateral connections in the primary visual cortex.
2. Affective problems in MS can be modeled using tests of decision-making processes. For such purposes, we used the IGT, which is sensitive to the lesions of the ventromedial prefrontal cortex.
3. In a combined S-R sequence learning and context representation test, PD patients with basal ganglia dysfunctions show impairments in the S-R learning phase, whereas aMCI patients with medial temporal lobe pathology show relatively intact learning.
4. The S-R learning deficit is related to risk PD variants of the ASN gene.

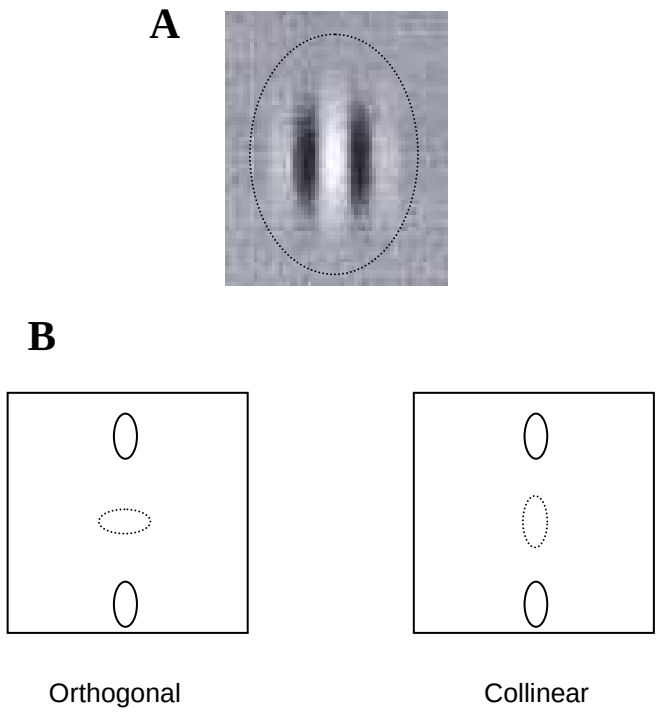
Materials and methods

The studies were approved by the university ethics committee and all participants gave their written informed consent. The studies were carried out in accordance with the Declaration of Helsinki.

I. Materials and methods in the first experiment

Participants**:** Twenty-two (n=22) young, visually unimpaired MS patients with minimal symptoms and thirty (n=30) healthy controls subjects participated in the study. Patients were recruited from the multiple sclerosis outpatient unit at the Department of Neurology, University of Szeged. Controls were staff members and their acquaintances. Inclusion criteria were definite diagnosis of multiple sclerosis according to the Poser criteria (Poser et al., 1983), Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) scores less than 2, less than 5 years since diagnosis, no evidence for visual impairments as measured with the 25-item National Eye Institute Visual Function Questionnaire (VFQ-25) (Balcer et al., 2000), Beck Depression Inventory (BDI) (Steer et al., 1993) scores less than 10, and the absence of other neurological, ophthalmologic, and psychiatric disorders. The level of fatigue was assessed with the Fatigue Severity Scale (FSS) (Krupp et al., 1989). In addition to magnetic resonance imaging and oligoclonal banding assay, patients underwent visual evoked potential (VEP) examinations to exclude optic neuritis. All participants had normal or corrected-to-normal visual acuity.

FIGURE 1. (A) Gabor patch. (B) Configuration of the target Gabor patch (dotted line) and the lateral flankers.



The flanker task**:** The tests were run binocularly. Stimuli were presented on a gamma-corrected ViewSonic PF815 monitor (resolution: 800 x 600 pixel; refresh rate: 100 Hz; VSG graphic card, version 5.02, Cambridge Research System Ltd, Rochester, UK). The monitor was controlled by an IBM-compatible PC. The viewing distance was 100 cm. The mean luminance of the display was 50 cd/m2 (Spectra Pritchard 1980A-CD photometer). The E-Prime software was used for stimulus presentation (Schneider et al., 2002). The stimulus field consisted of three Gabor patches (size: 0.15 degree) presented against a uniform gray background. The vertically or horizontally oriented target Gabor patch was flanked with two lateral Gabor patches (Figure 1). The luminance-contrast profile of Gabor patches was formed by the multiplication of a sinusoidal waveform with a Gaussian envelope. The sinusoidal waveform means periodically altering stripes with maximal and minimal luminance (‘‘white’’ and ‘‘black’’ stripes, respectively). This sinusoidal waveform was modified according to a Gaussian distribution of luminance, which resulted in maximal values in the center of the Gabor patch (Figure 1). Contrast was defined according to the Michelson-formula (the

absolute difference between the maximal and minimal luminance divided by their sum). The contrast of the target was 8%, whereas that of the flankers was 40%. The spatial frequency was 6.7 cycles/degree (spatial frequency: the number of cycles comprising a pair of stripes with minimal and maximal luminance under 1 degree of visual angle; wavelength: 1/spatial frequency). The center-to-center distance between target and flankers was 4λ. Before the experiment, participants observed the stimulus display to ensure that they were able to detect the low-contrast central target. First, participants were asked to press the space button on the computer keyboard. After this, a fixation display of 500-ms appeared. A central cross indicated the location of the subsequent target. After the fixation display, an interval of 40-, 60-, 80-, or 100-ms appeared during which the stimulus display was presented. Participants were asked to indicate whether the orientation of the target was vertical or horizontal pressing separate buttons on the computer keyboard (‘‘1’’ and ‘‘9’’). The next trial was initiated by the response. The order of stimulus displays with different exposure time was pseudo-randomized, that is, a maximum of 2 consecutive stimulus displays with the same exposure time may have occurred. Ten trials were administered at each exposure time. Performance was defined as the proportion of correctly detected target stimuli. Responses with reaction time exceeding 2000-ms were eliminated.

Contrast threshold**:** The setup for stimulus presentation and the parameters of the Gabor patches were the same as in the flanker task. First, participants were asked to press the space button on the computer keyboard. After this, a fixation display of 500-ms appeared with. A small central cross-indicated the location of the subsequent target Gabor patch for which contrast threshold was measured. After the fixation display, a brief interval of 80-ms appeared during which the target was presented. Volunteers were asked to indicate if they noticed the target with pressing separate keys on the computer keyboard (‘‘1’’ for yes, ‘‘9’’ for no). The next trial was initiated by the response. Contrast threshold was measured with Levitt’s staircase method. In the case of three consecutive correct responses (hits), contrast was decreased with 0.1 log unit, whereas in the case of one incorrect response (miss), contrast was increased with 0.1 log unit. Contrast threshold was the average of the last six reversals. The final threshold was the average of five independent measurements in separate blocks.

Background neuropsychology (Lezak, 1995):

(1) Attention and short-term memory: digit span forward and backward.

(2) Speed of information processing and divided attention: Symbol Digit Modalities Test.

(3) Selection and retrieval of linguistic material: verbal (FAS) fluency.

(4) Verbal declarative memory: California Verbal Learning Test-II.

Data Analysis: Kolmogorov-Smirnov tests were used to check data distribution. Contrast threshold data were logarithmically transformed. Neuropsychological performances of the patients and controls were compared with two-tailed t-tests. Repeated measures analysis of variance (ANOVA) was used for the flanker task data with group (multiple sclerosis vs. controls) as the between-subject factor and flanker orientation (vertical vs. horizontal) and exposure time (40-, 60-, 80-, and 100-ms) as the within-subject factors (2 by 2 by 4 design). Tukey's honest significant difference (HSD) tests were used for post hoc comparison. The level of statistical significance was $\alpha<0.05$.

II. Materials and methods in the second experiment

Participants: Twenty-one outpatients with relapsing-remitting MS (8 men, 13 women; mean age: 31.4 years, SD=9.8; mean education: 13.6 years, SD=7.6; mean duration of illness: 3.1 years, SD=1.1) and 30 healthy control volunteers (9 men, 21 women; mean age: 28.2 years, SD=8.2; mean years of education: 14.0 years, SD =9.8) participated in the study. There were no significant differences between the two groups regarding gender distribution, age, and years of education. Inclusion criteria were definite diagnosis of MS according to the Poser et al. (1983). MRI scanning was also performed in each patient. The Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) scores were 0 in the case of 3 patients, 1 in the case of 3 patients, 2 in the case of 13 patients, and 3 in the case of 2 patients (mean: 1.7).

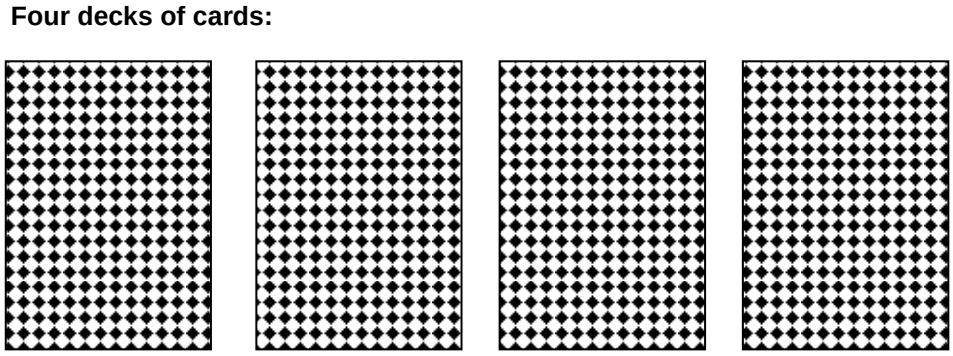
Background Neuropsychology: Neuropsychological testing included the following procedures: Wisconsin Card Sorting Test (concept formation and attentional set-shifting), Digit Span Forward and Backward (attention and short-term memory), Symbol Digit Modalities Test (speed of information processing and divided attention), Verbal Fluency (selection and retrieval of linguistic material) (Lezak, 1995).

IGT: The test was administered as described by Bechara et al. (2000) using a personal computer. Participants received standard instructions and were told that the aim of game is to win as much money as possible. Participants were not actually paid the money. In the ABCD version, four decks of cards labeled as A, B, C, and D were presented on the computer screen. Each deck contained 40 cards. The task was to click on a card from any of the decks using the mouse. After picking a card, the amount of money the participant won or lost was depicted on the computer screen, together with a smiley or a sad cartoon face and different sounds. There was a green bar on the top of the screen. Winning and losing money was indicated by an increase and a decrease of the length of the bar, respectively. When the money was added or subtracted, the cartoon face disappeared and the participant could select the next card. The

inter-trial interval was 6 sec. The game consisted of 100 trials. Participants always won $100 if they selected a card from deck A or B and always won $50 if they selected a card from deck C or D. The amount of lost money was $150, 200, 250, 300, or 350 for deck A (50% of the cards), $1250 for deck B (10% of the cards), $25, 50 or 75 for deck C (50% of the cards) and $250 for deck D (10% of the cards). If there was no loss (50% of cards for decks A and C and 90% for decks B and D), a sentence appeared on the computer screen stating that "You won $100 (or $50)." If there was a loss, a sentence appeared on the computer screen stating that "You won $100 (or $50), but you lost $X." The order of winning and losing cards was randomized and unpredictable. Altogether, decks A and B were associated with high immediate reward but even higher future punishment (Figure 2).

The layout and design of the EFGH version was similar. The four decks were labeled as E, F, G, and H. Participants always lost $100 if they selected a card from deck E or G and always lost $50 if they selected a card from deck F or H. The amount of received money was $1250 for deck E (10% of the cards), $25, 50 or 75 for deck F (50% of the cards), $150, 200, 250, 300, or 350 for deck G (50% of the cards), and $250 for deck H (10% of the cards). If there was no winning (50% of cards for decks F and G and 90% for decks E and H), a sentence appeared on the computer screen stating "You lost $100 (or $50)." If participants won some money, a sentence appeared on the computer screen stating that "You lost $100 (or $50), but you won $X". Altogether, decks E and G were associated with high immediate punishment but even higher future reward (Figure 2). For data analysis, the 100 trials were divided into five equal blocks. The dependent measure was the number of cards selected from advantageous minus disadvantageous decks as calculated for each block (*C+ D)-(A+ B*) in the ABCD version and (*E+ G)-(F+ H)* in the EFGH version).

FIGURE 2. The Iowa Gambling Test



Data Analysis: Kolmogorov-Smirnov tests were used to check data distribution. IGT results were analyzed with a group (MS vs. controls) by IGT type (ABCD vs. EFGH) by trials analysis of variance (ANOVA). Two tailed t-tests were used for post-hoc comparisons. Forward stepwise linear regression analysis was used to determine factors that predicted IGT performance. In this analysis, the dependent variable was the IGT performance after 100 trials and the independent variables were the WCST, digit span, digit symbol, and verbal fluency measures. Pearson's correlations coefficients were calculated between IGT performance and background neuropsychological measures. The level of significance was $\alpha<0.05$. Effects sizes (Cohen's d) were given for each comparison.

III. Materials and methods in the third experiment

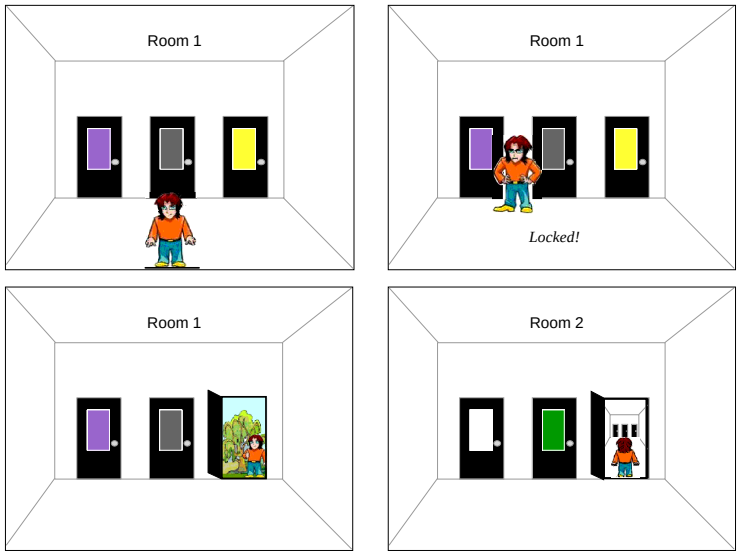
Participants: Twenty (n=20) healthy controls, fourteen (n=14) patients with aMCI, and sixteen never medicated-patients with PD (Hoehn-Yahr stages: I–IV, median: 2.8) participated in the study. The diagnosis of aMCI was established according to the Mayo

Clinic Alzheimer' Disease Research Center criteria (Petersen et al., 1999). Exclusion criteria were other neurological or psychiatric disorders, substance misuse disorders, head trauma, vascular lesions on routine head MRI scans, and medications affecting central nervous system functions. All subjects received background neuropsychological testing including verbal IQ, Mini-Mental State Examination (MMSE), Rey Auditory Verbal Learning Test (RAVLT), Boston Naming Test (BNT), and semantic fluency (SF) test (Lezak, 1995). These tests are especially sensitive for aMCI, and deficits on these tests correlate with the subtle pathology of the medial temporal, parietal, and frontal lobe.

The chaining task: The "Kilroy" chaining task was an updated version of the one presented in Shohamy et al. (2005). The subject's task is to guide an animated character through the rooms, to a goal point, the outside world. The task was run on a Macintosh computer, and programmed in the SuperCard language. On each trial of the experiment, the animated character (nicknamed "Kilroy") appears in a room with three doors; each door has a colored card (Figure 3). The rooms have a uniform white background, and are drawn using perspective lines, with three black doors appearing on the far wall. The doors appear about 2" high, and the colored cards are each 1" high by 0.5" wide, and outlined in white for visual clarity. The animated figure (Kilroy) appears about 2" tall. For each subject, the colored cards marking the doors in each of six rooms are selected from a set of eighteen unique colors, so that the same three colors appear each time Kilroy enters a particular room, but no color appears in more than one room during training. Thus, for example, room A might have red, green, and purple doors; room B might have yellow, blue, and brown doors; and so on. Spatial layout of these three colors on the doors (left, center, right) is randomized on each trial, so that the correct answer (left, center, right) varied across trials in a room; only the location of the color card indicated which was the correct response. Colors were highly discriminable and assignment of colors was randomized across subjects. In each room, the subject uses the computer mouse to move the cursor to click on one of the doors. When the subject selects a door, a few additional drawings of Kilroy appear to approximate a rough animation showing Kilroy turning, walking to the door, and trying to open it. If the subject's choice is incorrect, the door is "locked" and Kilroy cannot open it; he puts his hands on his hips and makes a disappointed face, and the word "Locked!" appears on the bottom of the screen. Kilroy then moves back to the center of the room, and awaits the subject's next choice. If the subject's choice is correct, Kilroy opens the door and steps through. If this room was at the end of the chain, Kilroy reaches the outside, where he turns and gives a thumbs-up sign; if the room was at an earlier stage of the chain, Kilroy steps through into the next room and, once there, waits

for further instructions. In either case (correct or incorrect response), the outcome appears on the screen for 1 s; there is then a 0.33 s interval before Kilroy appears at the bottom of the screen again, ready for new instructions. There is no limit on response times. One trial consists of Kilroy traversing a full sequence of rooms until (eventually) reaching the outside. The length of this sequence increases from one to four rooms over the course of training. A trial is scored as correct if the subject chooses the correct door on the first opportunity for every room in the chain; however, a subject may make one or more errors on a trial by choosing an incorrect door one or more times before choosing the correct door, in each of one or more rooms in the chain. This means that a subject could make more than one error per trial. Each learning phase continues until the subject completes four consecutive correct trials or to a maximum of fifteen trials. If a subject fails to reach criterion within the maximum number of trials for any phase, that phase is terminated, further training and probe phases are skipped, and the subject proceeds directly to the last (retraining) phase of the task.

FIGURE 3. Sample screen events during the “Kilroy” chaining task



The subject is seated in a quiet testing room at a comfortable viewing distance from the screen. Before the test, the subject is informed that the aim of the game is to help a cartoon figure get out of the house as many times as possible. The following instructions appear: “Welcome to the experiment. In this experiment, you will see a character named Kilroy who is trying to get out of the house. Each room in the house has three doors, and each door has a colored card on it. On each trial, two of the doors are locked, and one door is unlocked. In

each room, click on the color card of the door that you think is unlocked. If you are correct, Kilroy will get outside. Good luck!" The test then consisted of the following parts:

1. *Practice*. The Practice Room appears, with three colored doors, and Kilroy in his "waiting-for-instructions" position at the front bottom of the screen. If the subject chooses the correct door, Kilroy makes it outside and the trial is concluded. Every trial terminates with Kilory eventually reaching the outside. The practice phase continues until the subject makes four consecutive correct trials (i.e. chooses the correct door on the first response in each of four trials).

2. *Sequence training*. At this point, new instructions appear: "You've successfully finished practice! Now Kilroy will be put in some new rooms. Again, in each room, two doors are locked and one door is unlocked. Each time, click on the door that you think is unlocked. Sometimes, Kilroy will have to go through more than one room to reach the outside. Good luck!" Kilroy now appears in his "waiting-for-instructions" position in Room 1. This phase is identical to the Practice phase, except that three new colored cards are used. Here, subjects have to learn to open the correct door (A). Once this is learned, phase 2 begins, in which Kilroy appears in Room 2, which contains three new colored cards; here, choice of the correct door (B) leads Kilroy to Room 1, where a correct answer leads him outside. Once this is learned, subjects work through phase 3 (door C in Room 3 leads to Room 2 and so on) and phase 4 (door D in Room 4 leads to Room 3 and so on) until, by the end of phase 4, subjects should be choosing the correct door in each room: D→C→B→A→reward.

3. *Probe phase*. Next comes a probe phase, unsignaled to the subject. At the start of a trial, Kilroy appears in Room 4. Correct responses will, as usual, allow him to progress through the sequence of rooms and reach the outside. Now, however, the colored cards are switched. In each room, one of the three cards is always the correct answer in that room, at that point in the sequence; one of the cards is always a choice that was correct in a different room; the third card (distracter) is a choice that was never correct in any room. Thus, in Room 2, Kilroy might be presented with a choice between card B, card A, and card X. Card B is the correct choice, and should be chosen by a subject who had learned the chain: that is, what choice to make at each step in the sequence. But a subject who had merely learned non-sequential stimulus-response associations might choose A, since that is a stimulus that had been directly associated with reward in the past. The probe phase contained six trials, each trial consisting of a trip through the usual four rooms. In the probe phase, the participant may commit three types of errors. "Reward error" is when the participant chooses the door at the end of the chain which had previously been directly associated with reward, but chooses it at

the wrong point in the sequence (i.e., choosing door A in any room other than Room 1). "Chaining error" is when the participant chooses any other previously correct door (B, C, or D) but chooses it at the wrong point in the chain (e.g., choosing door C instead of door B in Room 2). "Distracter error" is when the participant chooses a door (e.g., X or Y) that has never been right at any point in the sequence.

4. *Retraining phase*. Finally came a retraining phase, in which subjects are required to learn a new room with three new colored cards, one of which leads directly to the outside. The purpose of this phase was to determine whether any learning deficits observed on the sequence learning or probe phase were due to fatigue effects or other non-associative factors. At the end of the test, the subject sees a screen reporting the total number of trials on which Kilroy got out, which is equal to the total number of trials (regardless of intervening errors).

Data analysis: First, data were entered into Kolmogorov–Smirnov tests and Levene's tests in order to check the normality of distribution and homogeneity of variance, respectively. In the case of normal distributions and homogeneous variances, parametric tests were used, whereas if data deviated from normal distribution or variance was not homogeneous, non-parametric tests were included (Kruskal–Wallis analysis of variance (ANOVA) and Mann–Whitney U-tests). ANOVAs were followed by F-tests for planned comparisons and Tukey's HSD tests for post-hoc comparisons. The level of significance was set at $\alpha<0.05$.

IV. Materials and methods in the fourth experiment

Participants: Two hundred-four healthy volunteers were recruited from the community using newspaper advertisements and through acquaintance networks. Exclusion criteria were history of neurological or psychiatric disorders, psychoactive substance dependence and any other medical condition that can affect central nervous system functions.

Genotyping: Genomic DNA was extracted from venous blood samples. Ten SNPs in the 30-region (block B) of SNCA gene were genotyped (rs356180, rs356169, rs2572323, rs356219, rs356220, rs356165, rs356204, rs3822086, rs356203 and rs356168). These SNPs show linkage disequilibrium and previously six haplotypes were identified (Mueller et al. 2005). Four of these haplotypes (TAGACAGCAT, CAGACAGCAT, CCGACAACAC and CAGACAACAC) are associated with decreased risk of PD, and two of the six haplotypes (TCAGTGACGC and CAGGTGATGC) are associated with increased risk of PD (Mueller et al., 2005). Genotyping was performed using the matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry method (Sequenom, San Diego, CA, USA). The haplotype carrier status of individual participants was determined using the Bayesian

method (PHASE v2.0.2) (Stephens and Donnelly 2003). Altogether, 134 cases with protective haplotypes and 70 cases with risk haplotypes were identified. Six polymorphic alleles (-2 = 263 bp, -1 = 265 bp, 0 = 267 bp, 1 = 269 bp, 2 = 271 bp, 3 = 273 bp) of the Rep1 promoter region were identified, as described previously (Farrer et al., 2001; Xia et al., 2001). Polymerase chain reaction (PCR) was used to amplify the promoter region of the ASN gene (8748 bp upstream of exon 1, accession no.: U46896; fluorescently tagged reverse primers: Fam 5'-CCTGGCATATTTGATTGCAA-3' and 5'-GACTGGCCCAAGATTAACCA-3'). PCR products were treated by capillary electrophoresis and were analyzed using the GENOTYPER software (Applied Biosystems, Foster City, CA, USA).

Neuropsychology: In addition to the chaining task, participants received a battery including tests of executive functions/working memory (WCST, verbal fluency, Letter-Number Sequencing Test) and sensory-motor skill learning (mirror reading and pursuit rotor) (Lezak, 1995).

Data analysis: The distribution of the data was checked using Kolmogorov–Smirnov tests. Data were normally distributed. Two-tailed Student's t-tests were used to compare the mean number of errors from the training and probe phases of the chaining task in participants with protective and risk haplotypes. A two-way analysis of variance (ANOVA) was used to investigate the effect of haplotypes on errors in different phases of training phase (from one to four associations). In this ANOVA, risk vs. protective haplotypes were the between-subject factor, and training phase was the within-subject factor. Another two-way ANOVA was used to investigate the effect of haplotypes on errors in the training phase and in the probe phase. In this ANOVA, risk vs. protective haplotypes were the between-subject factor and training vs. probe phase was the within-subject factor. Student's t tests were used for post-hoc analysis and for the analysis of background neuropsychological measures. Pearson's product moment correlation coefficients were calculated between errors in the chaining task and background neuropsychological measures. The level of significance was set at $\alpha<0.05$.

Results

I. Results of the first experiment: vision in MS

In the neuropsychological, VEP, and contrast threshold data there were no significant differences between the patients and the controls ($p>0.1$) and these did not correlate with the flanker task performance ($p>0.5$) (Table 1). The ANOVA conducted on the orientation detection performance revealed significant main effects of group [$F(1,50)=7.39$, $p<0.05$], flanker orientation [$F(1,50)=22.22$, $p<0.0001$], and exposure time [$F(3,150)=119.96$, $p<0.0001$]. The 2-way interaction between group and exposure time was significant [$F(3,150)=7.33$, $p<0.0005$]. The controls performed better when collinear flankers were presented [$F(1,50)=5.33$, $p<0.05$], similarly to the patients [$F(1,50)=17.89$, $p<0.001$]. Critically, the 3-way interaction between group, flanker orientation, and exposure time was significant [$F(3,150)=5.16$, $p<0.005$], suggesting that the difference between the patients and the controls was influenced by both flanker orientation and exposure time. Tukey HSD tests indicated significantly lower orientation detection performances in the patient group as compared with the control group in the orthogonal condition at 40-ms exposure time ($p<0.01$), whereas the other comparisons did not reveal statistically significant between-group differences ($p>0.1$) (Figure 4). In the critical orthogonal condition at 40-ms exposure time, 18 of the 22 multiple sclerosis patients (81.8%) performed below the 95% confidence interval of the control mean. The confidence intervals of the patients and controls did not overlap (Figure 4).

The ANOVA conducted on the reaction time data indicated no significant main effect of group ($p=0.63$). The main effects of flanker orientation [$F(1,46)=23.66$, $p<0.0001$] and exposure time [$F(3,138)=15.20$, $p<0.0001$] were significant. The controls responded faster when collinear flankers were presented [$F(1,46)=21.40$, $p<0.001$], similarly to the patients [$F(1,46)=6.32$, $p<0.01$]. The interaction between group and exposure time was significant [$F(3,138)=3.46$, $p<0.05$], but the post hoc tests revealed no significant between-group differences ($p>0.05$). The remaining interactions did not reach the level of statistical significance ($p>0.2$). An analysis of linear trend revealed a significant group by exposure time interaction [$F(1,48)=4.3$, $p<0.05$]. Although the controls showed decreasing reaction time along with increasing exposure time in both collinear and orthogonal conditions ($p<0.05$), the patients did so in neither condition ($p>0.5$) (Figure 5).

Table 1. Clinical, demographical, and neuropsychological data

	MS patients (n=22)	Controls (n=30)
Age (years)	28.5 (6.3)	28.2 (8.2)
Gender (male/female)	7/15	9/21
Education (years)	14.6 (3.2)	14.2 (6.8)
Duration of illness (years)	2.5 (2.0)	-
FSS	1.9 (1.3)	2.1 (1.3)
BDI	4.8 (2.3)	5.0 (2.4)
DSF	8.3 (1.3)	8.1 (1.2)
DSB	7.1 (1.2)	6.9 (0.9)
SDMT	56.8 (8.5)	54.6 (8.6)
Verbal fluency	49.5 (9.6)	46.0 (9.0)
CVLT	54.1 (11.3)	54.6 (9.2)
Contrast threshold (%)	4.9 (1.7)	5.0 (1.9)
VEP amplitude	L: 10.1 (3.5) R: 9.9 (3.1)	-
VEP latency	L: 102.6 (6.5) R: 103.8 (5.9)	-

Data are mean (SD). All between-group comparisons shown in the table were non-significant ($p>0.1$, t-tests). MS – multiple sclerosis, FFS – Fatigue Severity Scale, BDI – Beck Depression Inventory, DSF – digit span forward, DSB – digit span backward, SDMT – Symbol Digit Modality Test, CVLT – California Verbal Learning Test

FIGURE 4. Performance in patients with MS and controls (*p<0.01, Tukey's HSD test)

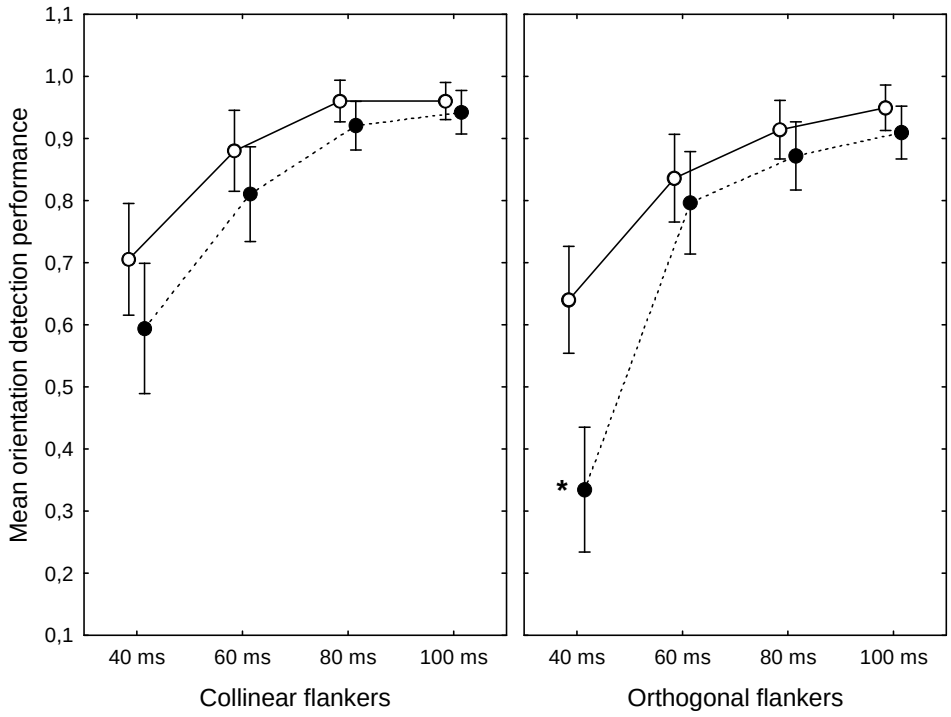
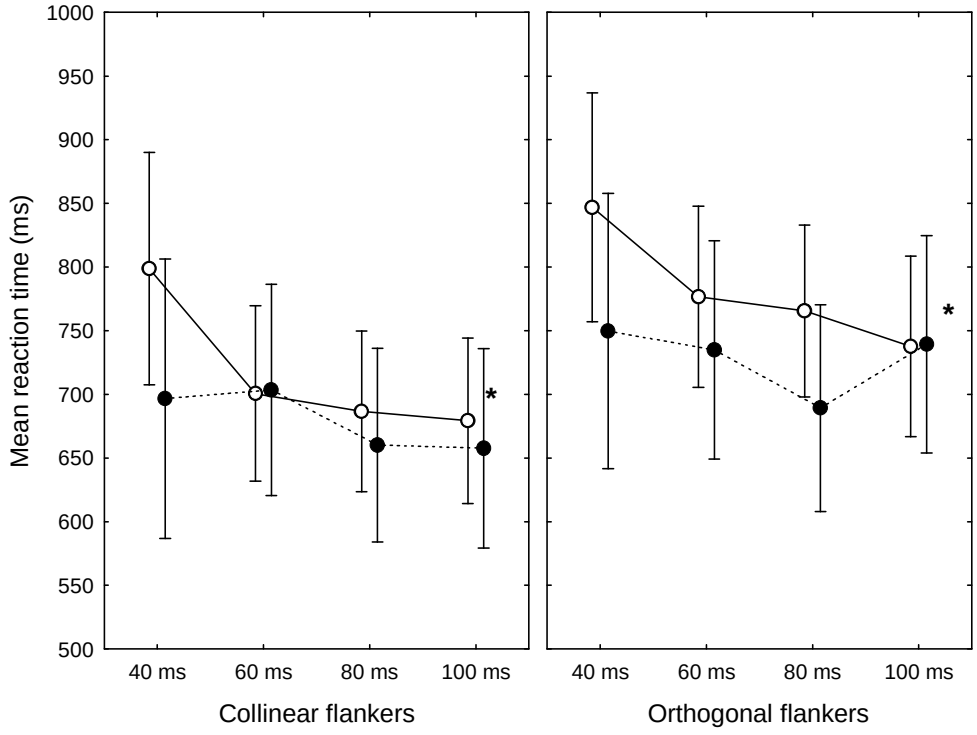


FIGURE 5. Reaction time in patients with MS and controls (*p<0.05, ANOVA group by exposure time interaction)



II. Results of the second experiment: decision-making in MS

The results of the background neuropsychological tests are shown in Table 2. The patients with MS displayed impaired performances on tests of executive functions, attention, speed of information processing, and verbal retrieval.

Table 2. Neuropsychological results

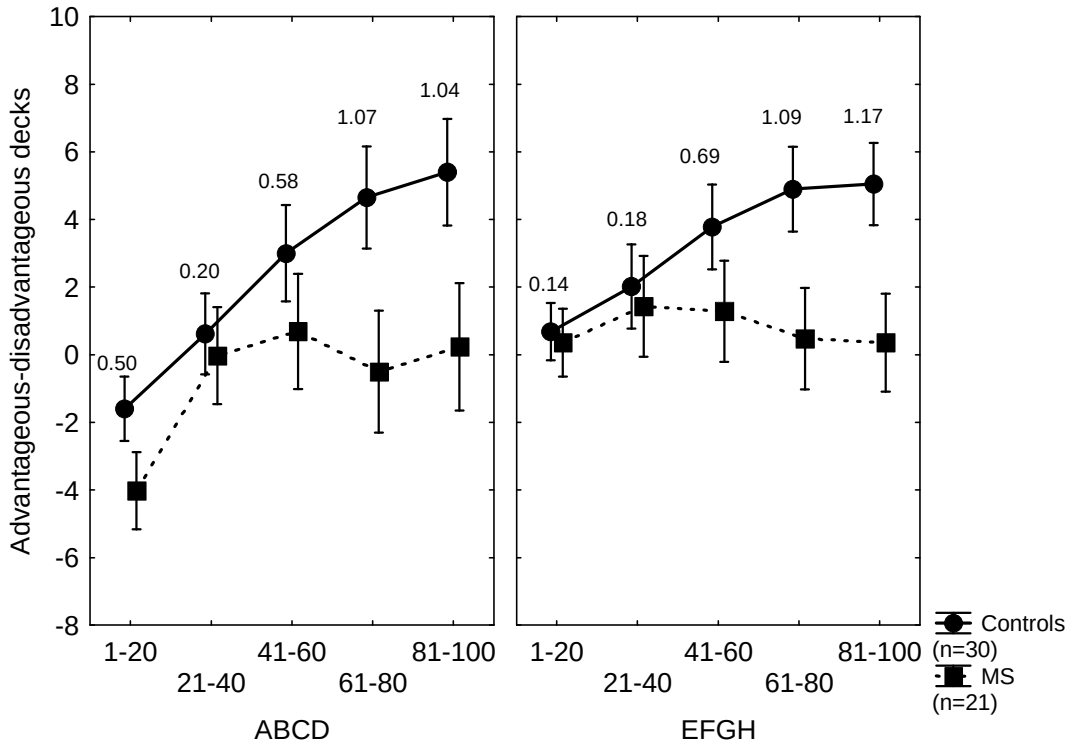
	Multiple sclerosis (n=21)	Controls (n=30)	t	p	*d*
WCST categories	4.2 (1.3)	5.2 (0.9)	-3.15	0.003	0.82
WCST perseverative errors	15.5 (7.3)	8.8 (4.2)	4.16	0.0001	1.03
Digit span forward	7.3 (1.4)	8.1 (1.2)	-2.09	0.04	0.60
Digit span backward	5.9 (1.4)	6.9 (0.9)	-3.06	0.004	0.80
Symbol digit	47.7 (10.2)	54.6 (8.6)	-2.64	0.01	0.70
Verbal fluency	40.0 (9.2)	46.0 (9.0)	-2.33	0.02	0.63

Mean values (standard deviation) are compared with two-tailed t-tests. WCST – Wisconsin Card Sorting Test

The IGT results are shown in Figure 6. Kolmogorov-Smirnov tests did not indicate deviations from normal distribution in the patient and control groups ($p>0.2$). The ANOVA revealed significant main effects of group [$F(1,49)=22.15$, $p<0.001$], IGT type [$F(1,49)= 11.41$, $p<0.01$] and trials [$F(4,196)= 30.02$, $p<0.001$]. There were significant interactions between group and trials [$F(4,196)= 11.51$, $p<0.001$] and between IGT type and trials [$F(4,196)= 6.51$, $p<0.001$]. The remaining interactions were not significant ($p>0.5$). The t-tests indicated that the MS patients made significantly less advantageous decisions than the

controls in the ABCD task after 1–20 [t(49)= -3.28, p< 0.01; power= 0.41], 41– 60 [t(49)= -2.01, p< 0.05; power=0.51], 61–80 [t(49)= -4.40, p< .001; power>0.9], and 81–100 trials [t(49)=-4.22, p<0.001; power>0.9]. Similar differences were found in the EFGH task after 41– 60 [t(49) = -2.57, p< .05; power= 0.66], 61–80 [t(49)= -4.55, p< .001; power> 0.9], and 81–100 trials [t(49)= -4.99, p< 0.001; power>0.9] (Figure 6). The linear regression analysis revealed that the WCST perseverative errors, digit span, symbol digit, and verbal fluency scores did not predict ABCD and EFGH task performances after 100 trials (p>0.4). There were no significant correlations among ABCD and EFGH task performances and background neuropsychological parameters (r<0.3). These results were the same when data from the patients and controls were separately analyzed and when data from the two groups were collapsed.

FIGURE 6. Mean number of cards selected from advantageous minus disadvantageous decks. Positive scores reflect advantageous strategy (overall gain), whereas negative scores reflect disadvantageous strategy (overall loss). Numbers represent effect size *(d)* for each between-group comparison. Error bars indicate 95% confidence intervals.



III. Results of the third experiment: chaining in PD and aMCI

Table 3. Demographical parameters and background neuropsychology

	Controls (n=20)	PD (n=16)	aMCI (n=14)
Age (years)	69.3 (9.5)	68.4 (8.7)	71.0 (10.3)
Male/female	14/6	11/5	8/6
Years of education	12.5 (2.3)	13.0 (5.1)	12.9 (4.6)
Verbal IQ	107.2 (10.4)	109.9 (11.6)	108.0 (12.9)
MMSE	28.7 (1.2)	28.8 (1.5)	27.2 (1.4)
RAVLT	50.5 (3.2)	48.8 (4.4)	40.1 (5.5)
BNT	53.3 (3.9)	51.7(3.0)	48.9(5.0)
SF	17.6 (3.8)	16.3 (3.4)	13.4 (3.8)

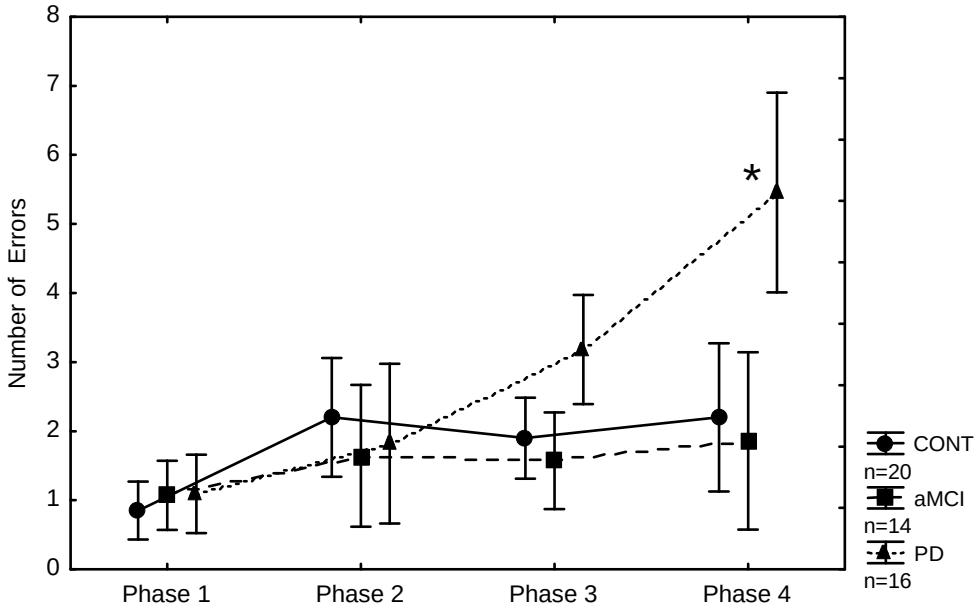
PD – Parkinson's disease, aMCI – amnestic mild cognitive impairment, MMSE – Mini-Mental State Examination, RAVLT – Rey Auditory Verbal Learning Test, BNT – Boston Naming Test, SF – semantic fluency

The three experimental groups did not differ in age, years of education, or verbal IQ ($p > 0.1$) (Table 3). The Kruskal–Wallis ANOVA conducted on the MMSE scores revealed a significant main effect of group ($H(2)=10.62$, $p=0.005$). As compared with controls and patients with PD, patients with aMCI showed significantly lower MMSE scores (Mann–Whitney U-tests, $Z=2.82$, $p=0.005$ and $Z=2.73$, $p=0.006$, respectively). There was no significant difference between controls and patients with PD ($p > .5$) (Table 1). The ANOVA conducted on the RAVLT scores revealed a significant main effect of group ($F(1, 47)= 25.38$, $p<0.0001$). Tukey's HSD tests indicated that patients with aMCI displayed lower RAVLT scores as compared with controls ($p<0.001$) and with patients with PD ($p<0.001$). There was no significant difference between controls and patients with PD ($p>0.4$). The ANOVA conducted on the BNT scores revealed a significant main effect of group ($F(1,47)=5.22$, $p< 0.05$). Tukey's HSD tests indicated that patients with aMCI were impaired as compared with controls ($p<0.05$), but not as compared with patients with PD ($p>0.1$). Controls subjects and patients with PD did not differ ($p>0.5$). The ANOVA conducted on the fluency scores revealed a significant main effect of group ($F(1,47)=5.57$, $p<0.05$). Tukey's HSD tests indicated that patients with aMCI were impaired as compared with controls ($p<0.05$), but not

as compared with patients with PD ($p>0.08$). Controls subjects and patients with PD did not differ ($p>0.5$).

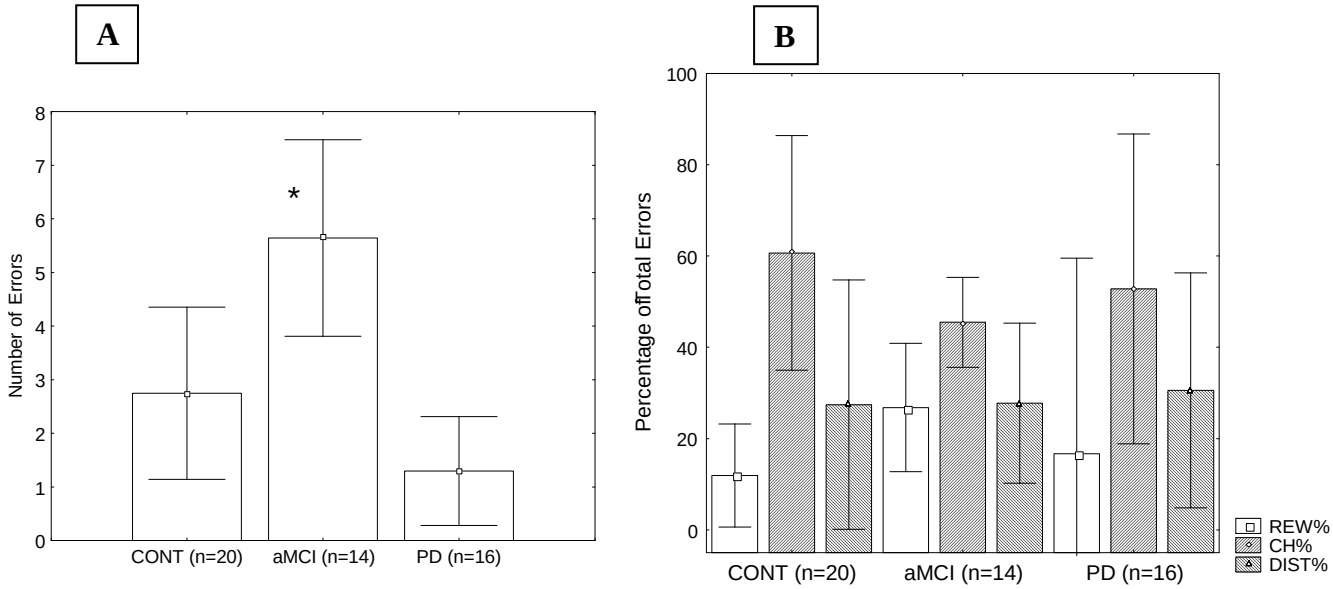
The ANOVA conducted on the number of errors in the four training phases of the chaining task revealed a significant main effect of group ($F(1,42)=8.87$, $p<0.001$) and training phases ($F(3,126)=11.30$, $p<0.0001$). The interaction between group and training phases was significant ($F(6,126)=3.75$, $p<0.01$). However, this interaction was not significant when controls were compared with patients with aMCI using an F-test for linear trend ($p = .4$). In contrast, the group by training block interaction was significant when controls were compared with patients with PD ($F(1,42)=13.04$, $p<0.001$) and when patients with aMCI were compared with patients with PD ($F(1,42)=14.63$, $p<0.001$). Tukey's HSD tests confirmed that patients with PD were impaired in this phase of the chaining task as compared with controls ($p<0.01$) and with patients with aMCI ($p <0.005$). According to the Tukey's HSD tests conducted on the group by training phase interaction, this difference was significant only in the fourth training phase ($p<0.005$). Control subjects and patients with aMCI did not differ ($p>0.4$) (Figure 7).

FIGURE 7. Mean number of errors in the four phases of the training phase of the "Kilroy" chaining task. Error bars indicate 95% confidence intervals (CONT – controls, aMCI – amnestic mild cognitive impairments, PD – Parkinson's disease, *$p<0.005$ (CONT vs. PD and aMCI vs. PD), Tukey's HSD tests)



The ANOVA conducted on the number of errors in the context-dependent probe phase revealed a significant main effect of group ($F(1,42)=6.75$, $p<0.01$). Tukey's HSD tests revealed that patients with aMCI committed more errors than controls ($p<0.05$) and than patients with PD ($p<0.005$). Control subjects and patients with PD did not differ ($p>0.4$) (Figure 8A). However, the absence of a group difference in total number of errors on the probe phase might conceivably mask a difference in the types of errors made by each group on the probe phase. To examine this, we analyzed the different types of errors in the probe phase ("reward", "chaining", and "distracter" errors). The ANOVA revealed no significant main effect of group ($p=0.6$), indicating that the distribution of different types of errors were similar across groups (Figure 8B). Finally, on the retraining phase, the control group averaged 1.1 errors (SD 1.7), the PD group averaged 1.2 errors (SD 1.0), and the aMCI group averaged 1.1 errors (SD 0.9); these group differences fell short of statistical significance (ANOVA, $p>0.5$).

FIGURE 8. A. Mean number of errors in the context-dependent probe phase of the "Kilroy" chaining task. B. Mean percentage of different types of errors in the probe phase of the "Kilroy" chaining task. Error bars indicate 95% confidence intervals. REW – reward, CH – chaining, DIST – distracter, CONT – controls, aMCI – amnestic mild cognitive impairments, PD – Parkinson's disease



IV. Results of the fourth experiment: ASN and chaining associative learning

Figure 9 shows the number of errors in the training phase (stimulus-reward learning of the chaining sequence). Participants with 3'-block risk haplotypes committed more cumulative errors during the training phase (mean number of errors: 2.3, SD=1.7) compared with participants carrying protective haplotypes (mean number of errors: 1.6, SD=0.8) [$t(199)=-3.81$, $p<0.001$]. As the length of the sequence increased (from phase 1 to phase 4), the mean number of errors also increased [main effect of phase: $F(3,597)= 20.96$, $p<0.001$]. The effect of haplotypes was also significant [$F(1,199)= 14.55$, $p<0.001$]. Participants with risk haplotypes committed more errors in phases 2, 3 and 4 compared with participants carrying protective haplotypes ($t>2.4$, $p<0.05$). In the probe phase, participants with protective haplotypes performed worse (mean number of errors: 2.3, SD= 2.6) than participants with risk haplotypes (mean number of errors: 1.5, SD= 2.0) [$t(195)=2.30$, $p<0.05$; ANOVA interaction between haplotypes (protective vs. risk) and task phase (training vs. probe): $F(1,195)=14.74$, $p<0.001$). The percentage of chaining errors was 70.5% (SD=58.6) in the case of participants with risk haplotypes, whereas this value was 81.9% (SD=47.4) in the case of participants with protective haplotypes ($p>0.1$). This indicates a tendency for participants with protective haplotypes to choose previously correct doors but to choose them at the wrong point in the chain. However, because of the large standard deviations, the difference did not reach the level of statistical significance. There were no significant differences between male and female participants, and there was no gender by haplotypes by task phase interaction ($p>0.1$). There was no significant correlation between age and performance in the training phase (participants with protective haplotypes: $r=0.02$ and participants with risk haplotypes: $r= 0.11$) and in the probe phase (participants with protective haplotypes: $r=0.09$ and participants with risk haplotypes: $r=0.08$). Participants with protective and risk haplotypes did not differ in WCST, verbal fluency, mirror reading and pursuit rotor.

We found no significant correlations between errors in the training or probe phase of the chaining task and background neuropsychological measures ($r<0.2$). The distribution of the six polymorphic variants of the Rep1 promoter region is shown in Table 4. ANOVAs revealed that these polymorphic variants had no significant effect on the number of errors in the training phase and in the probe phase ($F< 1$, p 0.5).

FIGURE 9. Mean number of errors in the training phase (stimulus-reward learning) in participants with protective and risk ASN haplotypes. Error bars indicate 95% confidence intervals.

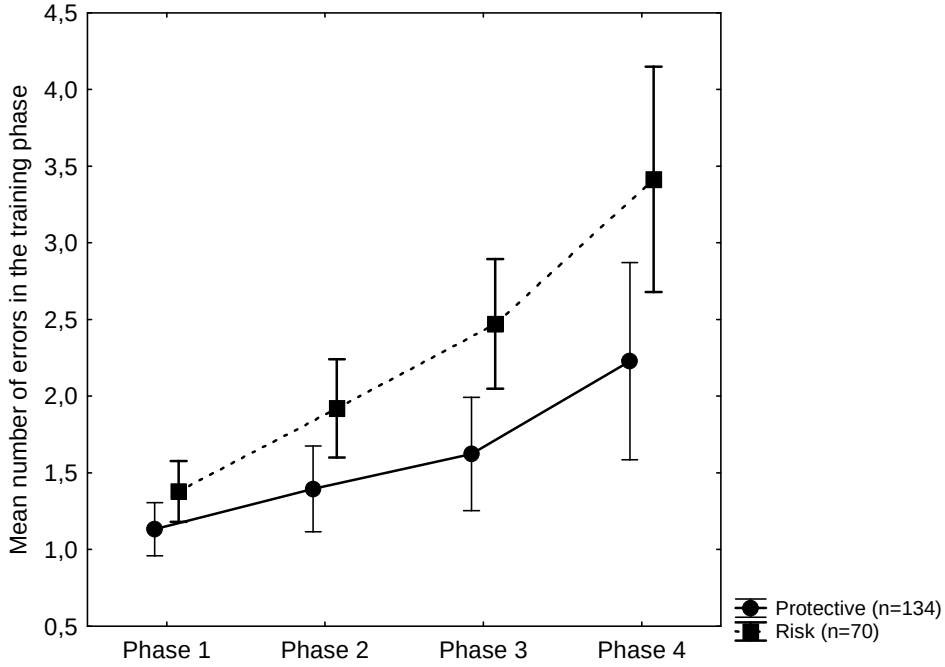


Table 4. The effect of Rep1 polymorphism on cognitive sequence learning

Polymorphic alleles	-2 (263 bp)	-1 (265 bp)	0 (267 bp)	1 (269 bp)	2 (271 bp)	3 (273 bp)
Percentage of participants	0%	2.0%	40.2%	51.5%	5.9%	0.5%
Mean number of errors, training phase	-	1.9 (SD=1.2)	1.7 (SD=0.9)	1.5 (SD=1.0)	1.7 (SD=1.2)	1.8 (SD=1.5)
Mean number of errors, probe phase	-	2.0 (SD=2.4)	1.9 (SD=1.7)	1.9 (SD=2.1)	1.9 (SD=2.0)	1.8 (SD=1.8)

DISCUSSION

General summary and discussion

The results of this series of studies in MS, PD, and aMCI revealed a unique pattern of cognitive task performance in these patients, which can be interpreted in parallel with the critical neuronal structures affected in these disorders. First, contrary to our hypothesis, we did not find perceptual integration deficits in patients with MS, which is against the hypothesis of impaired lateral connections in early visual areas. However, patients with MS showed significantly slowed visual information processing, which was confined to the orthogonal flanker condition at the shortest exposure time. Second, we demonstrated signifcantly altered decision-making in MS in both reward- and punishment-guided conditions, which may indicate the impairment of emotion-related brain areas such as ventromedial prefrontal cortex, insula, and amygdala. Third, unmedicated patients with PD displayed impaired chaining associative learning performance, which can be explained by dysfunctional feedback-prediction processing in the basal ganglia. Patients with aMCI displayed the opposite pattern of performance with relatively sufficient chaining learning and impaired context representation, which may point at a deficit of the medial temporal lobe. Finally, and perhaps most interestingly, the PD risk haplotype of the ASN gene was associated with a lower efficacy of chaining learning relative to the protective haplotype, which is consistent with the results obtained in PD and may indicate a genetic background of impaired feedback processing.

Visual dysfunctions in MS

The results revealed that patients with MS showed intact visual contrast sensitivity and neuropsychological functions, whereas orientation detection in the orthogonal condition was significantly impaired. At 40-ms exposure time, collinear flankers facilitated the orientation detection performance of the patients resulting in normal performance. These data suggest that young MS with mild symptoms, low level of depression and fatigue, spared VEP, contrast sensitivity, and neuropsychological performance showed robust and selective impairments in the orientation detection task; in the orthogonal condition at short exposure time (40 ms), their performance remained below the 50% chance level. The spared VEP and visual contrast sensitivity are against the demyelinating pathology of the foveal retino-cortical

pathway in this sample (Pula and Reder, 2009), and therefore the deficit uncovered by the flanker task cannot be detected by conventional clinical methods. The patients did not respond slower than the controls during the flanker task, suggesting preserved psychomotor speed. We speculate that the deficit during the detection of briefly presented stimuli may be a consequence of subcortical pathology, possibly related to thalamic atrophy (Benedict et al., 2004).

Decision-making in MS

In young, non-depressed, relatively highly functioning patients with MS, we found impaired decision-making on the ABCD and EFGH versions of the IGT. The difference between patients and controls was more pronounced in the later phase of test, which suggests that poor decision-making is a consequence of impaired learning across trials and not of generalized cognitive impairments. Although executive dysfunction is characteristic for MS and may contribute to impaired IGT performances, in our study it was not associated with IGT performances. These findings are consistent with the results of Kleeberg et al. (2004). However, in the Kleeberg et al. (2004) study only the ABCD version of the IGT was used, and therefore it remained undetermined whether the deficit was due to hypersensitivity to reward or to impaired ability to evaluate long-term outcomes of decisions. According to our results, patients with MS show a similar performance to that found in patients with ventromedial prefrontal damage: their decisions are guided by recent outcomes irrespective of gain or loss. It is of particular interest that decision-making abnormalities were present in patients who did not show psychiatric and psychoactive substance-related disorders, which have been shown to disrupt decision-making cognition (Bechara et al., 2001; Rogers, 2003). We may speculate that dysfunctions in the IGT reflect subclinical pathology, which may be a progenitor of later full-blown disorders. Kleeberg et al. (2004) found associations between IGT performance and impaired emotional dimensions of behavior as measured by anticipatory skin conductance responses. Roca et al. (2008) demonstrated that impaired IGT performance is related to pathological alteration in fronto-subcortical fiber tracts in MS. According to Simioni et al. (2008), decision-making is generally spared in early MS, but patients who will show a relapse perform worse than controls. Decision-making abilities decline during the course of MS and are related to general health status and emotional well-being (Simioni et al., 2009). However, the intriguing nature of this decline is that it remains isolated as it is not

related to relapse rate, disability progression, general cognitive and behavioral changes (Simioni et al., 2009).

Chaining associations in PD and aMCI

Our results are consistent with the view that patients with PD show substantial learning deficits on tasks requiring trial-by-error, feedback-based stimulus-response learning, especially when sequences or chains of associations must be acquired (Shohamy et al., 2005). The degree of deficit depends on task demands, medication effects, and on the severity of symptoms (Swainson et al., 2006; Schmitt-Eliassen et al., 2007; Filoteo et al., 2007). The work by Shohamy et al. (2005) considered PD patients who had been withdrawn from their normal dopaminergic medication for a period of about 12 hours, and were thus in a relatively dopamine-depleted state; however, this paradigm could not rule out long term consequences of dopaminergic medication, such as neuroplastic changes in synapses and receptors in the striatum. Since our patients with PD had never received dopaminergic medications, their learning deficit could not be associated with long-term changes in the striatum. L-DOPA and dopamine agonists may improve learning from reward, but at the same time, they have a negative impact on the processing of negative feedback (Frank et al., 2004; Bódi et al., 2009).

In contrast to our patients with PD, patients with aMCI exhibited intact learning on the training phase of the chaining task. In general, patients with aMCI exhibit prominent episodic memory impairment, and sophisticated neuroimaging and neuropsychological methods reveal subtle alterations in medial temporal lobe (Trivedi et al., 2006; Sarazin et al., 2007), reflecting a high vulnerability for Alzheimer's disease which develops in 12% of these patients per year (Petersen et al., 1999; Gauthier et al., 2006). The preserved learning in our aMCI sample is consistent with other findings demonstrating that medial temporal lobe damage generally does not impair the ability to learn simple, non-declarative stimulus-response associations (Knowlton et al., 1996; Myers et al., 2003). The most interesting finding was that, in contrast to patients with PD who exhibited normal performance during the context-dependent probe phase of the chaining task, patients with aMCI committed significantly more probe errors than controls. The probe phase was intended to verify that participants had learned the correct door in its correct place in the sequence, encoding not only the correct door but also its context (the room in which it occurred). The deficit of context representation in aMCI is consistent with medial temporal lobe dysfunction, because this region is important in the representation of context, especially in the case of higher-order associations (Ergorul and Eichenbaum, 2006).

However, it is important to note that, in most aMCI patients, brain abnormalities are not entirely limited to the medial temporal lobe. Therefore, our data cannot completely rule out the possibility that context representation problems in the aMCI group are due to the dysfunction of other structures.

ASN and chaining associations

The data suggest a double dissociation between stimulus-reward and context-dependent cognitive sequence learning in participants with risk and protective haplotypes of ASN associated with PD. Healthy participants with risk haplotypes exhibited less efficient chaining learning, which is similar to that found in patients with unmedicated PD (see above), but in the patients, inefficient learning was much more pronounced than in healthy volunteers with risk haplotypes. Because L-DOPA improved stimulus-reward learning of chaining sequences in patients with PD (Shohamy et al., 2005), it is plausible to hypothesize that the risk haplotypes of ASN are associated with decreased dopaminergic transmission and reward signal in the basal ganglia. In an animal model, Oksman et al. (2006) demonstrated that the lack of ASN sensitized the reward system. A splice variant of ASN (NACP112) lacks exon 5 (Ueda et al., 1994), which is located within the investigated 30-block haplotypes. This could influence the expression of the splice variant, leading to altered dopaminergic transmission and reward sensitivity. However, the biological relevance of risk and protective ASN haplotypes investigated in our study is not elucidated, and therefore all inferences on molecular correlates are speculative at this stage of research.

A more unexpected and intriguing finding was that the risk haplotypes were associated with better performance during the context-dependent phase of the chaining task, the probe phase designed to verify that participants learned the correct door in its correct place in the sequence. Because the context-dependent phase of sequence learning may be related to the medial temporal lobe, including the hippocampus, the issue is how ASN and dopaminergic signals may affect the functioning of neurons of this brain structure. Dopaminergic pathways also exist in the medial temporal lobe, and hippocampal activity is modulated by positive feedback (reward) during classification learning (Seger and Cincotta, 2005). According to Lisman and Otmakhova (2001), the dentate and CA3 hippocampal regions could store and recall memory sequences in context. These authors showed that dopamine reduces the direct cortical input to CA1 while having little effect on the CA3 region, which is important in sequence and context learning. Therefore, it is possible that ASN has an important effect on

the interaction between CA3 and CA1 regions by the modulation of dopaminergic transmission. This may result in altered storage and recall memory sequences in context.

Regardless of the mechanism of action, it is somewhat unexpected that risk haplotypes for PD influenced cognitive sequence learning, given that for a long time it has been postulated that motor functions are first affected. Buhmann et al. (2005) showed motor reorganization in asymptomatic carriers of a mutant Parkin allele, providing a model for presymptomatic parkinsonism. The presymptomatic period can last five or more years (Fearnley and Lees, 1991), during which neuronal compensation develops to adapt to gradually declining striatal functions. Further studies are warranted to explore how polymorphisms of PD-associated genes affect these processes, as potential biomarkers of early diagnosis, together with other parameters such as olfactory problems and REM sleep disturbances (Marek and Jennings, 2009).

Limitations

The most important limitation of these studies was that functional neuroimaging methods were not used, and therefore all inferences regarding the affected brain structures remained indirect. However, several tests used in our studies have been investigated and validated by functional brain imaging and electrophysiological techniques in previous studies. Second, in many studies, the sample size was small. However, we intended to include only highly functioning patients with firm diagnosis at the beginning of the illness, which markedly limited our options to recruit more volunteers. In the statistical analyses, we carefully checked the power of each test and quality of data in order to minimize the likelihood of false positive or negative findings. Taking into consideration these limitations, we hope that the results of these studies elucidate new aspects of MS, PD, and aMCI, and in the future they may contribute to the development of new diagnostic tests and behavioral-molecular biomarkers.

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References

Balcer LJ, Baier ML, Kunkle AM, Rudick RA, Weinstock-Guttman B, Simonian N, Galetta SL, Cutter GR, Maguire MG. Self-reported visual dysfunction in multiple sclerosis: results from the 25-Item National Eye Institute Visual Function Questionnaire (VFQ-25). Mult Scler 2000; 6: 382-385.

Bechara A, Tranel D, Damasio H. Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. Brain 2000; 123: 2189-2202.

Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE. Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 2001; 39: 376-389.

Benedict RH, Weinstock-Guttman B, Fishman I, Sharma J, Tjoa CW, Bakshi R. Prediction of neuropsychological impairment in multiple sclerosis: comparison of conventional magnetic resonance imaging measures of atrophy and lesion burden. Arch Neurol 2004; 61: 226-230.

Bobholz JA, Rao SM. Cognitive dysfunction in multiple sclerosis: a review of recent developments. Curr Opin Neurol 2003; 16: 283-288.

Bódi N, Kéri S, Nagy H, Moustafa A, Myers CE, Daw N, Dibó G, Takáts A, Bereczki D, Gluck MA. Reward-learning and the novelty-seeking personality: a between- and within-subjects study of the effects of dopamine agonists on young Parkinson's patients. Brain 2009; 132: 2385-2395.

Buhmann C, Binkofski F, Klein C, Buchel C, van Eimeren T, Erdmann C, Hedrich K, Kasten M, Hagenah J, Deuschl G, Pramstaller PP, Siebner HR. Motor reorganization in asymptomatic carriers of a single mutant Parkin allele: a human model for presymptomatic parkinsonism. Brain 2005; 128: 2281-2290.

Cappa SF. Cognitive Neurology. An Introduction. World Scientific Publishing, London, 2001.

Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TC. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 2005; 123: 383-396.

Chiaravalloti ND, DeLuca J. Cognitive impairment in multiple sclerosis. Lancet Neurol 2008; 7: 1139-1151.

Eichenbaum H, Mathews P, Cohen NJ. Further studies of hippocampal representation during odor discrimination learning. Behav Neurosci 1989; 103: 1207-1216.

Ergorul C, Eichenbaum H. Essential role of the hippocampal formation in rapid learning of higher-order sequential associations. J Neurosci 2006; 26: 4111-4117.

Farrer M, Maraganore DM, Lockhart P, Singleton A, Lesnick TG, de Andrade M, West A, de Silva R, Hardy J, Hernandez D. Alpha-Synuclein gene haplotypes are associated with Parkinson's disease. Hum Mol Genet 2001; 10: 1847-1851.

Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991; 114: 2283-2301.

Feinstein A. The neuropsychiatry of multiple sclerosis. Can J Psychiatry 2004; 49: 157-163.

Filoteo JV, Maddox WT, Salmon DP, Song DD. Implicit category learning performance predicts rate of cognitive decline in nondemented patients with Parkinson's disease. Neuropsychology 2007; 21: 183-192.

Frank MJ, Seeberger LC, O'reilly RC. By carrot or by stick: cognitive reinforcement learning in parkinsonism. Science 2004; 306, 1940-1943.

Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de Leon M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B & International Psychogeriatric Association Expert Conference on mild cognitive impairment. Mild cognitive impairment. Lancet 2006 ; 367 : 1262-1270.

Kapadia MK, Ito M, Gilbert CD, Westheimer G. Improvement in visual sensitivity by changes in local context: parallel studies in human observers and in V1 of alert monkeys. Neuron 1995; 15: 843-856.

Kleeberg J, Bruggimann L, Annoni JM, van Melle G, Bogousslavsky J, Schluep M. Altered decision-making in multiple sclerosis: a sign of impaired emotional reactivity? Ann Neurol 2004; 56: 787-795.

Knowlton BJ, Mangels JA, Squire LR. A neostriatal habit learning system in humans. Science 1996; 273: 1399-1402.

Kovács I, Julesz B. Perceptual sensitivity maps within globally defined visual shapes. Nature 1994; 370: 644-646.

Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989; 46: 1121-1123.

Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33: 1444-1452.

Lezak M. Neuropsychological Assessment. Oxford University Press, Oxford, 1995.

Lisman JE, Otmakhova NA. Storage, recall, and novelty detection of sequences by the hippocampus: elaborating on the SOCRATIC model to account for normal and aberrant effects of dopamine. Hippocampus 2001; 11: 551-568.

Marek K, Jennings D. Can we image premotor Parkinson disease? Neurology 2009; 72 (7 Suppl): S21-6.

Mueller JC, Fuchs J, Hofer A, Zimprich A, Lichtner P, Illig T, Berg D, Wullner U, Meitinger T, Gasser T. Multiple regions of alpha-synuclein are associated with Parkinson's disease. Ann Neurol 2005; 57: 535-541.

Myers CE, Shohamy D, Gluck MA, Grossman S, Kluger A, Ferris S, Golomb J, Schnirman G, Schwartz R. Dissociating hippocampal versus basal ganglia contributions to learning and transfer. J Cogn Neurosci 2003; 15: 185-193.

Lycke J, Tollesson PO, Frisen L. Asymptomatic visual loss in multiple sclerosis. J Neurol 2001; 248: 1079-1086.

Oksman M, Tanila H, Yavich L. Brain reward in the absence of alpha-synuclein. Neuroreport. 2006; 17: 1191-1194.

Passamonti L, Cerasa A, Liguori M, Gioia MC, Valentino P, Nisticò R, Quattrone A,Fera F. Neurobiological mechanisms underlying emotional processing in relapsing-remitting multiple sclerosis. Brain. 2009 May 6. [Epub ahead of print]

Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E Mild cognitive impairment: clinical characterization and outcome. Arch Neurol 1999; 56: 303-308.

Peterson JW, Trapp BD. Neuropathobiology of multiple sclerosis. Neurol Clin 2005; 23: 107-129.

Polat U, Sagi D. The architecture of perceptual spatial interactions. Vision Res 1994; 34: 73-78.

Poldrack RA, Rodriguez P. How do memory systems interact? Evidence from human classification learning. Neurobiol Learn Mem 2004; 82: 324-332.

Poser CM, Paty DW, Scheinberg L, McDonald WI, Davis FA, Ebers GC, Johnson KP, Sibley WA, Silberberg DH, Tourtellotte WW. New diagnostic criteria for multiple sclerosis: guidelines for research protocols. Ann Neurol 1983; 13: 227-231.

Pula JH, Reder AT. Multiple sclerosis. Part I: Neuro-ophthalmic manifestations. Curr Opin Ophthalmol 2009; 20: 467-475.

Reichenberg A, Mill J, MacCabe JH. Epigenetics, genomic mutations and cognitive function. Cogn Neuropsychiatry 2009; 14: 377-390.

Roca M, Torralva T, Meli F, Fiol M, Calcagno M, Carpintiero S, De Pino G, Ventrice F, Martín M, Vita L, Manes F, Correale J. Cognitive deficits in multiple sclerosis correlate with changes in fronto-subcortical tracts. Mult Scler 2008; 14: 364-369.

Rogers RD. Neuropsychological investigations of the impulsive personality disorders. Psychol Med 2003; 33: 1335-1340.

Salmon DP, Filoteo JV. Neuropsychology of cortical versus subcortical dementia syndromes. Semin Neurol 2007; 27: 7-21.

Sarazin M, Berr C, De Rotrou J, Fabrigoule C, Pasquier F, Legrain S, Michel B, Puel M, Volteau M, Touchon J, Verny M, Dubois B. Amnestic syndrome of the medial temporal type identifies prodromal AD: a longitudinal study. Neurology 2007; 69: 1859-1867.

Schmitt-Eliassen J, Ferstl R, Wiesner C, Deuschl G, Witt K. Feedback-based versus observational classification learning in healthy aging and Parkinson's disease. Brain Res 2007; 1142: 178-188.

Schneider W, Eschman A, Zuccolotto A. E-Prime User’s Guide. Psychology Software Tools Inc., Pittsburgh, 2002.

Seger CA, Cincotta CM. The roles of the caudate nucleus in human classification learning. J Neurosci 2005; 25: 2941-2951.

Shohamy D, Myers CE, Grossman S, Sage J, Gluck MA. The role of dopamine in cognitive sequence learning: evidence from Parkinson's disease. Behav Brain Res 2005; 156: 191-199.

Siegert RJ, Taylor KD, Weatherall M, Abernethy DA. Is implicit sequence learning impaired in Parkinson's disease? A meta-analysis. Neuropsychology 2006; 20: 490-495.

Simioni S, Ruffieux C, Kleeberg J, Bruggimann L, Annoni JM, Schluep M. Preserved decision making ability in early multiple sclerosis. J Neurol 2008; 255: 1762-1769.

Simioni S, Ruffieux C, Kleeberg J, Bruggimann L, du Pasquier RA, Annoni JM, Schluep M. Progressive decline of decision-making performances during multiple sclerosis. J Int Neuropsychol Soc 2009; 15: 291-295.

Squire LR, Stark CE, Clark RE. The medial temporal lobe. Annu Rev Neurosci 2004; 27: 279-306.

Steer RA, Rissmiller DJ, Ranieri WF, Beck AT. Structure of the computer-assisted Beck Anxiety Inventory with psychiatric inpatients. J Pers Assess 1993; 60: 532-542.

Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003; 73: 1162-1169.

Swainson R, SenGupta D, Shetty T, Watkins LH, Summers BA, Sahakian BJ, Polkey CE, Barker RA, Robbins TW. Impaired dimensional selection but intact use of reward feedback during visual discrimination learning in Parkinson's disease. Neuropsychologia 2006; 44: 1290-1304.

Trivedi MA, Wichmann AK, Torgerson BM, Ward MA, Schmitz TW, Ries ML, Koscik RL, Asthana S, Johnson SC. Structural MRI discriminates individuals with Mild Cognitive Impairment from age-matched controls: A combined neuropsychological and voxel based morphometry study. Alzheimers Dement 2006; 2: 296-302.

Ueda K, Saitoh T, Mori H. Tissue-dependent alternative splicing of mRNA for NACP, the precursor of non-A beta component of Alzheimer's disease amyloid. Biochem Biophys Res Commun 1994; 205: 1366-1372.

Vleugels L, Lafosse C, van Nunen A. Visuoperceptual impairment in MS patients: nature and possible neural origins. Mult Scler 2001; 7: 389-401.

Wishart H, Sharpe D. Neuropsychological aspects of multiple sclerosis: a quantitative review. J Clin Exp Neuropsychol 1997; 19: 810-824.

Xia Y, Saitoh T, Ueda K, Tanaka S, Chen X, Hashimoto M, Hsu L, Conrad C, Sundsmo M, Yoshimoto M, Thal L, Katzman R, Masliah E. Characterization of the human alpha-synuclein gene: Genomic structure, transcription start site, promoter region and polymorphisms. J Alzheimers Dis 2001; 3, 485-494.

Yechiam E, Busemeyer JR, Stout JC, Bechara A. Using cognitive models to map relations between neuropsychological disorders and human decision-making deficits. Psychol Sci 2005; 16: 973-978.

Yin HH, Knowlton BJ. The role of the basal ganglia in habit formation. Nat Rev Neurosci 2006; 7: 464-476.

Zakzanis KK. Distinct neurocognitive profiles in multiple sclerosis subtypes. Arch Clin Neuropsychol 2000; 15: 115-136.