The Automatic Brain

Studies on practice and brain function in healthy subjects and patients with schizophrenia

Tamara Ruth van Raalten

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Studies on practice and brain function in healthy subjects and patients with schizophrenia

Het Automatische Brein
Studies naar het effect van training op hersenfunctie bij gezonde mensen en patienten met schizofrenie

(met een samenvatting in het Nederlands)

Proefschrift

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Prof. dr. R.S. Kahn

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Chapter 1
Introduction

General Introduction

'Practice makes perfect' is a well known saying and refers to the beneficial effect of practice on performance and our limited capacity to process large amounts of information. The behavioral effects of practice are well-understood, however the neural mechanisms behind these behavioral changes remain largely unknown. This thesis aims at better understanding of the neurophysiology behind practice and its effect on our limited processing capacity. The main objective of this work is to investigate how practice changes brain activation in healthy individuals and how this can contribute to improved processing capacity.

People suffering from a psychiatric illness seem to have serious problems in processing the abundance of information present in our environment. In particular, patients with schizophrenia seem to be more limited in the amount of information they can process. The neural basis of limited processing capacity in schizophrenia is not clearly understood. A second objective in this thesis is to investigate whether decreased processing capacity in schizophrenia can be explained by a failure to relieve processing demands with practice.

Automatization

Background

Novel tasks are typically effortful, slow, prone to errors, easily interrupted by other tasks and need a considerable amount of control. Practice however allows automatic behaviors to develop gradually with repeated exposure. These behaviors typically involve quick and stereotyped reactions that are difficult to suppress, but at the same time allow other tasks to be executed as well. The dual-processing theory of information processing [1] attempts to account for the qualitative different profiles of automated and controlled performance. This theory proposes the concept of automatization, which refers to the transition from controlled to automated processing that gradually develops with experience.

An important prerequisite for automatization to develop is that the association between a stimulus and its contingent response remains constant over the course of practice. If stimulus-response associations change frequently there is little behavioral improvement, even after extended periods of practice [1]. Behavioral studies have demonstrated the profound decreases in reaction times and improvements in accuracy as a result of automatization [1]. In addition, automatization has been shown to be important for dual-task performance [2]. Together, this suggests an important role for automatization in improving our capacity-limited processing system with practice.

This thesis builds on previous studies that investigated the neural basis of automatization and its putative role in human processing capacity [3,4]. In these studies was suggested that working memory plays an important role in the development of automatization and its beneficial effect on limited processing capacity.

Working memory

Many daily routines, depend on the ability to actively remember and modify the goals we are pursuing [5]. For instance, when shopping for groceries you may keep in mind a list of products you want to buy. At the same time you need to keep track of the products in your basket, to avoid double purchases. This requires to keep information active that is no longer physically present, and that has to be accessed and updated frequently. The ability to do this is known as working memory.

Baddeley [6] introduced the concept of working memory as a cognitive system comprising independent modules for storage and executive control. According to this idea, the working memory system involves a central executive that is responsible for the coordination of limited attentional capacity and regulation of the information stored in the visuospatial and phonological memory buffers. The first studies investigating the neural basis of working memory involve electro-physiological studies in monkeys [7,8]. They demonstrated that prefrontal neurons remain active in the period between a presented cue and the execution of a contingent response. This work indicated an important role of the prefrontal cortex in working memory. More recently, neuroimaging studies of working memory have linked Baddeley's 'central executive' to a distributed network of brain regions, including the dorsolateral prefrontal cortex [5,9]. Other important regions in the working memory network involve the parietal cortex, the anterior cingulate, the premotor cortex and the cerebellum. Together these regions are important for active maintenance of task goals, and for using these goals to control and adapt behavior under ambiguous or novel circumstances [5,10,11,12].

Neural correlates of automatization

Automatization not only improves performance, but also reduces activity across regions in the working memory network [3]. This is consistent with a variety of neuroimaging studies that report decreases in brain activity as a result of practice [13,14,15,16,17]. The decline in activity is most prominent in regions that are thought to underlie cognitive control mechanisms. This has been postulated to

serve as a 'scaffolding' mechanism for learning new tasks [18]. In a previous study, it was shown that the drop in activity in working memory regions after practice was linked to how well one could concurrently perform an additional cognitive task [4]. This suggests an important role for working memory in the development of automatization and the ability to improve processing capacity with practice. The nature of activity changes in working memory regions and how they contribute to processing capacity however are still poorly understood.

An important question relates to the progress of activity changes over the course of practice. Automatization involves distinct behavioral components related to perceiving and responding to stimuli. It is unclear whether automatization follows similar courses in activity decreases for these behavioral components.

A second issue pertains to whether decreases in brain activity after practice are accompanied by compensatory changes in activity elsewhere in the brain. Some tasks allow for a change in cognitive strategy with practice [13]. Novel and practiced performance of these tasks activate different brain regions. This indicates that practice can induce a shift in activity from working memory to other regions in the brain supporting practiced performance [15,19,20]. Alternatively, for other tasks the same set of regions is active before and after practice. With practice activity shifts from one region to another within the same network. This indicates that practice can diminish contributions of initially active regions while other regions in the same network gain in importance for task execution [5,13]. There is no clear indication that automatization leads to enhanced recruitment of other brain regions, leaving open the question which brain regions perform the task after practice.

Schizophrenia

Clinical profile of schizophrenia

Schizophrenia is a chronic mental illness, with a lifetime risk of developing the disorder of about one percent [21]. The disease has a severely incapacitating effect on patients, affecting cognition, emotion and social behavior. Schizophrenia was first described by Emil Kraepelin in 1896 [22] with the classifications of a group of mental disorders as 'dementia praecox'. Kraepelin described patients with this disorder as having delusions and hallucinations. Later Eugene Bleuler renamed this disorder in the group of schizophrenias [23]. With this new term he tried to better classify the disorder, which he believed was characterized by a separation between the emotional part and the rational part of the brain.

The clinical profile of schizophrenia is heterogeneous, with a large variety in symptoms and in the degree of deterioration. Symptoms are generally divided into

positive, negative and disorganized profiles [24]. Positive symptoms refer to abnormal behavior that is not present in healthy individuals, such as delusions and hallucinations. By contrast, negative symptoms indicate a loss of function, emotion or movement. Disorganized symptoms typically involve disrupted speech, thought and behavior. The Diagnostic and Statistical Manual of Mental Disorders-IV (DSM) [25] is the most commonly used classification system for psychiatric symptoms. According to the DSM criteria for schizophrenia, at least some of these symptoms should be present for at least six months to establish a diagnosis. The heterogeneity on the symptom level may obscure more homogeneous deficits in the cognitive spectrum [26,27]. A cognitive deficit may lead to various expressions of clinical symptoms in different individuals.

Working memory dysfunction in schizophrenia

Currently, cognitive deficits are considered a core characteristic of schizophrenia that may be at the basis of clinical symptoms [27]. Patients with schizophrenia display a wide range of deficits, including problems with attention, memory and 'executive functioning' [28]. A fundamental deficit in working memory may underlie this wide range of cognitive problems in schizophrenia [26].

Patients with schizophrenia typically perform poor on tests of working memory function, especially when task load (e.g. the number of items that has to be memorized) is increased. Neuroimaging studies show that patients with schizophrenia activate the same set of regions during working memory performance, but exhibit abnormal levels of brain activity [29,30,31,32,33,34,35]. When task performance of patients is within normal boundaries, levels of brain activity are relatively increased [29,30,34]. This shows that patients inefficiently recruit working memory regions, as brain activity in patients peaks with lower task load than in healthy control subjects. However, when patients with schizophrenia perform poorly on working memory tasks they show decreased levels of activity compared to control subjects [36]. This decline in activity indicates that working memory is no longer engaged when task load is beyond their processing capacity. It was therefore postulated that patients cannot process as much information as efficiently as healthy subjects and consequently reach the limitations of their capacity sooner than healthy individuals [37]. Although this research links severely limited processing capacity in schizophrenia to working memory dysfunction, its neural basis still remains poorly understood.

Functional Magnetic Resonance Imaging

Background

In the early nineties the first studies were published that activation of the brain could be non-invasively visualized with a Magnetic Resonance Imaging (MRI) scanner. It was shown that the MR signal close to blood vessels and in perfused brain tissue decreased with a decrease in blood oxygenation. Because the oxygenation level of blood was used as a natural agent for detecting brain activation, this technique was called 'Blood Oxygenation Level Dependent' functional MRI (BOLD-fMRI) [38]. fMRI allows researchers in cognitive neuroscience to visualize brain activity related to performance of a cognitive task. This mapping of brain activity occurs with a moderate time resolution (i.e. seconds) and a high spatial resolution (i.e. millimeters). fMRI is a noninvasive method. This is a major advantage compared to other techniques, as there is no need for injections of radioactive ligands such as is used in PET and SPECT imaging. A downside of fMRI however is its sensitivity to movement. Subjects are therefore not allowed to move their body and especially their head during a scan session. Although movement can partially be corrected for by realignment of the functional time-series data, it can affect the outcome of the statistical analysis and cause activation artifacts. The restriction of movements also limits the types of paradigms suitable for fMRI, as manual responses are required to measure behavioral performance. Another downside is the noise generated by the MRI scanner. This limits the possibility to present auditory stimuli and to collect verbal responses.

How does BOLD-fMRI work?

Functional MRI images are obtained using an MRI scanner. This is a large magnet with a field strength of around 1,5 to 7 Tesla for use in research with human subjects. The acquisition of scans typically involves continuous series of scans of the brain, each lasting between one and a few seconds. A scan consists of several thousand data points. Each point is derived from a cube of brain tissue (also called a voxel). The series of scans is stored as a time-series of 3D volumes. Each voxel in the volume is associated with a series of intensity values. This represents the fMRI signal. The basis of this signal originates in protons. When a subject is placed inside the scanner (i.e. the magnetic field) a slight minority of the protons will align with the field (B_0). The signal measured by an MRI scanner is based on the emission of electromagnetic radiation from the nuclei of these protons (hydrogen atoms), which are excited by a radio frequency (RF) pulse. After excitation the protons will return to their original state. The type of pulse sequence determines the set of factors that affect the basis of the signal. Some sequences are differentially

sensitive to the type of tissue the protons are in, e.g. gray matter, white matter, cerebrospinal fluid or blood. In fMRI, the pulse sequence is sensitive to blood dynamics; blood flow, blood volume and oxygenated state. Transient changes in blood dynamics affect the fMRI signal. This can be detected by a receiver coil, which is placed very close to the head of the subject. fMRI thus enables visualization and measurement of transient changes in blood dynamics in the brain.

Neural activity and BOLD-fMRI

FMRI images are referred to as images representing brain activation. It is important to realize however that BOLD-fMRI is not a direct measurement of neuronal activity. The BOLD signal measures relative changes in the distribution of (de)oxygenated blood. Changes in brain activation are accompanied by changes in blood flow. This causes an increase in the level of oxygen in the blood in a particular brain region. Oxygenated hemoglobin is diamagnetic and thus exerts little effect on the regional magnetic field. By contrast, deoxygenated blood is paramagnetic, which disturbs the regional magnetic field. The proportion of deoxygenated and oxygenated hemoglobin constitutes the basis of the fMRI signal. The exact relationship between neuronal activity and fMRI signal, also known as the neurovascular coupling, is quite complicated. This involves multiple vascular, metabolic and neural processes, some of which are still poorly understood. However, research in this field has substantially increased knowledge of the relationship between fMRI signal and underlying neural activity. With simultaneous recordings of the fMRI signal and various measures of the electrical activity of neurons in monkeys during visual stimulation it has been shown that the fMRI signal is predominantly determined by local field potentials (LFP) [39]. LFP are slowly varying gradient potentials, arising from the input of the dendrites of neurons and associated with local information processing. This work has major implications for the interpretation of fMRI results. Considering these observations, one can with greater confidence ascribe the BOLD signal to a change in local field potentials in post-synaptic neurons. It should be cautioned however, that the signal-to-noise ratio of neural recordings direct from the brain are typically much greater than the accompanying BOLD signal. This means that the absence of an fMRI signal does not necessarily indicates that neural activity is absent in a particular brain region.

Transcranial Magnetic Stimulation

Background

Over the past decades functional MRI has proved to be a powerful approach to detect changes in brain activity associated with cognitive functions. A shortcoming

of this technique however is that the results are based on correlations between the modeled cognitive event and the observed BOLD response. It is therefore not possible to infer causal relationships between regional function and behavior. A new method was introduced in 1985 by Barker et al. [40] that involved direct but noninvasive stimulation of the human cortex using a pulsed magnetic field. Magnetic stimulation can temporarily disrupt cortical activation and hence allows investigation of causal brain-behavior relationships. This method, named Transcranial Magnetic Stimulation (TMS) is increasingly becoming popular in cognitive neuroscience to examine direct contributions of brain regions to a particular cognitive function [41].

How does TMS work?

TMS is based on Faraday's principles of electromagnetic conduction [42]. A pulse of current flowing through a coil of wire generates a magnetic field. A change in the magnetic field strength will induce a second current in a nearby conductor. The rate of change in the field determines the size of the induced current. In a TMS investigation a stimulation coil is held over a participant's head. A brief current runs through the coil generating a magnetic field that passes through the subject's scalp and skull to the underlying cortex. This time-varying magnetic field induces a current in the participant's brain which stimulates the neural tissue. Magnetic stimulation can be applied in single pulses or in a series of pulses, known as repetitive TMS or rTMS.

TMS can be used in conjunction with other neuroimaging techniques such as fMRI. For instance, functional maps of brain activity can be coregistrated to the head to guide TMS coil location to regions of interest for assessment of regional function and its timing [41]. TMS can also provide functional information of neural activity associated with hemodynamic changes measured with fMRI or regional blood flow measured with PET [41,43]. Knowledge about the exact mechanism by which TMS induces its effect on neural activity however is limited [41,42]. It is not yet possible to determine the depth of stimulation in the brain or the exact spatial resolution. Also it is not yet known which neural elements are most sensitive to stimulation in a particular area. Nevertheless most TMS investigations assume that stimulation of a particular area disrupts neural activity, which consequently interferes with cognitive processing. If this causes a deterioration in performance this indicates that the stimulated brain area is critical for behavior.

Outline of the thesis

The research in this thesis builds on previous work that suggests an important role for working memory in the development of automatization and the ability to improve processing capacity with practice. As mentioned before, the nature of these contributions and how they relate to processing capacity are still poorly understood. The first objective of this work is to investigate how automatization changes brain activation in healthy individuals to better understand how this may increase processing capacity. The studies on automatization in healthy volunteers are described in **chapters 2-4**. A second objective of this thesis is to investigate whether automatization can explain limited processing capacity in schizophrenia. **Chapter 5** describes the study where a putative link between automatization and processing capacity in schizophrenia is examined. Finally, **chapter 6** provides with a summary and general discussion.

Research Questions

Much remains unknown about the trajectory of activity decreases induced by practice for distinct behavioral components of automatization. The first question in this thesis pertains to the course of function-specific effects of automatization related to encoding and response selection. In **chapter 2** an event-related fMRI design is used to isolate effects of practice on brain function related to encoding and response selection. Based on the domain-general effects (i.e. task-independent) of practice reported in previous studies we hypothesize that the course of activity decreases in the working memory system is the same for encoding and response selection, while both may show independent courses in activity changes in function-specific networks (i.e. visual or motor regions).

A second question concerns whether automatization induces compensatory increases in activity in other brain regions, as activity decreases in working memory regions. In **chapter 3** we investigate three possible scenarios. First we investigate if practice leads to a change in activated brain areas, which would induce a shift from one set of regions to another set. Secondly, we examine if activity shifts within the initially active network(s); from cognitive control regions to perceptual and motor systems. Third, we investigate the scenario that the same networks are involved from beginning to the end of training, and the only change is an improvement of the efficiency with which regions communicate; in which case brain activity would decline coherently in all regions. In this study we investigate changes in brain activity following practice of a working memory task in a large sample of healthy subjects. Whole-brain fMRI scans are acquired on a 3T scanner with an 8-channel

head coil to obtain a high sensitivity for signal changes. In addition, we make use of the variability in practice-induced effects on brain activity among subjects, reflecting the rate or speed at which automaticity is achieved, to assess changes within and across networks. We hypothesize that regions responding to practice 'in concert' are correlated in their degree of signal change and thus will be part of the same network. Importantly, this approach allows to assess potential shifts of brain activity within networks, following practice. If particular regions gain significance with practice, one would expect those to correlate negatively with others that decrease in their involvement, even if activity declines in all regions.

Previous work has indicated the profound effects of automatization on brain activity in working memory regions. Yet much remains unknown about the functional importance of working memory in automatization. The third question in this thesis involves the critical role of working memory in establishing automatization. In **chapter 4** a combination of fMRI and transcranial magnetic stimulation is used to assess the functional contributions of two important working memory regions to the development of automatization. A cognitive paradigm is used to assess automatization, including a novel task with continuously changing information, a practiced task with consistent information and a control task with overlearned information. Subjects first participate in an fMRI session to localize the dorsolateral prefrontal cortex and parietal cortex on an individual basis, where activity decreases with practice. In separate sessions following fMRI, subjects perform the automatization paradigm while receiving stimulation over the left lateralized dorsolateral prefrontal and parietal cortices. We hypothesize that working memory enables the development of automatization. We expect that brief interruption of activity in these regions will disrupt retrieval of information that is kept active in mind. Based on our previous work we expect that this will significantly affect practiced performance, but not as much as for novel performance. We assume that the control task with overlearned information does not involve working memory retrieval and therefore will not be affected by stimulation. Hence, this allows us to test whether automatic behaviors become independent of cognitive control. Nonetheless, even when overlearned, performance in an experimental setting depends on an internally represented context of a task instruction in working memory. If working memory enables automatization by providing this context, it is also possible that brief disruption of working memory will affect performance on the control task.

The fourth question concerns whether inefficient brain function and limited processing capacity in schizophrenia can be explained by a deficit in automatization. In **chapter 5**, the results are presented of an fMRI study where a group of patients with schizophrenia and a group of healthy individuals were examined on an automatization paradigm and a dual-task that was performed outside fMRI. Assuming that patients with schizophrenia exhibit inefficient brain function, we hypothesize that they show a smaller decrease in working memory activity with practice compared to the control group. Based on the idea that patients have more limited capacity than controls, we also expect that patients with schizophrenia perform worse on a dual-task. Finally, we hypothesize that an inability to decrease activity with practice in schizophrenia, is related to reduced capacity to accommodate processing of two tasks simultaneously.

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Chapter 2
Practice induces function-specific changes in brain activity

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Abstract

Background

Practice can have a profound effect on performance and brain activity, especially if a task can be automated. Tasks that allow for automatization typically involve repeated encoding of information that is paired with a constant response. Much remains unknown about the effects of practice on encoding and response selection in an automated task.

Methodology

To investigate function-specific effects of automatization we employed a variant of a Sternberg task with optimized separation of activity associated with encoding and response selection by means of m-sequences. This optimized randomized event-related design allows for model free measurement of BOLD signals over the course of practice. Brain activity was measured at six consecutive runs of practice and compared to brain activity in a novel task.

Principal findings

Prompt reductions were found in the entire cortical network involved in encoding after a single run of practice. Changes in the network associated with response selection were less robust and were present only after the third run of practice.

Conclusions/significance

This study shows that automatization causes heterogeneous decreases in brain activity across functional regions that do not strictly track performance improvement. This suggests that cognitive performance is supported by a dynamic allocation of multiple resources in a distributed network. Our findings may bear importance in understanding the role of automatization in complex cognitive performance, as increased encoding efficiency in early stages of practice possibly increases the capacity to otherwise interfering information.

Introduction

Practice can have a profound effect on performance and underlying brain activity especially if a task can be automated. Tasks that allow for automatization typically involve repeated encoding of information that is paired with a constant response [1]. While previous studies have demonstrated the profound effects of automatization on working memory [2-4], much remains unknown about how automatization affects function-specific effects related to encoding and response selection in an automated task.

Decreases in working memory activity after practice have been reported in a wide range of cognitive tasks; such as verb generation [5], mirror reading [6,7], delayed response tasks [4,8] and motor sequence learning [9,10] and have been interpreted in terms of reduced demands on domain-general cognitive control resources that support early learning or novel task performance [2,11]. It has also been shown that practice-induced activity decreases are closely related to one's capacity to concurrently perform an additional cognitive task [3]. Better understanding of the mechanism behind automatization may explain how automatization can contribute to complex cognitive performance such as dual tasking.

To investigate function-specific effects of automatization we build upon our previous work in which we examined automatization by means of a Sternberg Task [12]. Performance of this task involves an encoding phase during which information is presented that is briefly memorized, and a response phase including a probe stimulus that requires a decision whether it matches the previously presented information or not. The blocked design we employed in our previous studies [3,4] did not allow investigation of function-specific changes in brain activity associated with encoding and response selection. In addition, it was not possible to assess changes in brain activity over the course of practice. To investigate function-specific changes in brain activity as a result of practice we used a pseudo-random event-related design in which encoding and response phases in a Sternberg task were controlled by means of m-sequences [13]. This is a novel method that allows for model-free measurement of BOLD signals and optimal separation of BOLD signals of rapidly displayed stimuli. Brain activity was measured at six consecutive runs of practice to measure changes in activity during encoding and response selection over the course of practice. Based on current theories of practice we hypothesize that the course of activity decreases in brain areas associated with working memory function is the same for encoding and response selection, while both functions may show independent courses in activity changes in function-specific networks.

Methods

Participants

Eleven right-handed subjects (M/F 6/5, mean age 33.0 (±2.9)) participated in the study. Before the functional MRI (fMRI) session, all subjects gave written informed consent to participate in the study, which was approved by the Intramural Review Board (IRB) of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health under protocol #00-N-0082. Participants were provided with earplugs to protect their hearing from the acoustic noise generated by the MRI gradient system.

Task

We based the task used in our study on a Sternberg task-paradigm [12] (figure 1). This task has been used extensively in fMRI studies and it has been shown to reliably activate regions associated with working memory [4,14-22]. It allows for trial-by-trial measurement of the level of performance (reaction time (RT) and error rate) to verify that subjects are executing the task as required.

In our experiment participants were instructed to memorize either one or five letters that were visually presented (memory set). To increase similarity between stimuli, all of the letters used in the task were consonants. The memory set was followed by presentation of a probe stimulus. Participants were instructed to decide as fast as possible whether the probe belonged to the memory-set (target) or not (non-target). Tasks were presented in eight runs of approximately five minutes with each run containing 68 trials of 4000 ms duration. Each trial started with an encoding phase during which the memory set was presented for 1500 ms. This was followed by a delay period of 1500 ms in which a fixation cross was displayed. The brief delay was followed by the response phase, which involved the presentation of a probe stimulus for 500 ms followed by another fixation-cross for 500 ms (figure 1). In the first two runs, the memory sets for each trial were randomly generated out of ten consonants. Because memory sets were novel in each trial these runs are denoted “novel task” (NT1 and NT2). In the following six runs all trials used the same fixed memory set. These runs are denoted “practiced task” (PT1 – PT6). The constant stimulus-response associations in’ PT are thus practiced in six runs allowing automatization to be established over time. The stimuli in PT were chosen from a different set of consonants than NT to prevent interference. The first NT run was used to select regions of interest (ROI) representing brain areas involved with

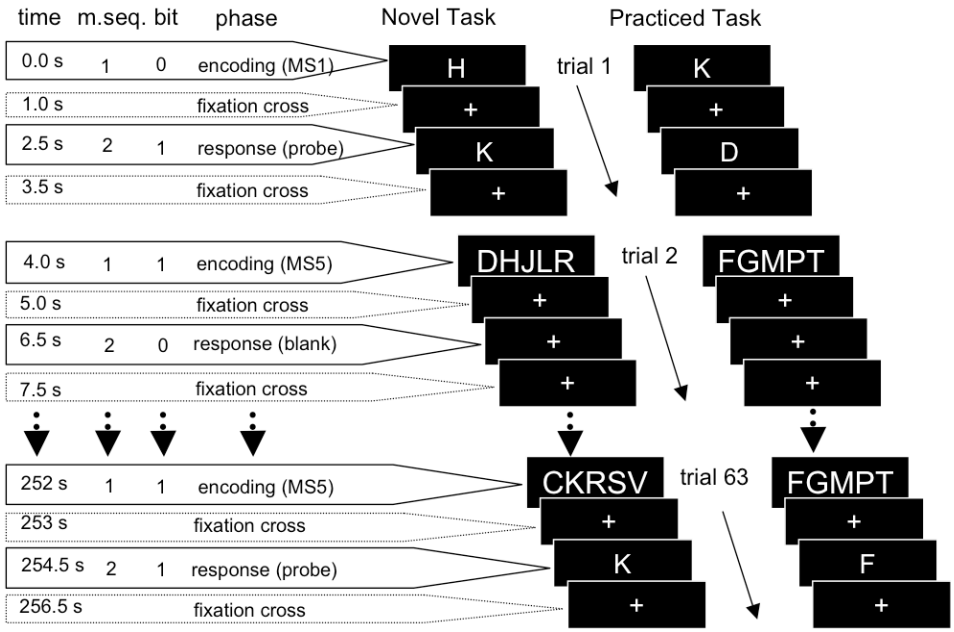

Figure 1. Cognitive Paradigm

The timeline is shown for the cognitive experiment. Two m-sequences (m.seq.) of 63 bits control the encoding phase (1) and the response phase (2). Each trial starts with the encoding phase followed by a brief delay and the response phase. Where bits are 0 (baseline); memory sets with 1 letter (MS1) are presented during the encoding phase and blank trials are presented during the response phase. Where bits are 1; 5-letter memory sets (MS5) are presented during the encoding phase and a probe stimulus during the response phase. In the novel task the letters presented during the encoding phase were different in each MS1 and MS5 trial. In the practiced task, the same five letters were repeated in each MS5 trial.

encoding and response phases. The second NT run was used to establish signal level for NT performance in the ROI's, which was used as reference for comparison of activity during PT.

The M-sequence

A 63 element binary m-sequence consisting of 32 positive and 31 negative bits was used to control the timing of the presentation of the task stimuli [13]. The primary sequence was used to control the encoding phase. Each bit of the sequence

belonged to one trial. If the sequence was negative then a one-letter memory set was presented (baseline condition for encoding phase). If the sequence was positive a five-letter memory set was presented. The sequence was shifted nine bits to create an independent but related sequence to control the response phase. If this sequence was negative no stimulus was presented (baseline condition for the response phase). If this sequence was positive a probe letter was presented. The m-sequence was extended by inserting a replica of the last five bits at the beginning of the sequence to allow removal of the initial BOLD transient, yielding an extended sequence of 68 bits. The uneven runs used the primary versions of the sequence, while the even runs used an inverted version (positive and negative bits switched).

Functional MRI

Data was acquired on a 3T GE MR system. Image signal-to-noise ratio (SNR) was boosted by employing multi-channel MRI with a custom-built helmet-type 16-channel receive array that fits tightly around the head [23], connected to a custom-built 16-channel MRI receiver [24]. A single-shot rate-2 sensitivity-encoded (SENSE) [25] echo-planar imaging (EPI) [27] was employed for fMRI acquisition. The EPI matrix size was 96 by 72, and the field of view (FOV) 224 mm^2, leading to a nominal in plane resolution of 2.3 mm^2. Slice thickness was 2.0 mm, with a slice gap of 0.3. Echo time (TE) was 32 ms, repetition time (TR) was 2000 ms, and flip angle 90 degrees. Tasks were presented in three runs of 290 functional scans with approximately one-minute period in between. A video projector presented stimuli on a small screen attached to the head-coil in the scanner. Participants could see the screen via a mirror also attached to the head-coil. Subjects were instructed to respond to each probe as quickly as possible by ion of the pushing a button with the index finger of the right hand to targets or with the middle finger of the right hand to non-targets.

Data preprocessing and statistical analysis

All fMRI data were analyzed off-line on a multimode Linux/PC reconstruction cluster using IDL™. Image reconstruction was performed as described previously and included direct Fourier transform of the ramp-sampled data, EPI ghost correction using a navigator echo [26] and SENSE unfolding as well as image intensity correction based on coil sensitivity reference maps derived from the array data itself [27].

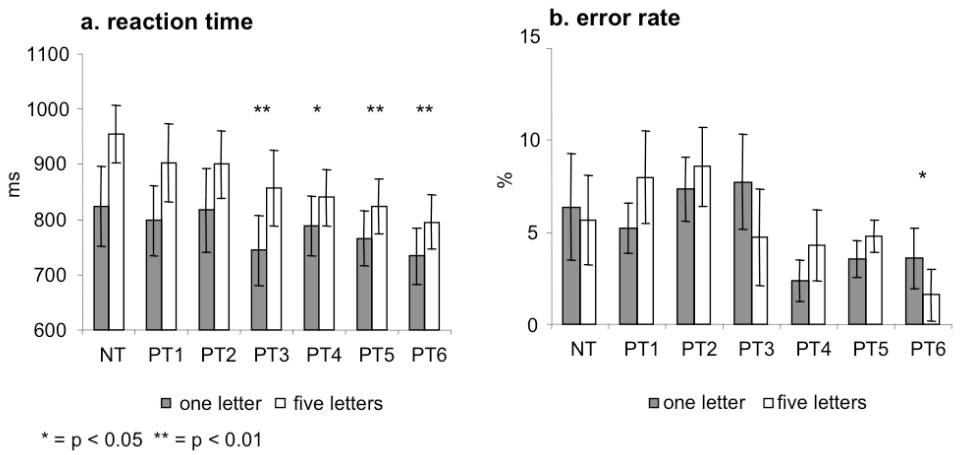

Figure 2. Performance

a. reaction time in milliseconds (left) and b. % error rate (right) for trials with one-letter memory sets and five-letter memory sets. Performance measures are displayed for novel task (NT) and each practice run (PT1-PT6).

First and second order trends were removed from the fMRI signal per voxel. After this, an outlier test was performed, which removed all time points larger than three standard deviations away from the mean. Trend correction for first and second order was repeated after outlier correction. The input function (primary m-sequence) was balanced to have a mean of zero. Because there were two scans per trial, the sequence was interleaved with zeros in order to have a sequence length equal to the number of scans. Analysis of brain activation was performed by calculating the cross-covariance of this input function with the image intensity on a voxel by voxel basis, for all 63 temporal shifts [13] by multiplication in Fourier domain. Covariance values were transformed into t-values by dividing each value by an estimate of the temporal noise level. The temporal noise value in the fMRI signal was estimated by calculating the temporal standard deviation in covariance values over shifts 20 to 63, where no covariance peaks related to our experimental paradigm were present. Subsequently, the correlation maps for ten shifts (or a 20 s period) following the expected correlation peak were spatially normalized to the MNI305 standard brain, as it was expected that the BOLD curve would be fully covered by this segment.

Table 1. Practice and Performance

a. reaction time	**overall (MS1 and MS5)**		**difference (MS1 vs MS5)**	
contrast	F(1,10)	p	F(1,10)	p
multivariate	**5.40***	**<0.01**	1.67**	0.17
NT-PT1	3.02	0.11	0.06	0.46
NT-PT2	3.16	0.11	2.33	0.16
NT-PT3	**17.5**	**<0.01**	0.25	0.63
NT-PT4	**6.37**	**0.03**	4.68	0.06
NT-PT5	**11.6**	**<0.01**	2.39	0.15
NT-PT6	**15.7**	**<0.01**	4.16	0.07

* df = (2.8,28.4) ** df = (4.3, 43.2) (Huynh-Feldt corrected)

b. error rate	**overall (MS1 and MS5)**		**difference (MS1 vs MS5)**	
contrast	F(1,10)	p	F(1,10)	p
multivariate	**2.91***	**0.05**	0.008**	0.93
NT-PT1	0.08	0.78	2.67	0.14
NT-PT2	1.23	0.30	0.79	0.40
NT-PT3	0.01	0.94	0.41	0.54
NT-PT4	3.10	0.11	1.59	0.24
NT-PT5	1.23	0.30	0.82	0.39
NT-PT6	**5.91**	**0.04**	0.09	0.77

* df = (2.8,28.4) ** df = (4.3, 43.2) (Huynh-Feldt corrected)

a. reaction time (top) and b. error rate (bottom). Measures were tested over all runs (1st row) and between novel task (NT) and each practice run (PT), across one-letter (MS1) and five-letter (MS5) memory set trials (1st column) and for MS1 trials vs. MS5 trials (2nd column). Significant results are displayed in bold.

These maps were transformed into group activity maps by testing the value in each voxel against zero over all subjects. Two covariance peaks were expected: The first peak related to the encoding phase with an onset at shift zero, the second peak related to the response phase with an onset at shift nine.

Regions of interest (ROI) for encoding phase and response phase were created by combining neighboring voxels that reached a threshold of $t > 3.71$ ($p < 0.0001$ uncorrected) in the group map of the NT1, at shift 2 and at shift 11 (corresponding to the fMRI signal at 4000 ms after PS presentation). These signals were analyzed using multivariate analysis (repeated measurements executed with SPSS ™ 11.0).

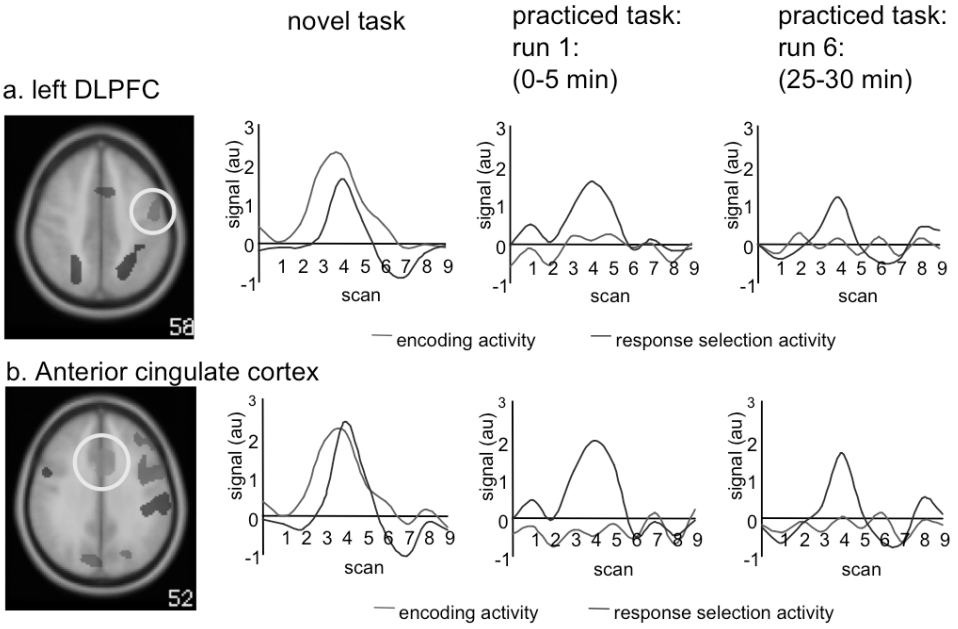

Figure 3. Heterogeneous effect of practice on regions activated by both encoding and response selection
Example of bold activity (arbitrary units) in regions activated by both phases: a. left DLPFC (top) and b. anterior cingulate cortex (bottom) during the novel task (left), after one practice run (middle) and six practice runs (right); showing the heterogeneous effects of practice for encoding and response selection.

Results

Performance

We examined the behavioral effect of practice for changes in performance over all practice runs and by comparison of each practice run with NT2. Overall task performance was averaged over responses in one-letter memory set (MS1) and five-letter memory set (MS5) trials and for the difference in performance between responses in MS1 trials and responses in MS5 trials. For reaction time (RT) there was a significant performance improvement over all runs ($F=5.40$, $p<0.01$). Practice runs three through six showed a significant improvement compared to the novel task (see table 1 and figure 2). There was also a significant overall improvement in error rate ($F=2.91$, $p<0.05$) and a significant improvement in the sixth run of practice compared to NT ($F=5.91$, $p<0.04$). The differences between MS5 and MS1 in RT and error rate were not significantly changed by practice (see table 1 and figure 2).

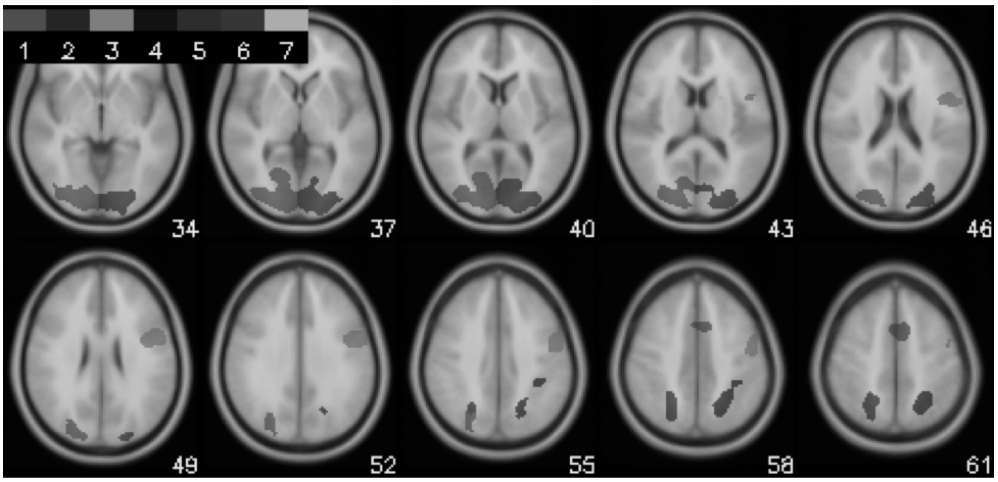

Figure 4. Encoding ROI's

ROIs showing activity related to encoding. The numbers in the color bar refer to the encoding phase ROIs (E1-E7) in table 2.

Table 2. Encoding ROI's

ROI	Region	abbr.	BA	NV	x	y	z	tmax
E1	right calcarine sulcus	Rcalc	18	2886	14	-94	2	12.87
E2	left calcarine sulcus	Lcalc	18	2334	-12	-94	-2	12.75
E3	dorsolateral prefrontal cortex	Ldlpfc	9	1044	-56	-2	42	6.94
E4	left superior parietal cortex	Lspc	7	693	-24	-58	46	6.46
E5	right superior parietal cortex	Rspc	7	475	24	-50	46	4.06
E6	Anterior cingulate cortex	SMA	24	472	-4	2	58	8.36
E7	left putamen	Lput	Na	112	-22	2	-2	3.53

Description of ROIs showing activity correlated with encoding phase. (Abbreviations: E = encoding; BA=Brodmann Area; NV=number of voxels in ROI (size of ROI); x, y, z = MNI coordinates of voxel with highest t-value in ROI; tmax: maximum t-value in ROI).

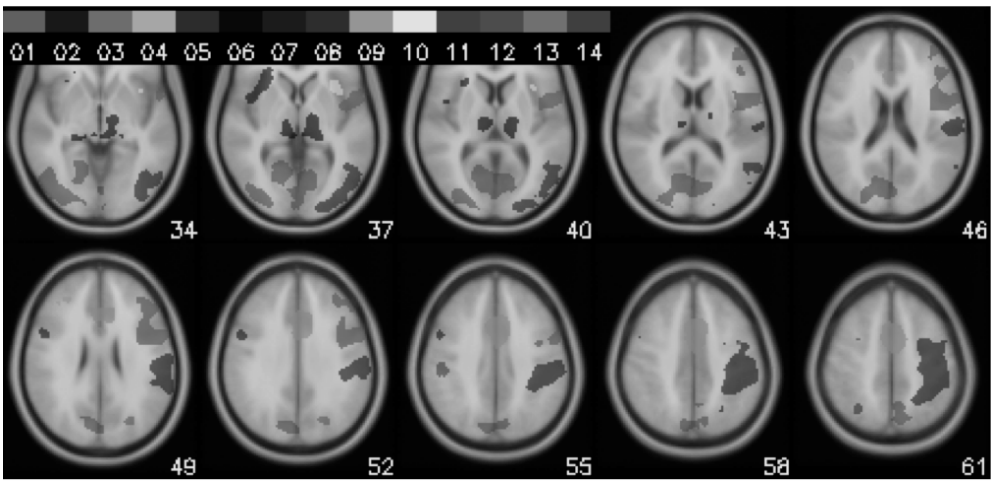

Figure 5. Response Selection ROI's

ROIs showing activity related to the response selection. The numbers in the color bar refer to the response selection ROIs (RS1-RS14) in table 3.

Table 3. Response Selection ROI's

ROI	Region	abbr.	BA	NV	x	y	z	tmax
RS1	right occipital cortex	Rocc	18/19	4054	18	-64	8	7.42
RS2	left primary sensorimotor cortex	Lpsmc	4	3723	-38	-22	54	11.84
RS3	left dorsolateral prefrontal cortex	Ldlpfc	9/46	2527	-52	2	14	8.05
RS4	anterior cingulate cortex	ACC	32	2105	-4	-4	56	10.11
RS5	left occipital cortex	Locc	19	1457	-52	-68	6	6.8
RS6	Thalamus	thal	Na	874	-14	-26	2	6.21
RS7	right operculum	Roper	45	348	30	20	0	5.03
RS8	right dorsolateral prefrontal cortex	Rldpfc	46	152	52	2	38	5.49
RS9	right ventrolateral prefrontal cortex	Rvpfc	47	149	40	30	20	3.94
RS10	left operculum	Loper	45	132	-40	12	4	5.54
RS11	right precentral gyrus	Rpcg	6	99	28	-2	54	6.48
RS12	right postcentral gyrus	Rpocg	2	96	54	-24	40	5.85
RS13	left cuneus	Lcun	19	95	-24	-76	30	6.06
RS14	right superior parietal cortex	Rspc	7	81	30	-58	48	4.13

Description of ROIs showing activity correlated with response phase. Abbreviations: RS = response selection; BA=Brodmann Area; NV=number of voxels in ROI (size of ROI); x, y, z = MNI coordinates of voxel with highest t-value in ROI; tmax: maximum t-value in ROI).

Overview of regions of interest

Encoding and response selection activated distinct cortical networks with limited overlap (see figure 3). Encoding ROIs are described in table 2 and figure 4. Response selection ROIs are listed in table 3 and figure 5.

Encoding

During the encoding phase bilateral regions in the occipital cortex and superior parietal cortex and the dorsal part of the anterior cingulate cortex were activated. In addition, there was activity in the left dorsolateral prefrontal cortex and the putamen.

Response selection

During the response phase there was also bilateral activity in the occipital cortex, but closer to the extrastriate and middle occipital gyrus, the DLPFC, the ACC, the operculum and the thalamus. In addition, ROIs were identified in the left primary sensorimotor cortex and the cuneus. In the right hemisphere we identified ROIs in the ventrolateral prefrontal cortex, the postcentral gyrus, the precentral gyrus and the superior parietal cortex.

Changes in activity related to practice

To examine function-specific effects of practice we tested activity averaged over all ROIs in the encoding and response selection networks (table 4 and figure 6) and in each individual ROI of the separate encoding network (table 5) and response selection network (table 6) for changes in activity across all practice runs and between each PT run compared to NT.

Effects of practice on encoding activity

The multivariate test for changes in activity (averaged over all encoding ROIs) shows a significant effect of practice across all six practice runs (table 5). Tests for changes in activity compared to the novel task show a significant decrease in all practice runs ($p<0.01$) (table 5). Separate tests for each ROI show significant decreases in bilateral visual cortex (E1 and E2) and left DLPFC (E3). Bilateral SPC (E4 and E5) and SMA (E6) show significant decreases in activity for all but the third practice run. Left PUT (E7) shows a significant decrease for all practice runs, except runs three and five (table 5).

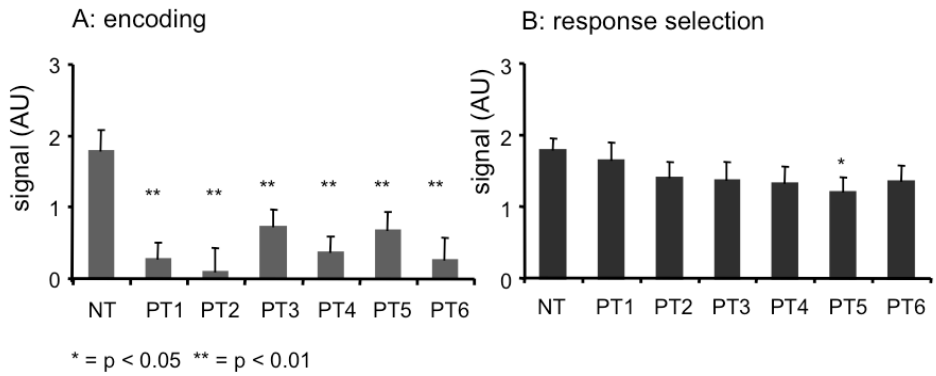

Figure 6. Practice and brain activity

a. activity in arbitrary units during encoding averaged over encoding phase ROIs (left) and b. activity during response selection averaged over response selection ROIs (right). Activity is displayed for novel task (NT) and each practice run (PT1-PT6).

Effects of practice on response selection activity

The multivariate test for changes in activity (averaged over all response selection ROIs) across all practice runs was not significant (table 6). In addition, there was no significant change in activity from the novel task at any practice run (table 6). In tests of separate ROIs (table 6), we found a significant decrease in signal compared to the novel task in the lPSMC (RS2) in practice run 5, in the lDLPFC (RS3) in runs 5 and 6, in the ACC (RS4) in runs 4, 5 and 6, in lOCC (RS5) in practice run 5, rDLPFC (RS8) in practice run 4 and 5, in lOPER (RS10) in practice run 5, and in rPCG (RS11) in practice run 4, 5 and 6.

In summary, practice reduced activity in function-specific regions associated with encoding and response selection. However, signal in encoding areas was reduced in all regions of the network after the first practice run, while in the response selection areas practice decreased activity only after the third practice run, and only in a subset of regions.

Table 4. Practice and Brain Activity

	encoding		**response selection**	
contrast	F(1,10)	p	F(1,10)	p
multivariate	**7.96***	**<0.01**	1.19**	0.32
NT-PT1	**31.97**	**<0.01**	0.38	0.55
NT-PT2	**19.67**	**<0.01**	0.30	0.16
NT-PT3	**18.23**	**<0.01**	2.48	0.15
NT-PT4	**18.51**	**<0.01**	3.59	0.09
NT-PT5	**14.24**	**<0.01**	4.97	0.05
NT-PT6	**36.39**	**<0.01**	2.99	0.11

* df = (6,60) ** df = (6,60) Huynh-Feldt corrected

Tests for significant effects of practice on encoding activity averaged over all encoding ROIs and response selection activity averaged over all response selection ROIs. Signals were tested over all runs (1st row) and between novel task (NT) and each practice run (PT). Significant results are displayed in bold.

Table 5. Practice and Encoding Activity

	multi-variate		NT-PT1		NT-PT2		NT-PT3		NT-PT4		NT-PT5		NT-PT6	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Rcalc (E1)	**4.93**	**<.01**	**17.0**	**0.00**	**10.6**	**0.01**	**19.5**	**<.01**	**14.6**	**<.01**	**9.56**	**0.01**	**21.3**	**<.01**
Lcalc (E2)	**7.29**	**<.01**	**27.9**	**0.00**	**12.7**	**0.01**	**28.7**	**<.01**	**13.7**	**<.01**	**20.5**	**0.00**	**31.9**	**<.01**
Ldlpfc (E3)	**6.16**	**<.01**	**15.8**	**0.00**	**15.2**	**<.01**	**6.88**	**0.03**	**19.7**	**<.01**	**10.1**	**0.01**	**23.7**	**<.01**
Lspc (E4)	**4.81**	**<.01**	**20.5**	**0.00**	**13.9**	**0.00**	3.86	0.08	**10.7**	**0.01**	**10.4**	**0.01**	**22.9**	**<.01**
Rspc (E5)	**3.62**	**<.01**	**11.0**	**0.01**	**12.5**	**0.01**	3.13	0.11	**6.33**	**0.03**	**11.1**	**0.01**	**12.6**	**0.01**
SMA (E6)	**7.46**	**<.01**	**10.2**	**0.01**	**12.5**	**0.01**	3.48	0.09	**12.7**	**0.01**	**6.61**	**0.03**	**18.8**	**<.01**
Lput (E7)	**3.14**	**0.01**	**6.3**	**0.03**	**10.3**	**0.01**	0.64	0.44	**5.13**	**0.05**	0.96	0.35	**6.7**	**0.03**

Multivariate tests for signals in ROIs related to encoding. Signals were tested over all runs (1st column) and between novel task (NT) and each practice run (PT). Significant results are displayed in bold. (For abbreviations see table 2).

Table 6. Practice and Response Selection Activity

	multi-variate		NT-PT1		NT-PT2		NT-PT3		NT-PT4		NT-PT5		NT-PT6	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Rocc (RS1)	0.89	0.51	0.28	0.60	2.48	0.15	0.48	0.51	0.19	0.67	0.69	0.42	1.71	0.22
Lpsmc (RS2)	1.37	0.34	0.84	0.38	1.57	0.24	3.69	0.08	3.67	0.09	**7.52**	**0.02**	1.07	0.33
Ldlpfc (RS3)	1.27	0.29	0.49	0.50	1.53	0.24	1.32	0.28	4.60	0.06	**9.60**	**0.01**	**5.60**	**0.04**
ACC (RS4)	1.49	0.20	3.47	0.09	1.14	0.31	3.27	0.10	**5.01**	**0.05**	**6.78**	**0.03**	**7.20**	**0.02**
Locc (RS5)	1.40	0.22	1.11	0.32	3.18	0.11	3.90	0.08	4.61	0.06	**4.83**	**0.05**	1.83	0.21
thal (RS 6)	1.59	0.19	1.00	0.34	0.70	0.42	2.84	0.12	0.67	0.43	1.90	0.20	0.11	0.75
Roper (RS7)	0.75	0.61	0.04	0.86	0.08	0.78	0.12	0.74	1.28	0.30	0.75	0.41	0.92	0.36
Rldpfc (RS8)	1.51	0.20	1.73	0.22	2.87	0.12	2.27	0.16	**7.66**	**0.02**	**8.10**	**0.02**	3.33	0.10
Rvpfc (RS9)	0.70	0.63	0.01	0.95	0.47	0.51	0.00	0.99	2.20	0.17	0.96	0.35	1.04	0.33
Loper (RS10)	0.96	0.45	0.01	0.94	0.01	0.91	0.78	0.40	0.90	0.37	**7.06**	**0.02**	0.88	0.37
Rpcg (RS11)	2.32	0.05	2.03	0.18	1.75	0.22	2.56	0.14	**7.77**	**0.02**	**14.59**	**0.01**	**5.06**	**0.05**
Rpocg (RS12)	1.12	0.37	0.07	0.79	2.15	0.17	0.42	0.53	2.16	0.17	3.00	0.11	0.05	0.83
Lcun (RS13)	0.53	0.78	0.01	0.96	0.44	0.52	0.70	0.42	0.30	0.60	3.10	0.10	1.10	0.32
Rspc (RS14)	1.33	0.26	4.72	0.06	0.15	0.71	0.00	1.00	1.58	0.24	0.00	0.99	0.22	0.65

Multivariate tests for ROI signals related to response selection. Signals were tested over all runs (1st column) and between novel task (NT) and each practice run (PT). Significant results are displayed in bold. (For abbreviations see table 3).

Heterogeneous effect of practice on encoding and response selection activity

Figure 3 illustrates the distinct effect that practice has on the encoding and response phase activity by showing the complete BOLD curves for left DLPFC and ACC. During the encoding phase, BOLD activity was practically absent in the first runs of practice in both regions (3a, 3b; red lines). For the response phase, BOLD activity is still visually detectable in lDLPFC (3a, blue line), and ACC (3b, blue line), up to the last run of practice.

Discussion

Summary

This study examined the effect of practice on brain activity associated with encoding and response selection. We used an optimized pseudo-random event-related design that isolated effects of practice in the encoding phase and response phase of a Sternberg task, at six runs of practice. Performance and brain activity at each practice run were compared to that of a similar task with novel stimuli. Our

behavioral results show that practice gradually but significantly improved performance, confirming automatization of task performance [1]. Practice promptly reduced activity across the entire regional network involved in encoding at the first run of practice, before response selection activity and performance were affected. Changes in response selection activity emerged at the third run of practice and were not present in all regions, but specific for ACC, left and right DLPFC, lPSMC, lOCC, rPCG and lOPER. Our results indicate that automatization can induce independent changes in function-specific brain regions over the course of practice.

Heterogeneous effects of practice on encoding and response selection

In the novel task, encoding and response selection activated regions associated with working memory in left DLPFC, and SMA/ACC and right superior parietal cortex. This common activation of the working memory network during different phases of novel Sternberg performance supports the notion of a scaffolding system that contributes to novel task performance [2]. However, practice induced different courses of activity reductions in working memory activity for the encoding and the response selection. Practice immediately reduced activity in the encoding network at the first run of practice in left DLPFC and ACC. In sharp contrast, response selection activity in these regions did not show any effect of practice over the course of three runs with repeated memory sets. This indicates that DLPFC and ACC were activated to an extent specifically needed for each phase at the different runs of practice. This divergent pattern of activity changes for encoding and response selection does not support the notion that domain-general control resources are reduced as the need to ‘‘scaffold’ task performance decreases with practice [2]. Consequently, these findings do not seem to support our hypothesis based on this idea. Our findings are more in line with the idea of decentralized theories of working memory function [28-30]. From this perspective practice may independently reduce working memory contributions to different phases of cognitive performance depending on the level of control necessary for each phase. The immediate reductions in activity associated with encoding possibly indicate that practice promptly reduces the need for working memory to support the transformation of visually presented stimuli into a neural representation that facilitates temporary storage of information during the delay [31-35]. In addition, the current data shows that a similar amount of practice can only marginally reduce working memory contributions to the response selection phase. This suggests that early in practice, working memory remains engaged to guide response selection based on earlier presented information [15,34]. The ability to automate task performance has been shown to be important for complex cognitive performance

such as the capacity to perform multiple tasks at once [1,3,36,37]. Our results suggest that early in practice reduced demands on encoding may increase one's capacity to process otherwise interfering information. However, performance of an additional task also deteriorates automated performance to some extent [3,37]. Our results indicate that this could be induced by conflicts at the level of response selection.

Automatization vs. other effects of practice on brain activity

Our findings are similar to other studies that have reported reductions in brain activity as a result of practice, representing increased efficiency of information processing [38,39]. However it should be noted that practice-induced activity changes in those studies were either not accompanied with improved performance [39], or selectively involved response selection [38]. Differences with our design are the type of stimuli used [39], and more importantly that stimulus-response associations in those studies changed over trials, which makes it difficult to compare with our findings. Neuroimaging findings of practice effects on brain activity have been inconsistent across studies [40]. The different effects of practice on brain function have been interpreted in terms of reorganization vs. redistribution of activity [40], changes in skill or strategy underlying task performance [41], item-specific or task-skill effects [42], or improved task proficiency [22,43,44]. We propose an alternative but important distinction between tasks that allow for automatization and those that cannot be automated, because stimulus-response associations continuously change over the course of practice. Here we show that automatization predominantly affects encoding early in practice even before performance improves.

Independent encoding and response phase networks

Our finding of distinct networks activated by encoding and response selection is in line with previous studies. The encoding phase in our study activated bilateral SPC. Many other studies have found this region to be activated by visual perception of stimuli in verbal working memory tasks [19,21,45-47]. It has been postulated that this region is important for encoding and temporary maintenance of information [48]. Response selection activated parts of the prefrontal-striatal-thalamic circuitry (thalamus, left VLPFC and right DLPFC) [49] that is involved in motor response modulation. These regions have been reported to be active during the response phase in delayed response tasks before [15,45].

We have designated the cognitive functions that we examined encoding and response selection, to emphasize the difference between the functions present in encoding and response phases of cognitive performance that can be automated. Naturally both task phases include many different processes. The encoding phase requires visual perception, encoding and short-term maintenance of the presented stimuli. The response phase also involves visual perception and encoding as well as response selection and execution. Based on the current design it is not possible to distinguish any of these processes within the current results, but we feel that the terms used, describe the most important function associated with the phase. We have restricted our analyses of brain activity to the encoding phase and response phase, while other studies also included the delay [15,19,21,47]. We decided not to separate the encoding phase from the delay, as it is difficult to separate these phases other than to vary the length of the delay period, which is not possible in an m-sequence design. Notably, current emerging views are that these phases activate the same brain systems [48]

Limitations

Due to limitations in the design practice trials with five-letter memory sets were interleaved with novel one-letter memory sets. This may have prevented continuous rehearsal of the practiced memory set and consequently slowed down the effect of practice on brain function. The period of practice in our study may therefore have not been sufficient to establish a potential relationship between activity and performance changes reported in other studies [22,38]. Reaction times on baseline trials (one-letter memory set) showed some improvement with practice. Although this may indicate that task performance became more proficient over time (i.e, regardless of whether stimuli were novel or practiced) it does not affect the main conclusion. The design used in our study yields different baselines for encoding and response selection activity. Encoding activity was based on the contrast between five-letter and one-letter memory sets, while response selection activity was derived from the average of all correct responses in one-letter and five-letter trials. This may have affected the level of activity for the different phases. The m-sequence analysis also provides an interaction activity map [13]. This map did not show any significant activity indicating that interaction effects of the encoding and response phase were small.

Conclusion

This study demonstrates that practice in a visually delivered cognitive task predominantly increases efficiency of encoding in primary visual, prefrontal and

parietal cortex. Changes in the cortical network related to response selection as well as performance improvement occur at a later state of practice. Our results indicate that automatization causes decreases in brain activity that are heterogeneous across functional regions and do not strictly track performance improvement. This suggests that cognitive performance is supported by a dynamic allocation of multiple resources in a distributed network. Our findings may further bear importance in understanding the role of automatization in complex cognitive performance, as increased encoding efficiency in early stages of practice possibly increases the capacity to otherwise interfering information.

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Chapter 3
Practice and the dynamic nature of working memory

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Abstract

One of the key features of working memory is its sensitivity to practice. For tasks that concern material that remains the same, practice leads to improved performance. Various studies have shown that brain activity in regions of the working memory system(s) declines with practice, and this is taken to indicate that performance becomes automatic. Performance is thought to become dependent on perceptual and motor systems rather than on working memory. However, it remains unclear how tasks are performed after practice, and which brain regions are involved. To investigate the nature of changes in brain activity, an fMRI study was conducted with 46 healthy volunteers, performing an item recognition task with novel and with practiced items. Brain activity levels were evaluated in brain regions and in networks of brain regions that exhibited correlated changes. All regions of the working memory system reduced activity with practice, and there were no regions that increased activity, indicating that practiced performance was not supported by other regions than the ones involved during the novel items. Multiple regions were found where activity was reduced in the novel item task (relative to a rest condition), and all of these became less deactivated with practice. Several networks emerged from correlation analyses, including a working memory network, two negative networks matching the default mode and a sensory network, a subcortical and a cortical motor network. There were no negative correlations between regions within networks, indicating that there were no intra-network shifts of activity. The working memory network was inversely correlated with the default mode network, and positively with the cortical motor network. These findings suggest that activity levels revert towards resting state levels with practice in all regions involved. We postulate that involvement of the working memory system becomes near-obsolete because the stimuli acquire a new feature which allows for their immediate categorization as targets or non-targets. This feature is only utilized when it is required by circumstances, in the context of the task.

Introduction

Many tasks humans perform become easier to conduct if they can be practiced. Performance improves in terms of speed and accuracy, and it becomes easier to conduct other tasks at the same time. Extensive research has shown that several conditions favor these benefits from practice, and that the behavioral changes are accompanied by changes in brain activity [1,2]. Foremost, the effect of practice depends on the nature of the task. Tasks involving movement sequences, which are abundant in many sports, lend themselves to training, or skill learning. Improvement typically proceeds in a continuous fashion, and can be achieved explicitly, i.e. with deliberate monitoring and guiding of the actions, or implicitly where one is not aware of what exactly is practiced. Cognitive tasks, where information needs to be processed and evaluated in a particular context, are also amenable to improvement through training, but here the rate and nature of performance improvement can take different forms. With tasks that can be accomplished through different strategies, improvements can be stepwise rather than continuous, as better strategies can be found and implemented. In this case a switch to another strategy is likely to be accompanied by a change in the brain networks used [2,3,4,5,6,7]. Practice then reduces activity in areas that initially coordinate the mental processes required to perform the task (i.e. cognitive control) while increasing activity in areas that were not active before practice [3,4,6,7,8,9]. Tasks that do not allow for multiple strategies can be practiced but here it matters greatly whether the material that needs to be processed remains the same (constant) or not (variable) [1,10]. For instance, reproducing series of sequentially presented random letters may become slightly easier with practice, but it will remain effortful and slow, compared to reproducing one and the same series of letters repeatedly. It is generally thought that improved cognitive performance on material that remains the same is linked to a shift from controlled processing, which mostly involves working memory, to automatic processing where sensory input is seemingly directly classified and acted upon through motor programs [10]. This form of learning, which covers concepts such as cognitive procedural learning, rule-based learning and automatization, plays an important role in everyday life. Even quite complex tasks such as driving a vehicle in traffic or typing on a keyboard, improve with practice and with adequate amount of practice can be performed concurrent with for instance a conversation.

Various studies have shown that brain activity declines with practice of constant material [1,11,12]. This decline is most prominent in regions that are thought to underlie cognitive control mechanisms, and which have been postulated to act as a scaffolding network for learning new tasks. The notion of a cognitive control system

as serving to shape brain functions to accommodate a novel task by means of monitoring and feedback concerning errors has strong support [1,13,14,15,16]. There is no clear indication that practice in these cognitive tasks leads to enhanced recruitment of other brain regions such as those involved in perception or motor output, which leaves open the question which brain regions perform the task after practice. Several scenarios can be considered. First, practice may increase the involvement of long-term memory in the task, linking perceptual properties of stimuli to particular motor responses [3,4,7,17]. Second, practice may enable direct connections between perceptual and motor systems [14,18,19,20,21], increasing their involvement. Third, practice may reduce involvement of the initial set of brain regions, but not completely, and performance would still involve the cognitive control network [21]. These scenarios represent different neural substrates underlying practice effects, all of which may be difficult to detect with fMRI. As mentioned, various fMRI studies have only observed an activity decline [11,22,23,24], but subtle shifts within or between networks may well have gone unnoticed due to limited statistical power associated with relatively low fMRI sensitivity for neural activity and small numbers of subjects. Hence, it remains unclear if a decline of activity in the cognitive control network is paralleled by subtle compensatory increases in task-related areas. Empirical examination of the three scenarios can elucidate the mechanisms by which automaticity, a fundamental aspect of brain function, is accomplished.

In the present study we investigate changes in brain activity following practice of a working memory task, applying an approach that maximizes sensitivity for subtle effects. We recruited a large sample of healthy subjects, and obtained whole-brain fMRI scans on a 3T scanner with an 8-channel head coil to obtain a high sensitivity for signal changes. In addition, we made use of the variability in practice-induced effects on brain activity among subjects to assess changes within and across networks. A brief period of pre-scan training, too short to achieve full automaticity, results in differences in performance across subjects that reflects the rate or speed at which automaticity is achieved, and can thus be used as a cross-sectional approximation of the practice effect.

It was reasoned that the different scenarios would be reflected in different findings. A shift from one set of regions to another set would signify that practice leads to a change in the brain areas engaged (scenario 1). Alternatively, activity could shift within the initially active network(s) from cognitive control regions to perceptual and

Table 1. Demographic variables

	N	*Mean*	*SD*	*Range*
Male	23			
Female	23			
Age (years)		30.8	9.50	20-51
EHI index		0.88	0.16	0.40-1.00
Years of Education		16.66	2.18	12-22

motor systems (scenario 2). The third scenario would predict that the same networks are involved from beginning to the end of training, and the only change is an improvement of the efficiency with which regions communicate, in which case brain activity would decline coherently in all regions.

We first addressed the question whether there are brain regions that become more active after practice. Next, we addressed the question whether practice causes a shift of activity within the networks involved in performing the task. For this we first needed to identify the networks and assess interactions within those. We reasoned that regions that respond to practice 'in concert' (i.e. are correlated in their degree of signal change) would be part of the same network. Importantly, this approach made it possible to assess potential shifts of brain activity within networks, following practice. If particular regions would gain significance, one would expect those to correlate negatively with others that decrease their involvement, even if activity declines in all regions.

Methods

Participants

46 healthy volunteers participated in the study (details given in table 1). All subjects were recruited through the Normal Volunteer Office of the National Institute of Health. Prior to scanning all participants were screened for a history of psychiatric and/or neurological illness, active substance abuse or dependence and significant abnormalities on a screening MRI examination. Handedness was assessed by

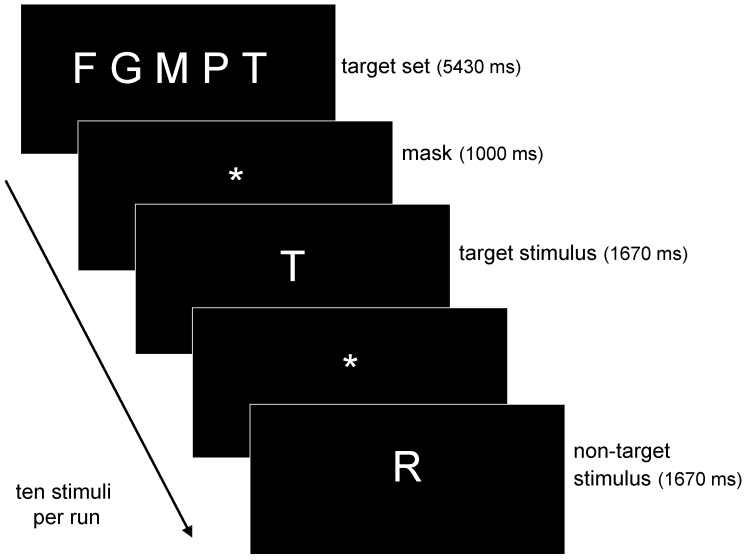

Figure 1. Cognitive paradigm

The temporal sequence is shown for the Sternberg automatization task. Each epoch starts with presentation of the target set and is followed by ten probes. Subjects press the left button of a button-box if the probe letter belongs to the set of targets and press the right button if the probe letter does not match the set of targets.

means of the Edinburgh Handedness Inventory [25]. The fMRI examinations were conducted under a protocol (95-M-0085) that was approved by the Institutional Review Board of the National Institute of Mental Health. All subjects gave written informed consent prior to participation in this protocol.

Materials

The task used in the fMRI experiment to test automatization is a modified version of the Sternberg paradigm (STERN). Subjects were instructed to memorize five letters that were visually presented (memory set, presented for 5600 ms). The memory set was followed by presentation of a series of ten individual letters (probes, each presented for 1000 ms followed by a fixation cross for 1800 ms), together forming an epoch. Participants were instructed to decide as fast as possible whether the

probes belonged to the memory-set (target) or not (non-target) (Figure 1). Overall, fifty percent of probes were targets and 50 percent were non-targets.

The task consisted of three conditions differing in type of memory set, being Novel (NT), Practiced (PT) and Control (CT), and rest periods of equal duration. Conditions consisted of 48 epochs each, divided over 12 fMRI runs. Thus, each fMRI run contained 4 epochs of NT, PT, CT and rest, which were presented in a semi-randomized order. In the NT the stimuli in the memory set in each epoch were randomly chosen from a set of ten consonants. In the PT one and the same memory set was presented in all epochs. This memory set was presented prior to the scan session where subjects practiced five runs of 100 PT probes during 25 minutes. In the CT the memory set consisted of one and the same vowel in all epochs, presented as an array of five (eg 'AAAAA') to maintain the same visual input as NT and PT.

To avoid interference, the stimuli used for memory sets and probes did not overlap between conditions. This was accomplished by reserving 10 consonants of the alphabet for NT and the remaining ten for PT. For CT two vowels were used, one of which always was the target (memory set) and the other the non-target.
The CT differed from the one used in previous studies where an arrow was used), and was chosen for its better match to NT and PT in terms of stimulus processing. This way, the CT essentially represents a fully automated version of the STERN task.

Procedure

The stimuli were generated by a standard desktop computer running Presentation software (www.neurobs.com) and presented through a projector on a through-projection screen. A mirror attached to the head coil enabled subjects to see the screen positioned near the feet. Responses were recorded through a fiber-optic response box. Subjects were instructed to press the right button in response to a target stimulus and the left button in response to a non-target stimulus. Accuracy (percent correct) was computed for each task, and mean reaction time (RT) was calculated over all correct responses per task. Mean RT and accuracy scores were tested with repeated measurements General Linear Model (NT, PT and CT as within-subject factors) and paired t-tests. Only RT was used for fMRI analysis, as accuracy was highly skewed towards 100% correct, and failed tests of normal distribution even after transformations.

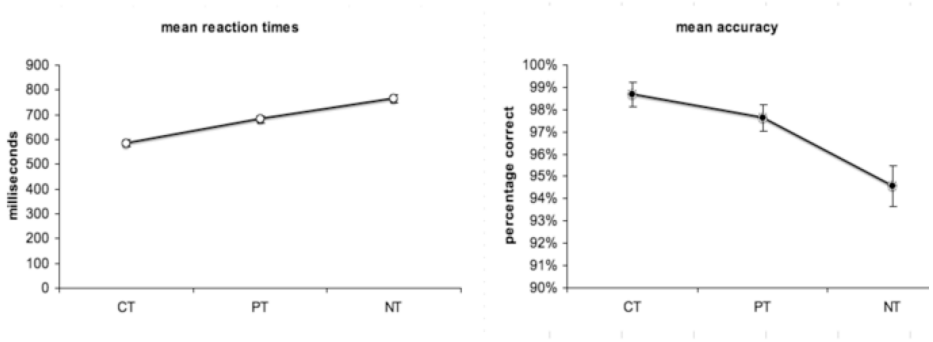

Figure 2. Performance

The graphs show behavioral fMRI-scan performance data. Mean reaction times (± SEM) of correct target responses and mean correct responses as percentage of all trials (± SEM).

fMRI acquisition

All fMRI studies were performed on a standard 3-T GE Scanner (General Electric Systems, Milwaukee WI). Whole brain fMRI data were acquired with a GE-EPI RT (Milwaukee, WI) sequence (24 contiguous slices, TE = 30, TR = 2000, flip angle = 90, FOV = 24, voxel size = 3.75 x 3.75 x 6, matrix = 64 x 64).

Data Preprocessing and Statistical Analysis

SPM2 software (Wellcome Department of Imaging Neuroscience, London http://www.fil.ion.ucl.ac.uk/spm) was used for image processing and statistical analysis. All EPI images were realigned to the first image in the series. EPI images were spatially normalized to a standard template. All normalized EPI images were spatially smoothed using an isotropic Gaussian filter (10 mm FWMH) and high-pass filtered. Task-related activity during NT, PT and CT was modeled as a block design using the general linear model in SPM2 with the canonical hemodynamic response function. Contrast maps were generated for each condition (NT vs Rest, PT vs Rest and CT vs Rest), for NT versus PT and for PT versus NT (primary contrasts of interest). All individual contrast images were then entered into second-level analyses with subject as a random factor. A more stringent threshold of t=5.5 corresponding with a p-value of 0.01 (corrected for multiple comparisons) was used to bilaterally separate inferior and superior frontal clusters of activity in the NT-PT contrast image. A more stringent threshold of t=5.5 corresponding with a p-value of 0.01 (corrected for multiple comparisons) was used to bilaterally separate inferior and superior frontal clusters of activity in the NT-PT contrast image. We determined

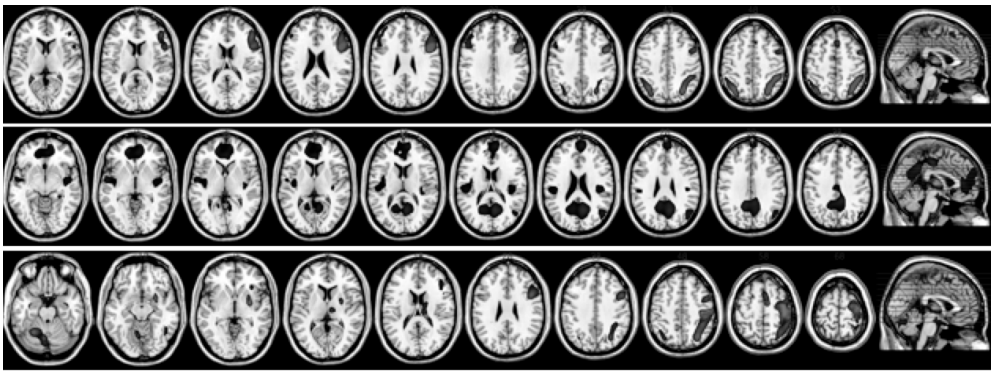

Figure 3. Regions of Interest

Group contrast images (average contrast map of all 46 subjects) showing the regions of interest. Contrasts were thresholded with t=4.5. The top panel shows the NT vs. PT contrast image. Red areas show the cortical network that was more active in NT (NT>PT). Blue areas show the cortical regions that were more active in PT (inverse contrast PT>NT). The bottom panel shows areas in red that were commonly active in the three task conditions: NT∩PT∩CT (i.e. the intersection of NT, PT and CT vs. rest). The numbers in the slices correspond to MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).

the loci of the MNI-coordinates of the activation peaks and subsequently used masks from the WFU pickatlas [26] to separate the adjacent clusters of activity within this large volume of activity. To identify the regions that are active during all three tasks we combined the NT versus rest, PT versus rest and CT versus rest group maps (NT∩PT∩CT) This analysis first involved separate one-sample t-tests for the each task versus rest contrasts. The three group contrasts were combined in xjView (http://people.hnl.bcm.tmc.edu/cuixu/xjView) to create a new map consisting of regions that were significantly active in NT, PT and CT (each $p<0.05$ family-wise error-corrected and more than 5 contiguous voxels). The NT∩PT∩CT contrast yielded an extensive cortical cluster of motor regions, extending from pre-motor to parietal regions with multiple peaks of activation (see table 2 and figure 3).

Three sets of ROIs were obtained, i,e., positive ROI's (NT>PT), negative ROI's (NT<PT) and common ROI's (NT AND PT > rest). Parameter estimates (b-values) were extracted with MarsBar [27] from individual NT, PT and CT contrast images, and were averaged for all voxels in each of the ROI's in the three sets.

Table 2. Regions of interest

	Region	Abbreviation	Brodmann area	Number of voxels	X	Y	Z	Maximum t-value
	Positive ROI's							
P1	Left Cerebellum	LCer	-	76	-52	-52	-20	7.76
P2	Left Inferior Frontal Gyrus	LIFG	44,45,47	62	-38	26	-5	7.37
P3	Left Middle Frontal Gyrus	LMFG	9,46	500	-49	15	25	12.08
P4	Left Parietal Cortex	LPar	7,40	231	-34	-64	50	11.60
P5	Medial Frontal Cortex	MFC	6,8	68	-4	23	55	6.53
P6	Right Cerebellum	RCer	-	34	38	-67	-35	6.33
P7	Right Inferior Frontal Gyrus	RIFG	45,47	62	28	26	-5	7.01
P8	Right Middle Frontal Gyrus	RMFG	9,46	184	49	23	35	6.18
P9	Right Parietal Cortex	RPar	7,40	147	38	-60	45	10.37
	Negative ROI's							
N1	Anterior Cingulate	ACC	24,32	412	15	49	0	8.52
N2	Left Hippocampus	LHip	35,36	36	-26	-30	-15	8.82
N3	Left Insula	LINS	13,	53	-41	-11	-5	7.04
N4	Left Middle Temporal Gyrus	LMTG	21,22,39	67	-49	-67	25	8.03
N5	Left Superior Frontal Gyrus	LSFG	8	51	-19	45	55	7.45
N6	Left Superior Temporal Gyrus	LSTG	37	5	-38	-15	-20	5.69
N7	Posterior Cingulate	PCC	23,29,31	575	-11	-60	25	10.15
N8	Right Insula	RINS	13,20,21,22,	19	45	-7	-20	7.35
N9	Right Superior Temporal Gyrus	RSTG	38,40,43	134	56	-4	-5	7.59
	Common ROI's							
C1	Left Thalamus	LThal	-	27	-11	19	10	7.90
C2	Right Cerebellum	RCer	-	241	30	-56	-30	12.28
C3	Left Putamen / Globus Pallidus	LPut/GP	-	56	-26	0	5	7.72
C4	Left Precentral Gyrus	LPreC	4	573	-38	-7	60	10.97
C5	*Left Premotor cortex*	*LPreM*	*6*		*-7*	*4*	*55*	*9.22*
C6	*Left Postcentral Gyrus*	*LPostC*	*5*		*-4*	*-22*	*60*	*9.95*
C7	*Left Parietal Cortex*	*LPar*	*7,40*		*-49*	*-30*	*55*	*7.90*

ROI correlation analyses

After determining the three sets of ROI's, we assessed correlations between regions within each ROI set, correlations across and between ROI sets and correlations between ROI's, sets and RT. Correlations were computed between regions within these sets, and between regions across sets, for the NT-PT values in ROI's. Significance thresholds for correlations were based on a Bonferroni correction for the total number of correlation computations, i.e. 9 positive ROI's, 9 negative ROI's and 7 common ROI's amounting to 271, at $p<0.05$ two-sided. Subjects differ in the average strength of activation, thereby potentially enhancing correlations between all ROI's. Therefore, all analyses were conducted with Partial

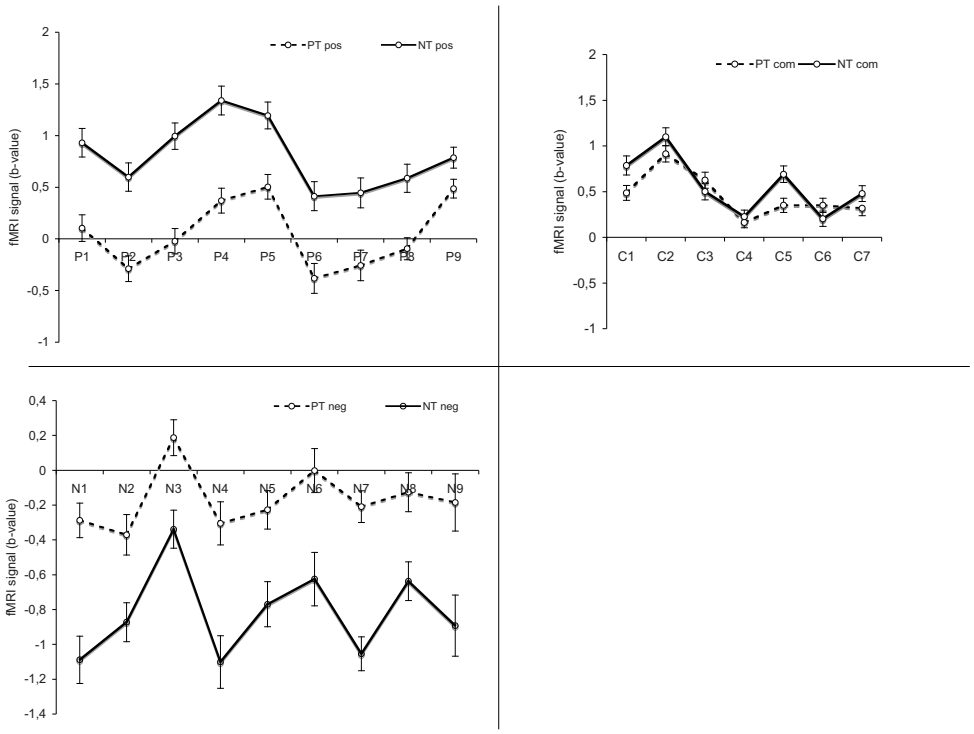

Figure 4. Practice and brain activity

The graphs show top left panel: The level of activity in the positive ROI's for NT and PT. For list of abbreviations see table 2). Activity was significantly enhanced during NT in all regions (*p<0.0001, uncorrected). 2. The level of activity in the negative ROI's. Activity during NT was significantly reduced in all regions (*p<0.0001, uncorrected) 3. The level of activity in the common ROI's. The y-axis shows mean parameter estimates (b-values) and represents level of activity.

correlation, using the grand mean b-value for all ROI's within a set across CT, PT and NT as covariate. Correlations patterns were then verified by means of factor analysis within ROI sets. Finally, for the networks defined by these tests, mutual relationships were assessed.

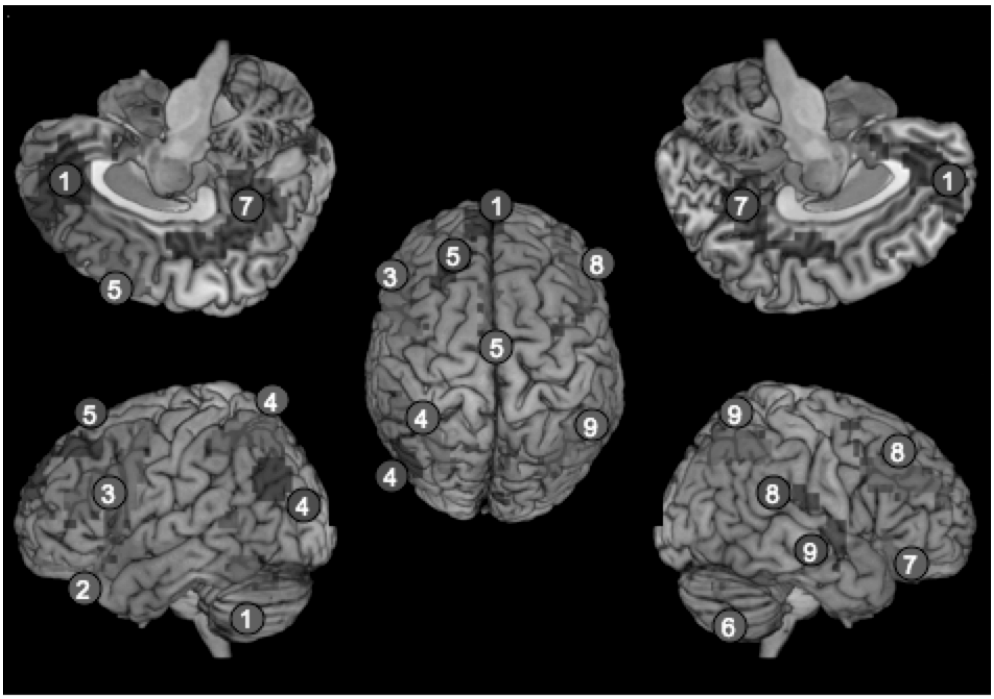

Figure 5. Positive and negative regions of interest

Surface representation of the positive (red) and negative (blue) regions of interest. Numbers correspond to those in Table 2. Negative regions 2, 3 and 6 are below the surface (see figure 3).

Results

Performance

Reaction time (figure 2) differed significantly between the three tasks, as indicated by a significant main effect of task ($F(2, 44)=170.53$, $p<0.0001$). Posthoc t-tests confirmed longer RT for NT than for PT and CT, and for PT than CT ($t(45)>11$, $p<0.001$). Accuracy (figure 2) was also significantly different in NT, PT and CT (main effect of task: $F(2,90)=6.50$, $p=0.002$). PT performance was better than NT ($t(45)=2.27$, $p<0.03$) but not better than CT. The graphs (figure 2) show that PT reaction time was halfway between NT and CT, indicating that full automatization had not yet been achieved.

Brain Activity

Positive regions of interest (NT greater than PT)

The contrast (NT>PT) shows areas that were more active during NT, including left cerebellar cortex, left inferior frontal gyrus, left middle frontal gyrus, left parietal cortex, left thalamus, medial frontal cortex right cerebellar cortex, right inferior

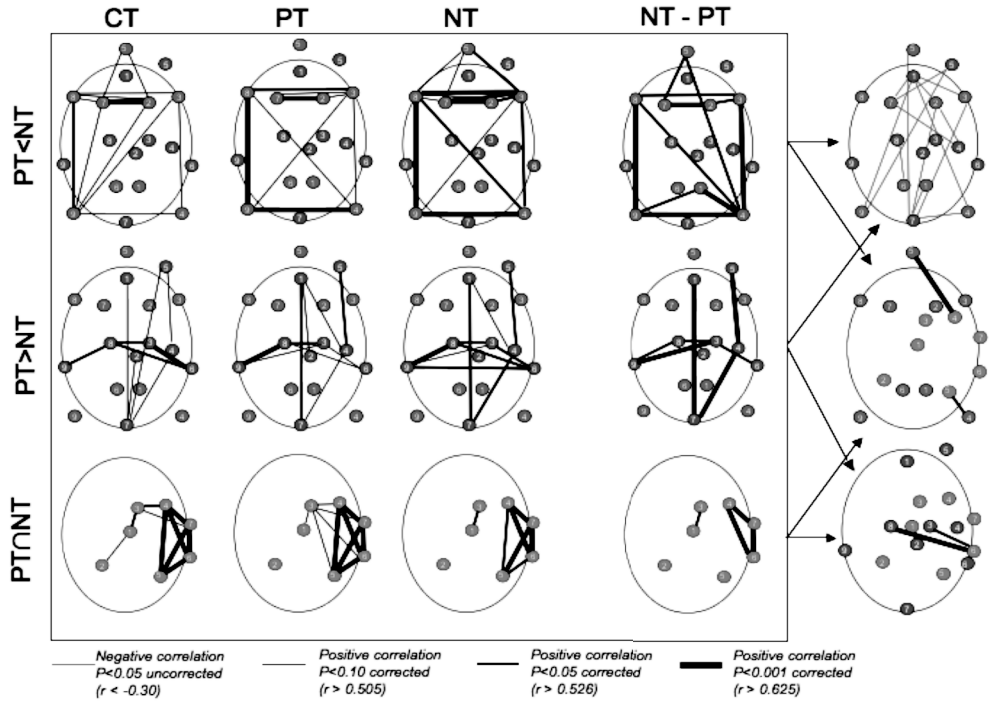

Figure 6. Interactions within and between regional networks

Correlations within regions (left 4 columns) and between (right column) regions. Left 3 columns display correlations of task versus rest values (not corrected for mean activity level, see text). Fourth column displays correlations of practice-induced signal change (NT - PT), corrected for mean activity of CT, PT and NT versus rest. Right column display correlations between regions across networks, corrected for mean activity of CT, PT and NT versus rest. Positive ROIs (NT > PT) are displayed in red, negative ROIs (NT < PT) in blue, and the common ROIs (NT> rest AND PT > rest) in purple. Numbers correspond to regions listed in Table 2. Right side is left hemisphere.

frontal gyrus, right middle frontal gyrus and right parietal cortex (see figure 3 and table 2). These areas are consistent with the cortical network supporting WM function [11,28,29,30,31] and replicate our previous findings of the difference in the level of activity in working memory areas between novel and practiced performance (figure 4).

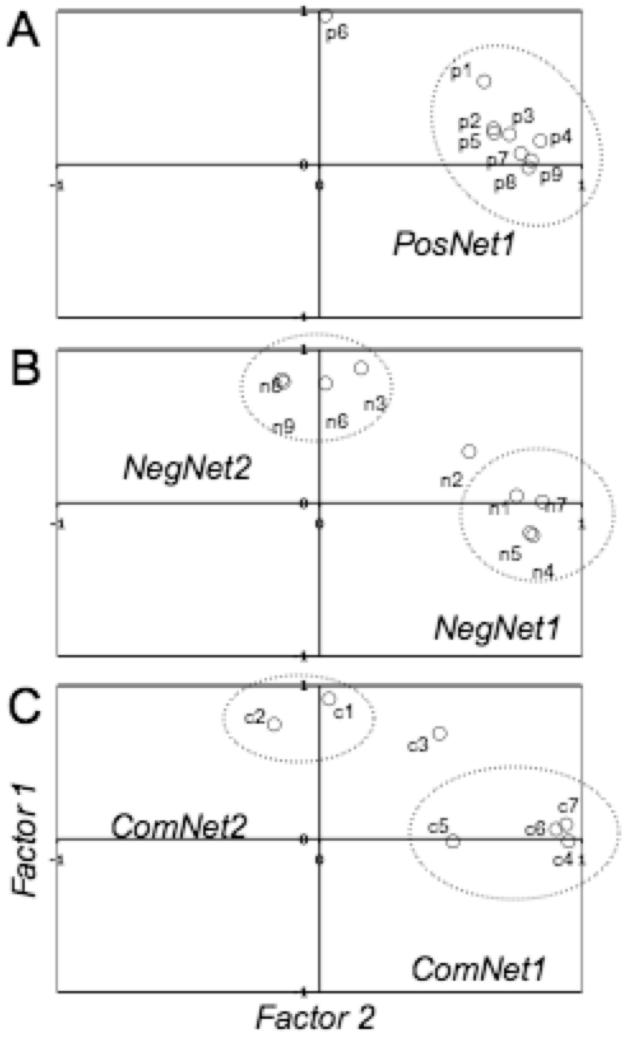

Figure 7.

Graphs depicting clustering of regions within their ROI sets, obtained by Principal Component Analysis (Varimax rotation with Kaiser normalization). For each ROI the loading on the two factors is shown. Explained variance for the 2 factors is 64% for the positive (A) and the negative (B) ROI set, and 71% for the common ROI set (C). Dotted ovals indicate the ROIs (and the network abbreviation) collapsed for further analyses.

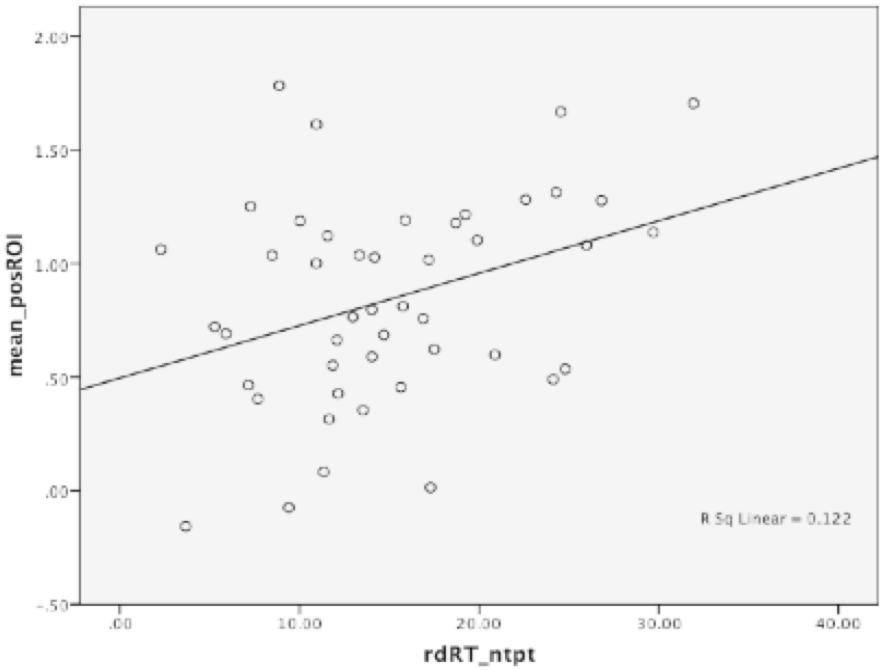

Figure 8. correlation between positive network activity and level of performance
Scatterplot of the decrease in reaction time on the Sternberg task following practice (NT-PT, displayed on the x-axis) versus the decrease in the positive Regions of Interest network following practice (y-axis).

Negative regions of interest (PT greater than NT)

Contrasting PT directly with NT revealed a set of regions including anterior cingulate cortex, left hippocampus, left insular cortex, left middle temporal gyrus, left superior frontal gyrus, left superior temporal gyrus, posterior cingulate cortex, right insular cortex, right superior temporal gyrus (table 2, figure 3). Importantly, signals in these ROI's exhibited negative values relative to rest during NT and returned towards baseline after practice (figure 4).

Regions common to NT and PT

To investigate whether automatization was associated with activity changes in task-related areas we determined the intersection of common NT, PT and CT activity (NT∩PT∩CT) to localize the regional overlap between the tasks. This resulted in a

network that partly overlapped with the working memory network as described in the previous section (see figure 3 and 4, and table 2). The common network involved left thalamus, right cerebellum, left putamen / globus pallidus, left premotor cortex, left superior parietal cortex, left postcentral gyrus and left precentral gyrus.

Networks

Correlations between and across regions within the ROI's identified in the three contrasts (figures 3 and 5) were computed, using partial correlation analysis correcting for mean activity level (average of all tasks versus rest contrasts) across all ROI's. Significance threshold was $p<0.05$ two-sided, Bonferroni corrected for all pairwise comparisons (i.e. 271). For each ROI set, a factor analysis was performed to evaluate presence of separate networks within ROI sets (Principal Components with Varimax Rotation). Figure 6 displays all the correlations within and across sets of ROI's for the NT versus PT contrast. Correlations for the separate task contrasts are displayed also, for comparison. In supplementary figure S1 the regions are displayed schematically, showing which regions are significantly different from rest for each task.

Positive regions of interest

Correlations were strongest between the regions known for their involvement in WM, being bilateral inferior and middle frontal gyrus, left and right superior parietal cortex, and medial frontal gyrus. Value distributions and scatterplots are show in supplementary figure S2. In addition, the left cerebellum/fusiform gyrus was also strongly correlated. Factor analysis confirmed existence of only one network (P1), encompassing all ROI's except for the right Cerebellum (see figure 7).

Negative regions of interest

Correlations were found in two apparently separate sets which did not evidence any relationship with each other. One set consisted of anterior and posterior cingulate, left middle temporoparietal cortex and left superior frontal gyrus (N1). The other consisted of bilateral insula/parietal regions and superior temporal gyrus (N2). One region, the left hippocampus, did not evidence any correlation with other regions. The existence of these two separate networks was confirmed by factor analysis (see figure 7).

Common regions of interest

Strong correlations were found between left premotor, precentral and postcentral cortex, forming a cortical motor set (C1). A second set consisted of the left putamen

and the thalamus (C2). The existence of these two separate networks was confirmed by factor analysis (see figure 7), be it that left parietal cortex was included in C1 and right cerebellum was included in C2.

Across Networks

Correlations between regions across ROI sets were few (figure 6). No correlations exceeded the threshold between the positive and the negative ROIs, suggesting that the sets are not strongly linked to each other. Common ROI's exhibit positive correlations with positive ROI's, i.e. between anterior cingulate and left premotor cortex, and within left parietal cortex. The common ROI in left postcentral gyrus correlated with the negative ROIs in insular cortex bilaterally.

Given indications that there were several distinct networks, correlations between networks (with values averaged across ROI's within networks as described above) were assessed (supplementary figures S3, S4). Corrected for the 5 comparisons and for overall activity (as above), P1 was negatively correlated with N1 ($r=-0.41$), and positively with C1 ($r=0.54$). C1 was also positively correlated with N2 ($r=0.48$). All other correlations were below $|0.18|$.

Correlations with performance

No regions of any of the 3 ROI sets correlated with reaction time or accuracy when correcting for numbers of comparisons. Of the networks, only P1 correlated with performance, both in reaction time ($r=0.32$, $p=0.03$, all other correlations below $|0.17|$), and accuracy ($r=-0.31$, $p=0.035$, all other correlations below $|0.18|$), indicating that a larger reduction in activity in P1 following practice coincided with a larger reduction in reaction time and a larger improvement in accuracy (figure 8).

Discussion

We investigated the nature of the changes in brain activity during a working memory task, following practice. The task does not allow for a change in strategy, as is indicated by the fact that performance improves in a continuous manner rather than stepwise [11]. The main question pertains to the regions that are involved in the initial and the trained phase. With practice, activity decreased in all regions associated with working memory, as was shown by previous studies with this or similar tasks [2,11,12,23,32,33,34,35,36]. A set of regions was found where the BOLD signal increased with practice, but in all of these the signal was negative relative to rest. Hence the novel task caused a strong decrease in activity in this set, which was ameliorated by practice. Activity in motor regions remained mostly

constant across tasks. Given the large sample size and the high sensitivity for BOLD signal change, the chances of failing to detect subtle changes (i.e. false negative findings) are minimized. The main finding hence supports the notion that practice does not lead to a shift from one network to another.

Shifts of involvement within the initially active network would be an alternative scenario, for instance an increasing dependence on perceptual or motor regions [14,16]. We assessed correlational structures within each of several sets of regions that emerged from the statistical image analyses, i.e. where signal decreased with practice (P1), where it increased (N1 and N2), and where signal was elevated during all tasks (C1 and C2). Partial correlation and factor analyses revealed strong correlations between regions within the sets, in how they respond to practice (Figure 7). Important for our study objective, there were no significant negative correlations within sets, which should be present if activity shifts between regions within a set. Hence, our findings also do not support the notion that specific regions within the initially active network(s) gain in involvement. The data do support the scenario where practice leads only to a decline of involvement of the WM system.

Regions of interest were determined by contrasting the novel with the practiced task, and by conjunction of all tasks. Cross-correlation (corrected for overall magnitude of activity) and factor analyses indicated that there were several distinct sets of correlated regions. Regions that were active during all three tasks included the motor regions. Factor analysis identified two separate networks, a neocortical and a subcortical network. The positive regions that exhibited a decrease in activity following practice, exhibited strong correlations with each other, except for the right cerebellum. The left cerebellum ROI extended into the fusiform gyrus, which is likely involved in decoding the lexicographic stimuli [37,38]. The left parietal cortex seems to be the most dominant node in the positive network in terms of strength and number of correlations. Parietal, dorsolateral prefrontal and anterior cingulate cortex are recognized as the critical brain areas for working memory [39], and reproducibly activate with the sternberg task [11,12,40]. Regions are more strongly active in the left hemisphere, in accordance with the notion that laterality is to some extent related to the type of stimulus material (i.e. verbal) [30]. Ventrolateral prefrontal cortex extended into the operculum bilaterally, along the posterior wall of BA 44. This set of regions has also been coined the ‘Cognitive Control Network’ (CCN) [13], exhibiting correlated activity both during rest and during performance of a WM task. On average, activity in the WM network decreased by about 90% after 20 minutes of practice. Activity in the network differed from baseline (rest) only during

the novel task, and not during the practiced or the control task. The decrease in activity following practice has previously been shown to partially predict dual-task performance [12], suggesting that the decrease represents a decline in demand for WM resources and a subsequent increase in resources available for other tasks.

Although we did not find evidence of new areas selectively involved in practiced performance, a network of regions emerged when the practiced task was contrasted with the novel task that exhibited strong task-induced deactivation that was attenuated after practice. Factor analysis indicated that there were two separate, uncorrelated, sets of regions. The first set comprises anterior cingulate, posterior cingulate, left temporoparietal cortex and left superior frontal cortex, and matches the network that is active during a conscious resting state and which is often referred to as the default mode network (DMN) [41,42,43,44]. Signals in the DMN regions are correlated when the subject is at rest, both in terms of low frequency coherence [43,45] and of pattern similarities as observed with Independent Component Analysis [41,46], and seed-voxel correlation analyses [47,48]. Although the functionality of the default network has been debated [49], it has been argued that regions in this network are involved in sensory and emotional processing [43,45] that may interfere with cognitive performance (e.g inner speech, self-referential thoughts) [42,44], and is therefore inhibited during demanding cognitive tasks. The DMN has been shown to decrease in signal proportionally to task difficulty [50], which is seen as evidence for reallocation of processing resources away from the DMN, toward the task-relevant regions. In the present study, practice reduced deactivation by 75%, but deactivation remained significant both during the practiced and the control task.

Factor analysis revealed a second set of negative regions consisting of bilateral auditory and posterior insular cortex (N2). This network may deactivate to inhibit processing of auditory (notably scanner noise) and of somatosensory input. Posterior insula is thought to process interoceptive input, thereby representing the ‘physiological condition of the body’ [51]. It has also been linked to ‘stimulus independent thought’ [52], which emerges when task demands decline. Mason found a correlation between activity in insular cortex and the amount of daydreaming subjects reported. The most posterior aspect, parietal operculum, matches the location of SII, the secondary somatosensory area. This region can be activated bilaterally by electrical stimulation of the right hand [53]. Interestingly, this network operates independent from N1 in the present study, and does not respond proportionally to cognitive demand. Practice reduced the magnitude of deactivation

in this network by 55%, but deactivation remained significant during the practiced task and during the control task.

As can be seen in figure 6, there were very few correlations between regions of different networks. There were, however, robust correlations between whole networks (supplementary figure 3 and 4). The P1 network exhibited clear correlations with N1 and the neocortical motor network. The decline of WM activity with practice was accompanied by a proportional attenuation of deactivation in N1. In other words, both networks moved towards resting state activity levels in concert. The neocortical motor network C1 decreased activity in concert with the P1, but it remained significantly active (compared to rest) during all tasks. This correlation may reflect the decrease in reaction time as practice reduces the need for controlled processing. The C1 network was also correlated positively with the second negative network N2. The subcortical motor network (C2) did not covary with any of the other networks.

The overall pattern may be summarized as follows: Initially, when the memory set is new a WM network is active, and a DMN and a sensory network are deactivated. With practice, WM network involvement declines, and the DMN network increases activity proportionally. Performance improves, and activity in the neocortical motor network declines, but moderately. The sensory network also becomes more active, but not proportional to the WM or DMN network. One reason may be that the degree of deactivation is dominated by subject-specific strategies, for instance by a preference for phonological versus visual maintenance of the memory set. Importantly, performance improvement is associated only and be it weakly, with the decline of activity in P1, not with any other network. The weak association between brain activity and performance is in line with our previous study, where the drop in brain activity in the WM network was shown to reflect an individuals ability to perform multiple tasks, and did not correlate with improvement in reaction time [12].

Based on the seminal work of Sternberg [54], we can assume that in the novel task the probe is compared to each of the items in the memory set. Sternberg showed that reaction time increases proportional to the number of items in the memory set. Recalling the memory set for comparison to the probe is accomplished by WM, and is thought to be achieved by bringing the set that is held available in one of the slave systems, into the central arena of WM, the central executive [55]. With practice of the same memory set, the steady decline in reaction time is thought to reflect facilitation of recalling the memory set for each probe, potentially by shifting

to retrieval from long-term memory [3,4,7]. One would expect to see an increase in activity in regions associated with long-term memory, such as hippocampus, but this was not observed in the present study. The items also become strongly associated with one another as to become a single 'chunk' of information [56,57]. However, faster retrieval does not necessarily explain why reaction time decreases the way it does. We have shown previously that brain activity associated with holding the memory set online decreases within a few minutes if not less, whereas brain activity associated with processing the probes declines very slowly over time, matching the improvement in reaction time better [40]. Alternatively, the process of comparing the probe to memory set items is accelerated. It is hard to explain why this would only occur for practiced items, unless the nature of the probes changes. We hypothesize that with practice, the need to sequentially compare the probe with memory set items declines because the internal representation of probes themselves acquire a new feature, namely that of being a target or a non-target. This feature is then utilized when circumstances require so, but not when the context is different.

From the present and previous studies it is clear that a cognitive control network [13] as well as a default mode network [48,50] respond proportionally to working memory load. The CCN is indispensable for new tasks, as evidenced by effects of virtual lesions [58,59], is called for to shape responses to stimuli and retreats once performance is automatic. This is in line with the notion of WM emerging from interactions between motor and/or sensory pathways and association areas such as the prefrontal and parietal cortex [14,16,60]. Moreover, if practiced performance is no longer correct (e.g. when rules are changed, and for instance targets become non-targets), the CCN is likely to re-engage, potentially to overrule automatic responding. Interestingly, part of the CCN, notably the parietal cortex, as well as the whole DMN are still involved in the control task, suggesting that both are engaged for the purpose of maintaining the particular context and for reducing interference from internal and external sources respectively.

One of the important questions that remain is how the context of the task is represented and which brain regions maintain it. The context of a task instruction is critical because the (practiced) stimuli only generate a motor response when called for by the task at hand. The alphabetical stimuli used in the present study are used extensively in everyday life and do not generate motor responses during reading. Another question is what makes the WM system disengage from the task. One could imagine that the WM system is 'bypassed' by immediate classification based on a newly acquired feature of the probes, and ceases coordination of processes.

These questions may require additional techniques such as virtual lesions, and more sensitive and detailed measures of brain activity such as electrocortical recording.

In conclusion, practice of a task with a fixed set of stimuli leads to a significant reduction in brain activity in working memory regions. We found no evidence of either a shift to another network, or a shift in activity within the initially active network. Regions of the motor system remained active during all tasks. Several sets of regions were observed that formed correlated networks, all of which reverted towards resting state activity with practice. We conclude that following practice the working memory network is no longer involved because stimuli have acquired a novel feature that allows for rapid classification.

Supplementary materials

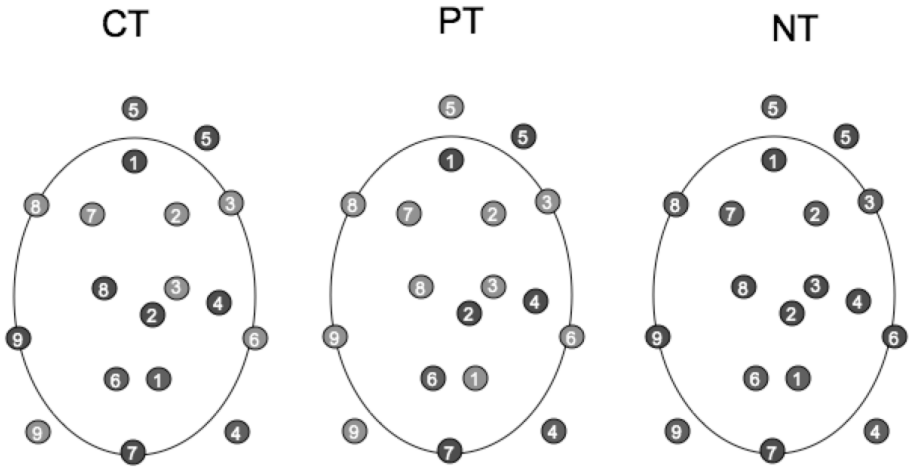

Figure S1

Color indication of significance of task versus rest. Positive ROIs (NT > PT) are displayed in red, negative ROIs (NT < PT) in blue. Colored regions are significantly (de)activated versus rest ($p<0.05$). Grey circles are not. Numbers correspond to regions listed in Table 2. Right side is left hemisphere. Common ROIs are not displayed, they are significant in all tasks.

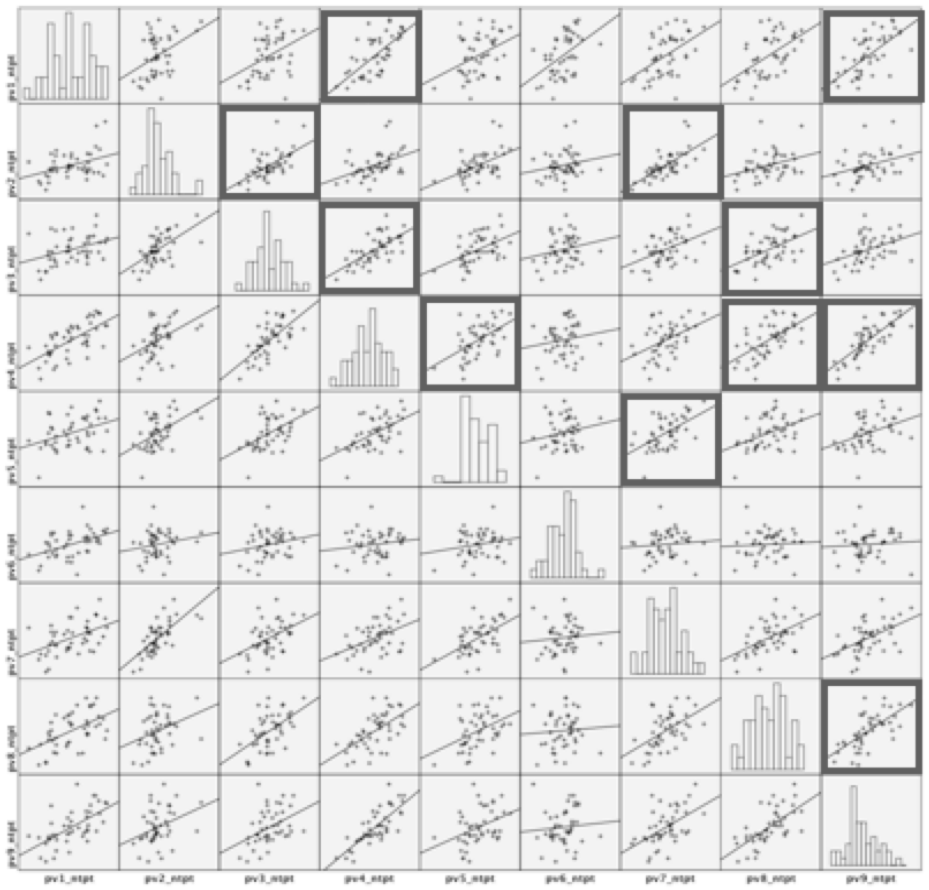

Figure S2

Scatterplots of positive ROI's versus each other, for the NT-PT change in brain activity. Red frames indicate significant correlations (see text for details). Diagonals display distributions of values across subjects, within ROI's.

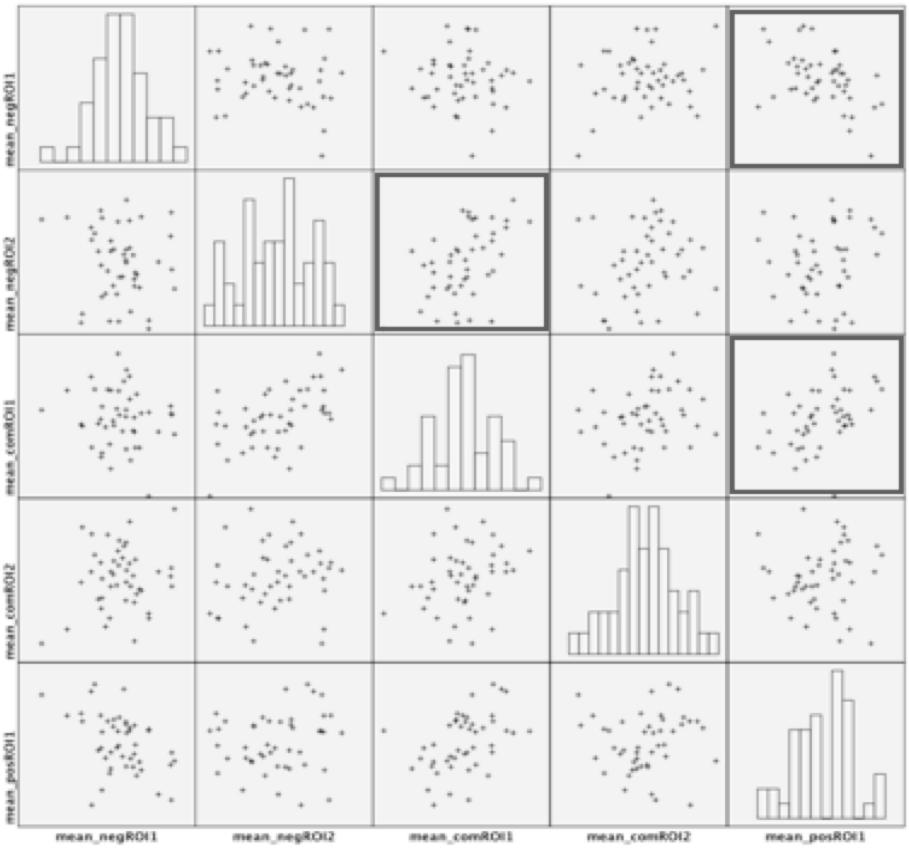

Figure S3

Scatterplots of networks versus each other, for the NT-PT change in brain activity. Red frames indicate significant correlations (see text for details). Diagonals display distributions of values across subjects, within networks.

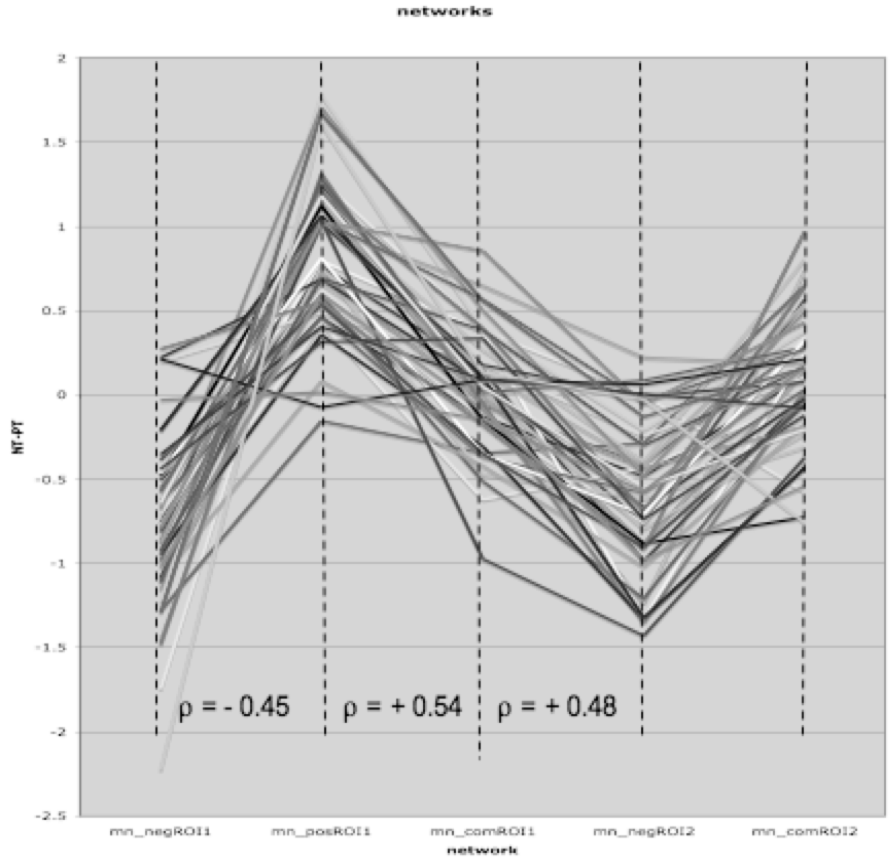

Figure S4

Mean levels of activity change (NT minus PT, displayed on y-axis) for all subjects. Values are shown for the 5 networks on the x-axis. Significant correlations between networks are displayed in the figure.

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Chapter 4
The functional importance of working memory in automatization; an fMRI-guided TMS study

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Abstract

Practice substantially affects performance and brain activity in working memory regions, especially when a task allows for automatization. Yet much remains unknown about the functional importance of working memory for the development of automatization. Here we show that working memory enables the development of automatization and is no longer required when this development is completed. In this study we use fMRI-guided transcranial magnetic stimulation to assess the critical role of working memory in automatization. A modified Sternberg paradigm was used with a novel task, a practiced task and a control task with overlearned information. Individual fMRI maps were used to localize dorsolateral prefrontal and parietal areas where activity decreases with practice. These regions were stimulated with real and sham magnetic stimulation to investigate the effects of brief disruption of working memory on task performance. Novel task accuracy for target responses was more susceptible to interference than practiced task accuracy. This shows that automatization induces a gradual decline in cognitive control. Accuracy of the control task with overlearned associations was not affected by brief disruption of brain activity. This suggests that with sufficient practice, performance may become independent of cognitive control. We propose that working memory enables the development of automatization by restructuring of task-relevant information that allows more efficient implementation of internal task goals.

Introduction

Practice substantially affects performance and underlying brain activity, especially when a task allows for automatization [1]. These tasks typically involve learning associations between sensory input and (motor) actions [1,2]. Previous studies have demonstrated the profound effects of automatization on brain activity in working memory regions [3,4,5]. Yet much remains unknown about the functional importance of working memory for the development of automatization.

Working memory refers to the active maintenance and utilization of internal task goals or rules, in support of complex cognitive performance [6]. Practice decreases activity in working memory regions across different types of cognitive tasks, including verb generation [7], mirror reading [8], delayed response tasks [3,9,10,11] and motor sequence learning [12,13]. This has been interpreted in terms of reduced demands on cognitive control that support early learning or novel task performance [2]. Practice-induced activity decreases in working memory have also been linked to one's capacity to perform multiple tasks at the same time [4]. Understanding the functional importance of working memory for automatization can clarify the neural mechanisms by which automatic behaviors are accomplished and how this contributes to increasing processing capacity with practice.

The objective of the present study is to investigate the functional importance of working memory in the development of automatization with practice. Here we build upon on previous work where we investigated the effects of automatization on brain function by means of a modified Sternberg paradigm [3,4,5]. This paradigm includes a novel task, that requires memorizing and responding to novel stimuli, a practiced task with stimuli that remain consistent over the course of performance and a control task with similar visual and motor requirements. In these previous studies we used functional MRI to localize changes in brain activity patterns associated with automatization. This however does not permit to determine the critical contributions of working memory to automatization. In the present experiment we use transcranial magnetic stimulation (TMS) to assess the critical role of working memory in automatization. TMS can briefly disrupt brain activity and thereby determine a causal relationship between cortical function and behavior [14].

The application of TMS in working memory research has increasingly gained interest in the past few years (see Motthagy [15] for a review). Recently a number of studies have confirmed the functional importance of left dorsolateral prefrontal and parietal cortices for (verbal) working memory [16,17,18,19]. These studies show

that brief disruption of neural activity in these regions significantly impairs working memory performance. In our previous work we consistently demonstrated that the left dorsolateral prefrontal and parietal regions are part of a set of brain regions where activity decreases with practice [3,4,5]. We therefore stimulate these regions in the present experiment to investigate the functional roles of these regions in automatization.

We hypothesize that during novel performance, prefrontal and parietal regions support the development of automatization. Magnetic stimulation was applied after presentation of a probe stimulus (probes were presented in a series after presentation of the memory set, see methods for details). We expect that this will briefly disrupt retrieval of the memory set that is kept active in mind. Consequently, the memorized stimuli may be temporary unavailable or inaccessible [20]. We expect that this will especially affect target stimuli, as they cannot be reliably compared to the temporarily unavailable memory set.

Our previous work and other studies show that brief practice predominantly increases encoding efficiency [5,10,21] and only modestly affects response selection [5]. This suggests that with practice, working memory is no longer required to modify or update task information, but may still be activated to access or retrieve task-relevant information to generate a decision. We therefore hypothesize that interference with working memory will significantly affect practiced task performance, but not as much as for novel performance.

We assume that the control task does not involve working memory retrieval, as subjects respond to arrows. This task may be considered fully automatic, as the relationship between stimuli and responses in this task is highly overlearned. Hence, it is possible that control task performance is independent of cognitive control [22,23]. Brief disruption of working memory may therefore not significantly affect performance on the control task. Nonetheless, even when overlearned, performance on the control task is determined by the context of a task instruction in an experimental setting. Theories on cognitive control suggest an important role of working memory in context representation [24,25]. If working memory enables automatization by providing this context, it is also possible that brief disruption of working memory will affect performance on the control task.

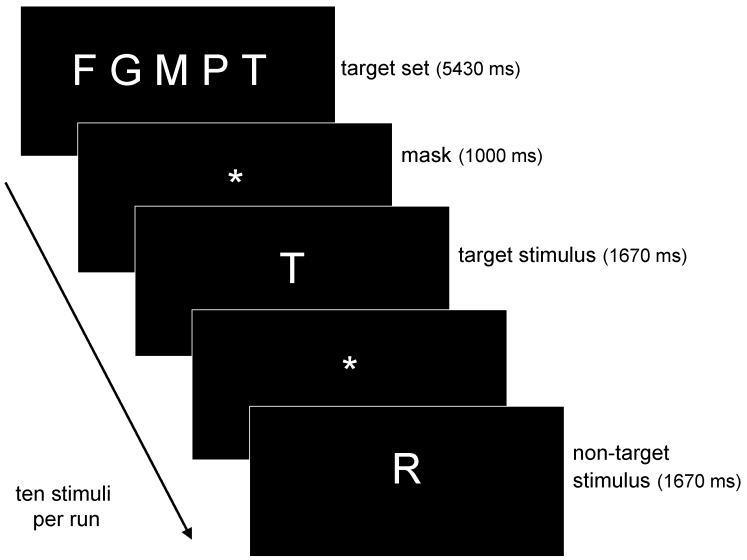

Figure 1.

The temporal sequence is shown for the Sternberg task. Each run starts with the presentation of the target set and is followed by ten probes. Subjects press a left button to targets and a right button to distracters. For NT, the target set was different in each run. The set with target letters was the same in each PT run. For CT the target set consisted of two arrows (< >) and probe stimuli were single arrows (< or >). The task involves 8 runs of NT, PT and CT in a pseudorandom order.

Material and Methods

Subjects

Twenty-one adult volunteers (11 males; mean age= 22.8 years; SD=3.03 years, range = 19-31 years) participated in the study after having given written informed consent. Subjects were recruited from the university campus through advertisement and rewarded for their participation. The M.I.N.I. International Neuropsychiatric Interview (M.I.N.I.) [26] was used to exclude subjects with a history of neurological illness, psychiatric disorders or substance abuse. All participants were tested for right-handedness using the Edinburgh Handedness Index (EHI) [27]. The study was approved by the local medical ethics committee, in accordance with the Declaration

of Helsinki (2004). Participants tolerated the TMS protocol well and did not report any lasting adverse effects.

Cognitive Task

The cognitive task involved a modified version of the Sternberg paradigm (see also Jansma et al. [3] and Ramsey et al. [4]). Subjects were instructed to memorize a set of five letters and subsequently respond to a series of ten probes to indicate whether they matched the memory set (target) or not (distracter), by making a left or right button press (Figure 1). The cognitive paradigm includes a novel (NT) and a practiced task (PT). For NT, the consonants in the memory set were randomly chosen from an array of ten consonants in each run. During PT memory sets involved a fixed set of consonants and were selected from the ten consonants that were not used in NT. The remaining five consonants served as distracter stimuli. In addition, a control task (CT) was included during which subjects responded to the symbols < and > by making left and right button presses respectively. Subjects first performed a training session to practice five series of 100 PT stimuli (approximately 25 minutes), to induce automatization.

Experimental Procedure

The experiment included three experimental sessions; one fMRI session and two separate TMS sessions for frontal and parietal stimulation on separate days. Each TMS session included real TMS and sham stimulation. Prior to each experimental session, subjects performed the training session. fMRI was always performed in the first session, to acquire an individual activity map that served to localize the regions for TMS stimulation. All task versions used in the experiment were programmed in Presentation 9.9. running on a Windows operating system. For both the fMRI and TMS sessions, the task was presented in eight runs of each condition (NT, PT, CT and rest) in a pseudorandom order. Each run included a memory set that was presented for 5000 ms, followed by a series of ten probes each lasting 1200 msec. During the interstimulus interval between probes an asterisk was presented for 1000 msec. Total task duration was approximately 15 minutes. Subjects were instructed to press the left button of a pneumatic MRI compatible push-button box when probes matched the memory set (targets) and the right button if the probe did not match the memory set (distracters). In the TMS sessions, subjects were instructed to press the M on a QWERTY keyboard to targets and the X to distracters. Both keys were clearly marked with an easily found ribbon to prevent searching of the keys during task performance. The left and right index finger rested on the keys during the entire session.

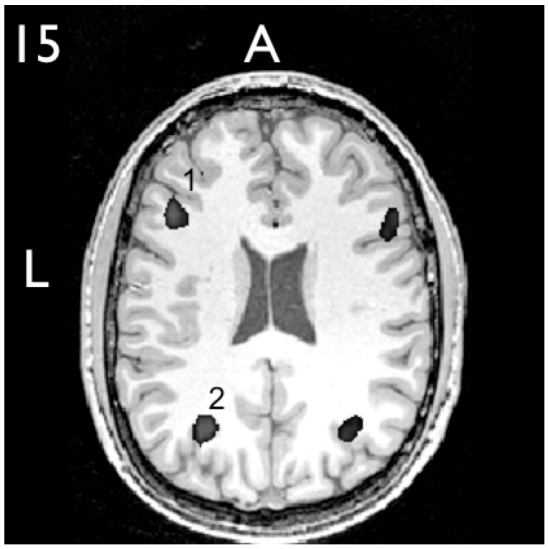

Figure 2.
An individual t-map in native space (single subject, t=3; p<0.05 corrected, L=left, A= anterior, z=15) is shown, displaying activation in the left prefrontal cortex (1) and left parietal cortex (2).

Three task versions with different PT sets were used for fMRI and the two TMS sessions. The same PT set was used during the fMRI session for all participants, in order to acquire reliable and consistent patterns of brain activity. Task versions for TMS were counterbalanced over the first and second stimulation session for the participating subjects. Reaction times (RT) of all correctly identified targets and percentage correct responses were recorded.

Functional Magnetic Resonance Imaging

FMRI was performed on a Philips 3T Intera scanner. Subjects were positioned supinely in the scanner. Head movement was reduced by using a strap around the forehead and foam padding. A mirror attached to the head coil enabled subjects to see a 1 m wide through-projection screen positioned near the feet at 2 m viewing distance. A video projector located outside the scanner room projected the tasks on the screen. A pneumatic push-button box with air pressure cables was used to record responses. A PRESTO SENSE pulse sequence was used for the acquisition of BOLD-sensitive images [28]. Functional images were acquired in two continuous runs of each 832 scans. All images were acquired with the following parameters TE = 32.4 msec., TR = 21.75 msec., voxel size = 4 x 4 x 4, 32 saggital slices and a scan duration of 500 msec. An additional functional image with high flip angle was

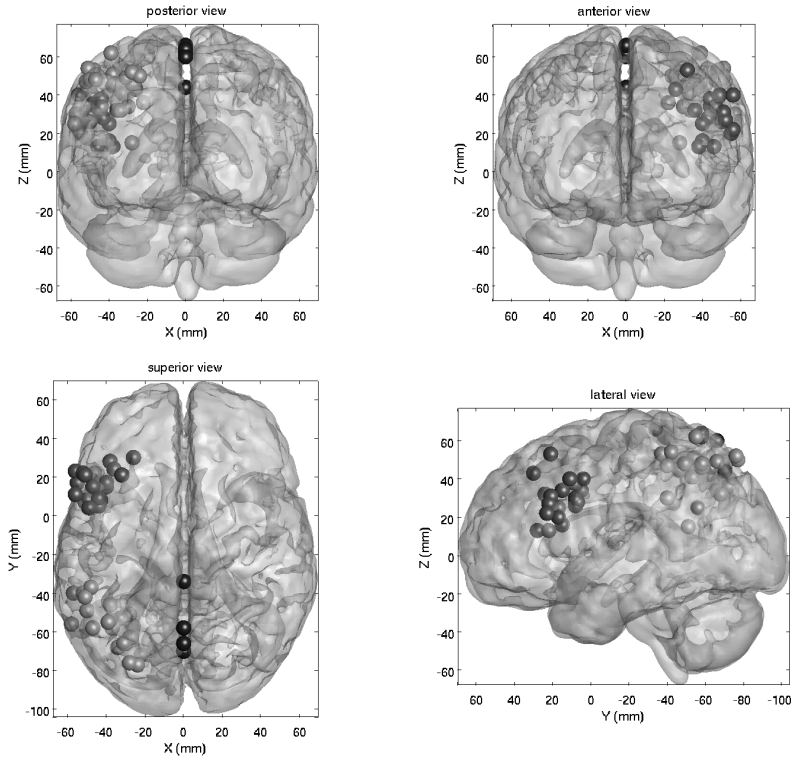

Figure 3.

The individual stimulation locations in the prefrontal (shown in red) and the parietal cortex (shown in green) are displayed in MNI space for all participants in the study. In blue the control region in the mid-saggital plane is displayed that was stimulated in five subjects. The top panel shows a posterior and an anterior view of the stimulation locations. The bottom panel displays a superior and lateral view of the individual stimulation locations.

acquired for registration purposes. A T1-weigthed anatomical image was acquired for spatial localization and for guiding TMS coil navigation.

All functional and anatomical data were processed in SPM5. After motion correction, individual statistical activation maps were generated for the three task conditions (NT, PT and CT, each compared to rest) using multiple regression analysis [29,30]. Individual NT-CT contrasts were generated to reveal working memory related activity and were used for TMS coil navigation to frontal and parietal areas. Individual t-maps (spatially smoothed with a Gaussian kernel with a

FWHM of 8 mm) and anatomical volumes were normalized into standard MNI-305 space [31].

Transcranial Magnetic Stimulation

A frameless stereotactic neuronavigator (NeNa) [32] was used for positioning of the coil above functionally relevant cortical areas. This device enables coregistration of the subject's head to his native MRI space by coregistering anatomical landmarks on the skin of the participants with the same landmarks as selected on a skin rendering based on their MRI scans in the NeNa software. Skin markers were measured with a 3D electromagnetic digitizer. TMS target coordinates were derived from the individual NT-CT fMRI contrast in native space and was entered into NeNa, where they were visible together with the brain and skin rendering. The TMS target coordinates were determined as the voxel with maximal activity in the dorsolateral prefrontal cortex and the superior parietal cortex. After coregistration of the real skin markers and MRI skin markers, the areas on the scalp directly overlying the TMS target coordinates of interest in the brain were marked on a tightly fitting swimming cap worn by the participant. This can be done in real time by moving the 3D digitizer over the scalp, which is visible on the NeNa screen together with the predefined TMS target coordinates.

Magnetic stimulation was applied using a Neopulse TMS device (Neotonic Inc. Atlanta) with an iron core coil. Before the experiment the individual motor threshold was determined, defined as the minimum intensity inducing a visible muscle twitch in the contralateral hand on at least five out of ten occasions (see Schutter et al. [33] for details on this procedure).
TMS was applied with a pulse intensity of 110% of the individual motor threshold. A train of TMS pulses was triggered by a computer. Five pulses were delivered separated by 100 msec. (i.e. 50-550 ms; 10 Hz), after the onset of a probe stimulus. Besides TMS, participants received stimulation with a sham coil to control for nonspecific effects induced by TMS (tactile and auditory sensations). The order of stimulation site (frontal and parietal) and stimulation type (TMS, sham) was counterbalanced, to prevent a bias due to learning, fatigue or habituation. Five subjects also received stimulation of a control region, located in at the midsaggital plane at about 57 mm on the anterior-posterior axis in MNI space.

Individual RT and target accuracy (proportion of correct responses) were obtained for each task condition (NT, PT, CT), type of stimulation (sham, TMS) and site of stimulation (frontal, parietal, control). All mean correct responses were entered in a

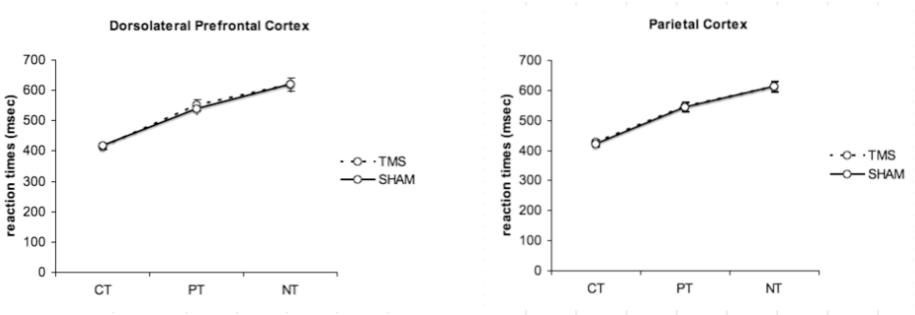

Figure 4.

The average reaction times (msec) of all subjects are shown for target responses. The left panel shows average reaction times of the three tasks during TMS (dashed line) and sham (solid line) stimulation of the dorsolateral prefrontal cortex. The right panel displays average reaction times of the three tasks during TMS and sham stimulation of the parietal cortex.

2 (site) x 2 (stimulation) x 3 (condition) repeated measures General Linear Model analysis.

Results

Region of interests

To localize working memory regions for transcranial magnetic stimulation we compared NT with CT images [3,4] in each individual subject (see material and method section for details). A single subject t-map in native space, representing brain activity in the prefrontal and parietal regions of interest is displayed in figure 2. Individual stimulation locations in the prefrontal and parietal cortex in MNI space for all participants are displayed in figure 3.

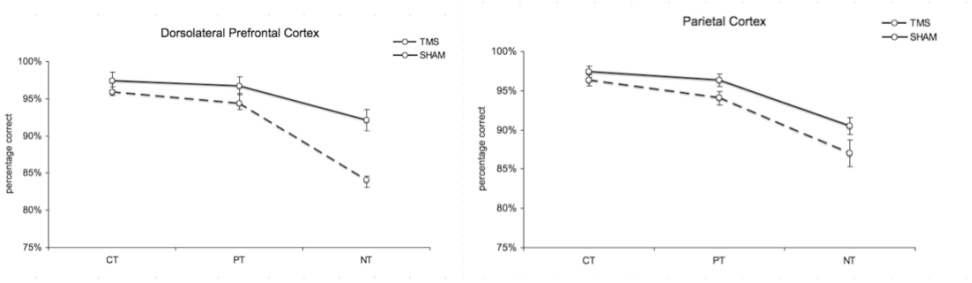

Figure 5.

The average accuracy (percentage correct responses) of all subjects is shown for target responses. The left panel displays accuracy for dorsolateral prefrontal TMS (dashed line) and sham (solid line) stimulation for the three tasks. The right panel shows accuracy of the three tasks for parietal TMS (dashed line) and sham (solid line) stimulation.

Frontal and parietal TMS

Reaction times

First, a GLM was performed on distracter reaction times. Besides the main effect task ($F(2,17)=74.72$, $p<0.0001$), there were no other significant main effects or interactions ($p>0.17$). Planned within-subjects contrasts show that PT reaction times were longer than CT ($F(2,17)=86,48$, $p<0.0001$) but not for NT ($F(2,17)=65.03$, $p<0.0001$). The GLM testing for an effect of TMS on reaction times did not reveal a significant main effect of stimulation ($F(1,18)=0.084$, $p=0.78$), or region ($F(1,18)=0.16$, $p=0.70$). There was only a significant main effect of task ($F(2,17)=56.35$, $p<0.0001$). Planned within-subject contrasts reveal that PT reaction times were significantly longer than CT reaction times ($F(2,17)=70.03$, $p<0.0001$) and that NT reaction times were significantly longer than PT reaction times ($F(2,17)=52.54$, $p<0.0001$). There were no significant interactions ($p>0.13$). Reaction times for target accuracy are displayed in figure 4.

Accuracy

First a GLM was conducted to test the effect of stimulation on distracter accuracy. Besides a main effect of task ($F(2,19)=16.30$, $p<0.0001$), showing that accuracy was different across conditions there were no other significant main effects or interactions ($p>0.14$). This confirms our hypothesis that brief disruption of working memory does not interfere with processing of distracter stimuli.

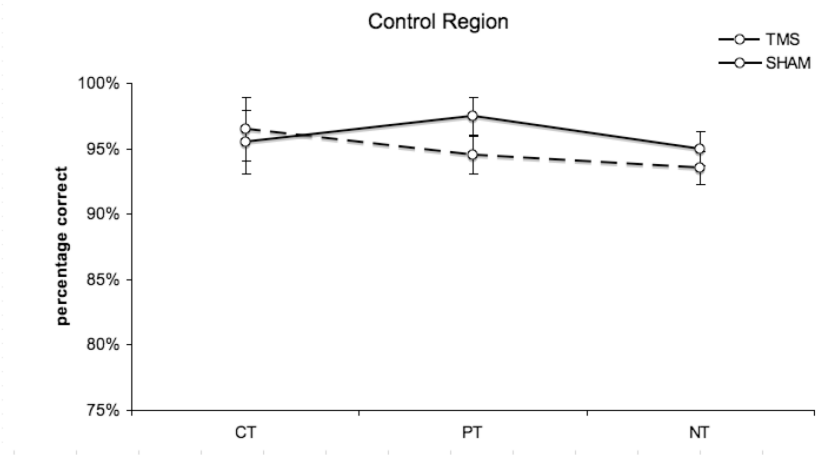

Figure 6.
This graph shows the average accuracy (percentage correct) for target responses for the three tasks during TMS (dashed line) and sham (solid line) stimulation of the control region in five subjects.

Below the results are discussed for target accuracy only (see also figure 5). First we tested the effects of frontal and parietal stimulation on target accuracy for the three tasks (NT, PT and CT). This analysis revealed significant main effects for stimulation ($F(1,20)=19,13$ $p=0.0001$), and task ($F(2,19)=39.66$, $p<0.0001$). The stimulation x task interaction was also significant ($F(2,19=4,43p=0.026$). Planned simple within-subjects contrasts show a deterioration of novel performance that is greater than practiced performance ($F(1,20)=8.09$, $p=0.01$) and also greater than performance on the control task ($F(1,20)=8.08$, $p=0.01$). Neither the main effect nor the interactions involving regions were significant ($p>0.16$). This shows that the effect of TMS on accuracy was similar for the stimulated regions in the prefrontal and parietal cortex.

Separate GLM's for NT, PT and CT were conducted to further examine the effect of TMS in each task condition. The multivariate main effect of TMS was significant for

NT ($F(1,20)=18.22$, $p<0.0001$) and PT ($F(1,20)=7.66$, $p=0.012$) but not for CT ($F(1,20)=0.98$, $p=0.33$). Planned within-subjects contrasts confirm that NT and PT accuracy were both significantly lower during TMS compared to sham stimulation.

To assess the possibility that stimulation in general affects performance, we stimulated a control region not involved in working memory, in five of our subjects. Target accuracy for the control region is shown in figure 6. First we tested whether TMS affected performance in the three tasks during stimulation of the control region. This GLM did not show significant main effects of TMS ($F(1,4)=2.58$, $p=0.18$) and task ($F(1,4)=1.01$, $p=0.46$). This suggests that stimulation of the control region, did not significantly affect performance. The GLM revealed a significant TMS x task interaction ($F(1,4)=9.05$, $p=0.002$, indicating that the effect of TMS on the control region was different for performance of the three tasks. We therefore separately tested the effect of TMS on performance of the three task conditions. The main effect of TMS was not significant in the separate GLM's conducted for CT ($F(1,4)=1$, $p=0.37$), PT ($F(1,4)=4.24$, $p=0.11$) and NT ($F(1,4)=0.64$, $p=0.47$). Next, we conducted a GLM on the NT target accuracy scores during stimulation of the three regions in these five subjects. This revealed a significant main effect for region ($F(2,3)=14,88$, $p=0.03$). Planned simple contrasts show that the effect of frontal stimulation on performance was significantly larger than stimulation of the control region ($F(1,4)=16$, $p<0.02$). In summary, TMS caused significant performance deterioration for NT and moderate deterioration for PT, but did not significantly affect CT performance.

Discussion

The present study used fMRI-guided TMS to investigate the functional importance of working memory in automatization. We hypothesized that working memory enables the development of automatization. We therefore predicted a larger disruption in novel performance than practiced performance. If working memory supports automatization by providing an internally represented context (e.g. task instruction), we also expect an effect of stimulation on performance on the control task with overlearned stimuli.

The results show a remarkable deterioration for novel performance when working memory was briefly disrupted. Interference with working memory induced a modest decline in practiced performance, and did not affect processing of overlearned information. This is consistent with our hypotheses regarding the functional importance of working memory for novel and practiced performance and indicates

that automatization enables a gradual decline in cognitive control. In addition, our data suggests that automated performance can occur independent of working memory.

Our data show that when performance is novel, working memory is important to support learning of associations between sensory information (e.g. alphabetical stimuli) and actions (selection of an appropriate response). This is in line with current models of practice [2] and cognitive control [25]. As expected, stimulation significantly interfered with target accuracy and not distracters. It confirms our idea that due to this brief disruption, the memory set with target letters was temporarily unavailable or inaccessible [20]. Target stimuli could therefore not be reliably matched to the memorized target set and were incorrectly classified as a distracter.

Interference with working memory only moderately affected practiced performance. This suggests that also after a brief period of practice working memory was activated. This is consistent with our previous work [5] and other studies [10,21] that indicate modest effects of brief practice on brain activation associated with response selection. We assume that also with some practice, the memory trace with target letters is reactivated when a response is required based on the contents of working memory. Our data further suggest that when processing is overlearned, working memory is no longer required. It should be noted however that the type of stimuli differed in the control task. We should therefore be cautious in interpreting the lack of an effect on control task performance. It may implicate however that with sufficient practice, task representations supported by working memory are no longer activated to guide task execution.

Our data suggest that working memory supports automatization by active maintenance and utilization of internally represented rules or goals (e.g. the set of target stimuli) for guidance of task execution. Our data further indicate that over the course of practice internal representations of task information become less important to support task performance. This is consistent with theories of categorization learning [34,35]. These theories assume that working memory is important for learning categories, by internally representing category structures (e.g. targets or distracters) and selecting responses based on category membership [34,35]. In their reviews both Seger [35] and Ashby [34] propose that categorization becomes increasingly independent of working memory with practice, as corticocortical connections between visual and motor cortices become strengthened. Both assume a putative role for the basal ganglia in this process.

The basal ganglia have been implicated in habit and stimulus-response learning [36]. They have been proposed to recode or restructure input received from cortical regions, into 'action chunks' [36,37]. This allows for more efficient implementation of 'performance plans' for learned behaviors [36]. Prefrontal and parietal regions have also been proposed to mediate 'chunking' strategies to more efficiently represent complex types of information [38,39]. The corticostriatal loop may therefore play an important role in the transition of controlled to automatic processing. In the present study, working memory thus may contribute to restructuring of the letter sequences in some arrangement (e.g. pseudo-words) to facilitate the development of automatization.

There was no evidence that working memory significantly contributed to performance of the control task. This task is similar in visual and motor requirements, but compared to the novel and practiced tasks, lacks the requirement to actively keep multiple items in mind. Also, the control task involves object information (arrows), while PT and NT involve verbal information. This could explain why interference with brain regions that have implicated in the phonological loop of working memory [40,41] did not affect control task performance. The dorsal prefrontal cortex however, has also been implicated in representing domain-general task information, such as task instructions, rules or goals [24,25]. The three tasks in the present experiment all involve arbitrary relationships between stimuli and responses that are determined by the task instruction (e.g. if you see A, press B). Our data thus indicate that automated performance that is based on a task instruction (i.e. that is not yet reflexive) can occur without significantly activating working memory.

It should be noted that other nodes in the working memory network might have compensated for the brief interruption in frontal and parietal activity. Current views of working memory emphasize that working memory should not be considered as a centralized executive processor that is located in a brain region, but rather emerges from interactions between the prefrontal cortex and posterior regions in the brain [25,42,43]. The prefrontal cortex has been found to coactivate with Broca's area, the premotor cortex and the parietal cortex in verbal working memory studies [3,40,41]. Also, Herwig et al. [44] has demonstrated critical contributions of the premotor cortex, rather than prefrontal and parietal cortex during the brief delay in a working memory task when verbal information should is actively maintained. It may thus be possible that in the present experiment other regions important for verbal working memory compensate for the disrupting effect on prefrontal and/or parietal

activation. This could explain that in the present study TMS interference with working memory did not result in a massive breakdown of performance.

Finally, there are some methodological considerations that should be taken into account. The TMS apparatus used in the current study involved different coils for real and sham stimulation. Although we did not tell participants about the different coils, they reported to be aware of the different effects of two types of stimulation. Performance of the control task however was not significantly affected by TMS. This argues against the idea that the data could have been biased towards more inaccurate performance during real stimulation because subjects knew about the interfering effects of TMS. Another consideration is that we only acquired data from a control region in a small sample of our subjects. This may limit the interpretation of the stimulation effects in the prefrontal and parietal regions. However, with the inclusion of the sham stimulation condition and the control task with overlearned information we argue that the results are robust enough to claim the importance of working memory for automatization.

Conclusion

The present study shows that automatization induces a gradual decline in cognitive control. When performance is novel working memory enables the development of automatization and with sufficient practice performance may become independent of cognitive control. We postulate that over the course of practice working memory, possibly in interaction with other networks in the brain, may engage in restructuring of information that facilitates the development of automatic behaviors that may eventually become independent of control.

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Chapter 5
Automatization and working memory capacity in Schizophrenia

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Abstract

Working memory (WM) dysfunction in schizophrenia is characterized by inefficient WM recruitment and reduced capacity, but it is not yet clear how these relate to one another. In controls practice of certain cognitive tasks induces automatization, which is associated with reduced WM recruitment and increased capacity of concurrent task performance. We therefore investigated whether inefficient function and reduced capacity in schizophrenia was associated with a failure in automatization. fMRI data was acquired with a verbal WM task with novel and practiced stimuli in 18 schizophrenia patients and 18 controls. Participants performed a dual-task outside the scanner to test WM capacity. Patients showed intact performance on the WM task, which was paralleled by excessive WM activity. Practice improved performance and reduced WM activity in both groups. The difference in WM activity after practice predicted performance cost in controls but not in patients. In addition, patients showed disproportionately poor dual-task performance compared to controls, especially when processing information that required continuous adjustment in WM. Our findings support the notion of inefficient WM function and reduced capacity in schizophrenia. This was not related to a failure in automatization, but was evident when processing continuously changing information. This suggests that inefficient WM function and reduced capacity may be related to an inability to process information requiring frequent updating.

Introduction

Cognitive dysfunction is a core characteristic of schizophrenia [1] and associated with deficits in working memory (WM). WM refers to the temporary maintenance and utilization of information [2] and is considered important for complex cognitive performance [3]. WM dysfunction in schizophrenia is characterized by inefficient prefrontal function as most patients exhibit excessive activity when performing a moderately difficult WM task [4,5,6,7,8,9]. When performing a more difficult WM task (i.e. with more information that has to be memorized and processed), they generally exhibit poor performance and lower levels of WM activity than controls [10,11,12,13], indicating that their WM capacity is quite limited [5,8,9]. In spite of structural abnormalities in the prefrontal cortex [14,15], and of genetic variation contributing to prefrontal activation levels [16,17,18,19] it is not clear what causes inefficient WM function and reduced WM capacity in schizophrenia.

Recently we demonstrated that the ability to reduce demands on WM with practice is closely related to the capacity to perform an additional task simultaneously in controls. If a cognitive task involves a constant relationship between stimuli and responses, practice can induce a shift from demanding to effortless processing, which is referred to as automatization. In a previous study we demonstrated that automatization is associated with improved performance and reduced activity in WM. In a related study we compared the difference in WM activity after practice to the ability to perform two tasks simultaneously. It was found that subjects with a larger reduction in WM activity after practice were better at performing two tasks concurrently [20]. This suggests that the drop in WM activity induced by practice may reflect an increase of available WM capacity to accommodate concurrent task performance.

Although behavioral tests of automatization indicate that schizophrenic patients improve performance with practice [21,22] several studies have shown that patients are either unable to process a second task concurrently [23,24] or need significantly more practice to achieve normal dual-task performance [21,22,23,24,25]. This suggests that schizophrenic patients may fail to reduce neural activity in spite of improved after practice and are therefore unable to liberate sufficient neural resources to perform additional tasks simultaneously.

This raises the question whether inefficient WM function and limited capacity in schizophrenia could be associated with a failure in automatization. To test this, brain

Table 1. Demographic and illness-related variables in healthy controls and subjects with schizophrenia

	Patients with schizophrenia				Healthy controls				
	N	*Mean*	*SD*	*Range*	*N*	*Mean*	*SD*	*Range*	*p-value* [a]
Male	14				13				-
Female	4				5				-
Age (years)		28.4	7.4	19.6-41.8		26.0	5.8	18.9-43.8	n.s.
EHI index		0.80	0.19	0.42-1.00		0.86	0.20	0.33-1.00	n.s.
PANSS (sum)									
Positive scale		1.77	0.57	1.00-2.83					
Negative scale		2.10	0.72	1.00-3.29					
General scale		1.59	0.32	1.06-2.19					
Length of Illness (years)		5.23	4.36	0.77-17.53					
Age of Onset (years)		23.17	5.04	15-33					
Medication (mg/day)									
Clozapine	7	145.36	179	0-400					
Quetiapine	1	700	-	700					
Risperidon	3	1.33	0.58	1-2					
Olanzapine	6	10.75	7.64	2-20					

[a] Significance of differences calculated by using t-tests and nonparametric Kolmogorov-Smirnov Z-test, two-tailed

activity was examined with fMRI using a verbal WM task with novel and practiced stimulus sets [26]. Subjects subsequently participated in a behavioral dual-task paradigm to measure their ability of concurrent task performance.

Methods and Materials

Subjects

Patients were recruited from the Department of Psychiatry of the University Medical Center Utrecht. DSM-IV diagnosis of schizophrenia was confirmed using The Comprehensive Assessment of Symptoms and History (CASH) [27] and severity of symptoms was assessed with The Positive And Negative Syndrome Scale (PANSS) interview [28]. Controls were recruited through advertisement and were rewarded for participation. The Mini International Neuropsychiatric Interview (M.I.N.I.) [29] was used to exclude controls with a history of neurological illness, psychiatric disorders or substance abuse. All participants were tested for right-handedness using the Edinburgh Handedness Index (EHI) [30]. Initially, 18 controls and 22 patients were scanned after signing an informed consent. In total four patients were

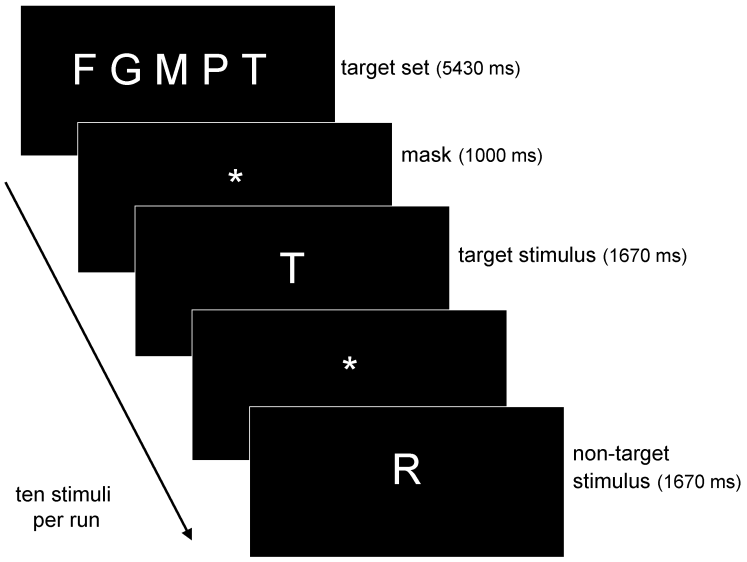

Figure 1.
The temporal sequence is shown for the STERN task. Each epoch starts with presentation of the target set and is followed by ten probes. Subjects have to press a button as fast as possible if the probe letter belongs to the set of targets.

excluded from the study; one could not complete the scan procedure due to technical problems with the scanner, three were excluded from analysis because their performance was at chance level. Presented results are based on the remaining cohort with 18 controls and 18 patients matched for age and gender. Demographic and illness-related details are shown in Table 1.

Assessment of automatization

The fMRI experiment to test automatization involved a modified version of the Sternberg paradigm (STERN) (see also [20,31,32]). Subjects were instructed to memorize a set of five letters and subsequently respond to matching probes (targets) (Figure 1). A novel (NT) and a practiced task (PT) were administered. In PT a fixed set of target and non-target stimuli was selected from an array of ten preset letters [33]. In NT, target and non-target letters were randomly chosen from

the ten remaining consonants that were not used in PT. In addition, a control task (CT) was included during which subjects made a button press when the symbol '<>' appeared. Prior to fMRI subjects performed five series of 100 PT stimuli (25 minutes) to induce automatization. During scanning an epoch (memory set and ten probes) lasted 32 s. The four tasks (NT, PT, CT and rest of equal epoch length) were presented eight times in a pseudo-randomized and counterbalanced order. Reaction times (RT) of all correctly identified targets and percentage correct responses for all stimuli were recorded.

Assessment of capacity: dual-task paradigm

After fMRI, STERN (five NT and five PT blocks) was performed concurrently with a selective attention task (SAT) outside the scanner. SAT involved detection of tones with a higher or lower pitch than a baseline tone (see also [20,31]). Task difficulty of SAT was standardized for each subject prior to the experiment, by adjusting pitch difference until the subject detected 80% of the deviant tones. The 200 ms tones (16% deviants randomly distributed) were presented in blocks of 25 seconds. STERN intertrial interval in the dual-task was 2500 ms with stimulus duration of 1500 ms. Although tones and letters frequently coincided, only on three out of 64 occasions a tone and STERN target overlapped. To prevent interference at the response level, subjects silently counted target tones and verbally reported the number after each series of 25 stimuli. STERN and SAT were also administered separately. STERN and SAT "performance cost" was defined as the difference in performance on the dual versus the single tasks and was also computed for NT and PT blocks separately.

fMRI procedure and acquisition

FMRI was performed on a Philips 1.5 T Intera scanner. Subjects were positioned supinely in the scanner. Head movement was reduced by using a strap around the forehead and foam padding. A mirror attached to the head coil enabled subjects to see a through-projection screen positioned near the feet. A video projector located outside the scanner room projected the tasks on the screen. A pneumatic push-button box with air pressure cables was used to record responses. To measure BOLD signal changes we used a three-dimensional navigated PRESTO pulse-sequence [34]. A single run of 384 scans was acquired over a period of 17 minutes. This was followed by one high-contrast scan for registration purposes and a 3-D anatomical scan for spatial localization (for scan parameters see [31,32]).

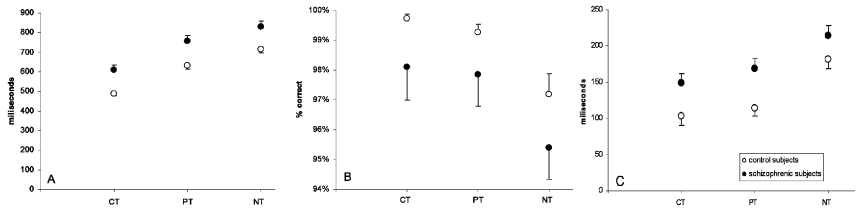

Figure 2.

Graphs show behavioral fMRI-scan performance data of STERN. Mean reaction times (± SEM) of correct target responses for both groups and mean correct (misses and false alarms) as percent of all trials (± SEM).

Data analysis: behavioral data

Individual mean and variance of RT calculated over all correct responses and percentage of correct responses of STERN during fMRI were tested with a general linear model (GLM) with repeated measurements, with practice (NT and PT) as within-subject factors and group (patients and controls) as between-subject factor. Dual-task performance cost was separately tested with a GLM with task (STERN and SAT) and practice (NT and PT) as within-subject factors and group (patients and controls) as between-subject factors.

Data Analysis: imaging data

After motion correction, fMRI signals were analyzed voxelwise, using multiple regression [35,36]. This resulted in individual activation t-maps for the three tasks contrasted with rest. Individual t-maps (spatially smoothed with a Gaussian kernel with a FWHM of 8 mm) and anatomical volumes were normalized into standard MNI-305 space [37]. A group t-map was calculated for NT-CT by testing the difference values in each voxel against zero. A value of 4.51 was used as an activity threshold, corresponding to a p-value of < 0.05 (onesided) Bonferroni-corrected for total brain volume. For all significantly active regions in the group map with a cluster size of at least ten voxels, a mean activity score (b-values obtained from the regression analysis) was obtained for NT, PT and CT for each subject. These final variables were entered into repeated measurements GLM analysis with practice (NT and PT) and region (listed in table 2) as within-subject factors and group (patients and controls) as between-subjects factor.

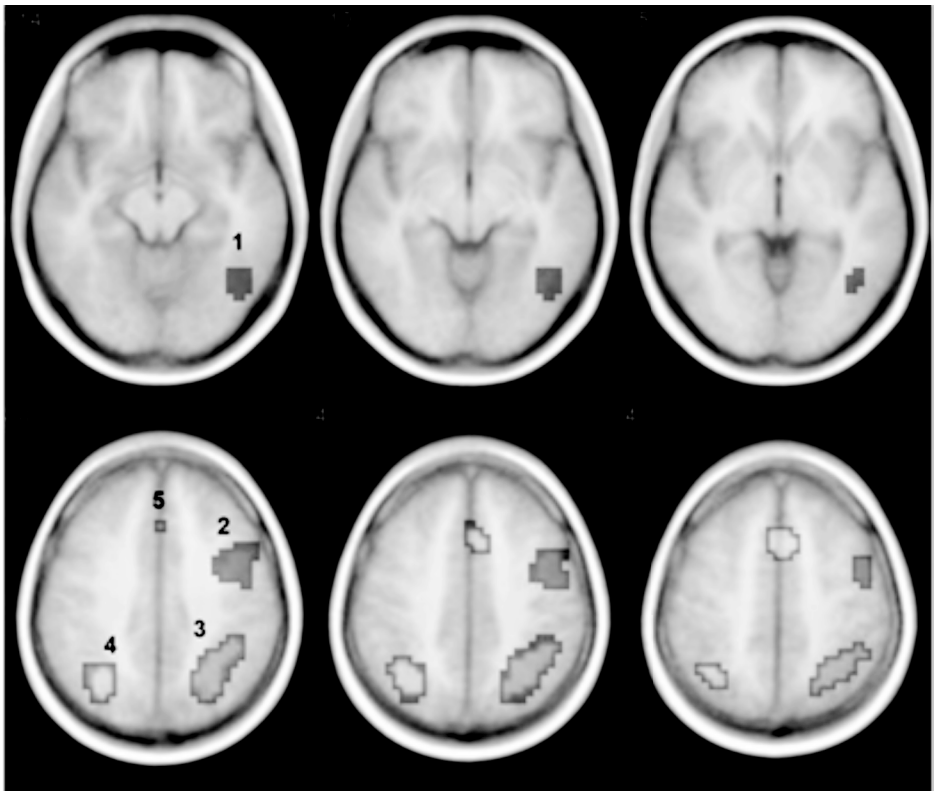

Figure 3.
Combined group map of patients and controls showing WM regions: 1. left fusiform gyrus LFG, 2. left prefrontal cortex LPFC, 3. left superior parietal cortex LSPC, 4. right superior parietal cortex RSPC, 5. anterior cingulate cortex ACC. The numbers in the slices correspond to MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).

Results

Practice and working memory

Effects of practice on working memory performance

The performance results are shown in Fig.2. NT reaction times were significantly longer than in PT in both groups (main effect of task [$F(1,32)=57.1$; $p<0.0001$]). Patients were overall slower than controls (main effect of group [$F(1,32)=13.9$; $p=0.001$]). There was no significant group by task interaction [$F(1,32)=0.12$; $p=0.65$], indicating that patients improved RT with practice to the same degree as controls.

Table 2. Working memory regions (STERN)

Region	Brodmann area	Number of voxels	X	Y	Z	Maximum z-value
Left Fusiform Gyrus (LFG)	37	14	45	-59	-11	10.53
Left Dorsolateral Prefrontal Cortex (LFC)	9/46	193	46	11	29	14.36
Left Superior Parietal Cortex (LPC)	7	133	33	-56	41	10.31
Right Superior Parietal Cortex (RPC)	7	56	-32	-60	42	8.77
Anterior Cingulate Cortex (ACC)	6/24	57	4	23	53	18.52

Although RT were more variable in schizophrenia across conditions (main effect of group [$F(1,26)=9.29$, $p=0.005$]), RT variability was larger in NT than in PT in both groups (main effect of task [$F1,26)=19.01$, $p<0.0001$]). There was no significant group by task interaction [$F(1,26)=0.76$, $p=0.39$], suggesting that the effect of practice on RT variability was similar in both groups. Patients and controls did not significantly differ in percentage of correct responses [$F(1,32)=2.45$, $p=0.13$]. The main effect of task shows that performance was more accurate after practice [$F(1,32)=13.98$, $p=0.001$]. The group by task interaction was not significant [$F(1,32)=0.10$, $p=0.75$]. This suggests that the effect of practice on accuracy was similar in both groups. In summary, although RT were generally slower and more variable in patients, practice improved performance to the same degree in both groups.

Overview of regions of interest

Regions in frontal (left dorsolateral prefrontal cortex (LPFC) and anterior cingulate cortex (ACC)) parietal (left superior parietal cortex (LSPC) and right superior parietal cortex (RSPC)) and visual cortex (left fusiform gyrus (LFG) reached significance in both groups and were used for further analyses (Table 2 and Fig. 3). These regions are the same in the previous studies with this task [20,31,32]. Patients did not activate additional regions.

Effects of practice on working memory function

To assess the effect of practice on WM activity mean b-values from individual NT and PT maps of the five ROI's were used in the analysis. Practice significantly reduced brain activity in all WM regions in both groups (main effect of practice

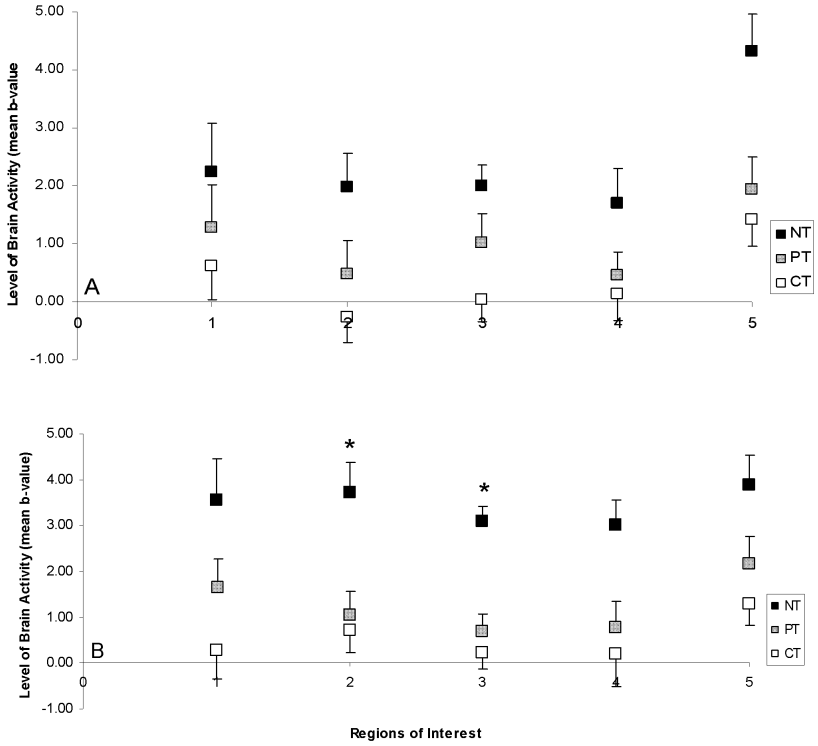

Figure 4.

The graphs show 1. the decrease in activity in WM areas, in response to reduced WM load after practice in both groups 2. excessive activity in LPFC and LSPC in patients with schizophrenia (* $p<0.05$) when processing novel information. The y-axis shows mean b-values and represents level of activity.

[$F(1,32)=142.10$; $p<0.001$] (Figs. 3 and 4). Practice induced a larger drop in brain activity schizophrenics (group by practice interaction [$F(1,32)=8.79$; $p=0.005$]). Post-hoc ANOVA indicates that this was due to excessive activity in schizophrenia during NT, especially in LPFC [$F(1,32)=5.04$; $p=0.03$] and LSPC [$F(1,32)=6.96$; $p=0.012$] (Figure 3)). These results indicate that patients excessively activated WM, especially left prefrontal and left parietal regions during NT, but were capable of normalizing levels of brain activity after practice.

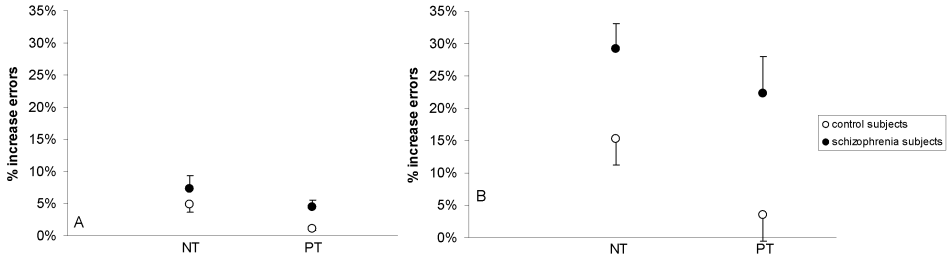

Figure 5.

STERN and SAT dual task performance cost (outside the scanner). The vertical axis of the graph shows the difference in accuracy between single and dual task performance of STERN and SAT respectively; a high value corresponds with higher performance cost. Patients show disproportionately higher performance cost than controls, especially on SAT (* $p<0.05$).

Practice and processing capacity

Dual task performance

STERN and SAT performance cost scores (Fig. 5) were calculated to assess WM capacity. Patients showed disproportionate performance cost for both SAT and STERN (main effect of group [$F(1,32)=10.53$; $p=0.003$]). SAT performance cost was higher than STERN (main effect of task [$F(1,32)=21.42$; $p<0.001$]), indicating that SAT performance was more influenced by simultaneous processing demands than STERN. The difference in STERN and SAT performance cost was more pronounced in schizophrenia [task x group interaction $F(1,32)=5.64$; $p=0.02$)]. Practice reduced perance cost in both tasks as was shown by a main effect of condition [$F(1,32)=9.03$; $p=0.005$]. Thus for both groups performance cost was larger for SAT than STERN, but practice reduced performance cost in both tasks. In addition, patients showed excessive performance cost for both tasks and especially for SAT.

Practice-related activity changes in WM and performance cost

The difference in WM activity after practice predicted STERN performance cost in controls ($r=0.58$, $p<0.02$) (Fig. 6). Thus, subjects with a larger drop in activity were better at maintaining STERN performance when a second task was added [20]. There was no such relationship in schizophrenia ($r=-0.16$, $p=0.54$) (Fig. 6) The difference between the correlations was significant: (Fisher z-transform, $z= 2.256$, $p=0.024$ [38].

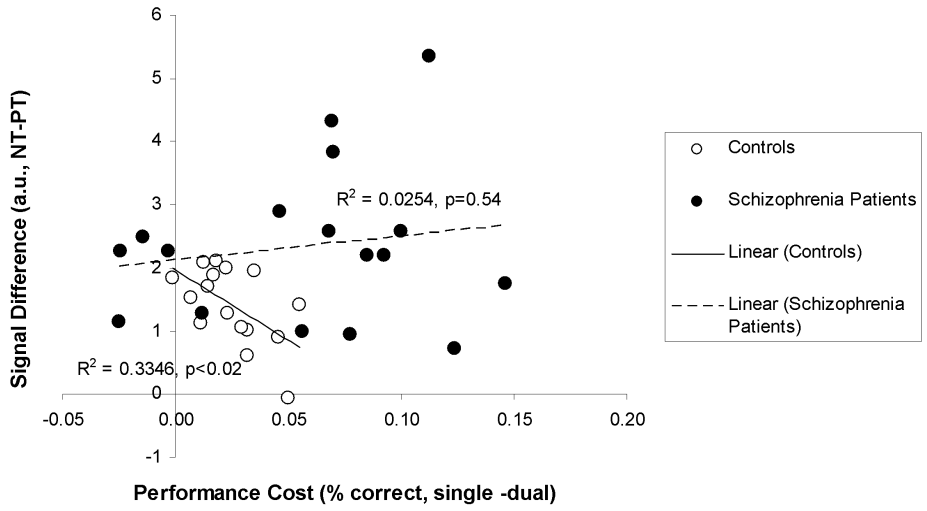

Figure 6.

Measures of WM efficiency and capacity. On the vertical axis, the difference in activity between NT and PT is shown as a measure of efficient WM function. A larger value indicates a larger reduction in brain activity; i.e. increased efficiency. The horizontal axis represents performance cost, which is inferred from comparing single task performance to dual task performance. A larger value corresponds with larger performance decrement in the dual task.

Discussion

To investigate whether inefficient WM function and reduced capacity in schizophrenia were associated with a failure in automatization, schizophrenia and healthy subjects performed a WM task with novel and practiced stimuli during fMRI and a dual-task outside fMRI.

Although RT were generally longer and more variable in schizophrenia, patients displayed normal accuracy on the novel WM task which was accompanied by excessive brain activity in WM regions. Practice improved performance in patients to the same extent as in controls and normalized excessive levels of activity. While practice reduced [39] performance cost in both groups, patients exhibited overall disproportionate performance cost in the dual-task, especially during tone-counting. The difference in WM activity after practice predicted STERN performance cost in controls but not in patients.

Excessive WM activity during novel WM performance in patients confirms the notion of inefficient WM function in schizophrenia [5,6,8,9,13,40]. Patients were able to improve performance and reduce inefficient activity down to normal levels with practice. This is in line with most behavioral studies [21,22], however not many studies have yet investigated the effect of practice on brain function in schizophrenia. Most studies investigated procedural learning [41,42]; a rule-based type of skill acquisition that is implicitly acquired through practice [42], and have reported abnormal patterns of brain activity associated with either intact [41] or impaired procedural learning [42]. A major difference with procedural learning however is that automatization does not involve implicit acquisition as memory sets were explicitly practiced during training.

Excessive performance cost in patients complements other studies reporting impaired dual-task performance in patients with schizophrenia [21,22,23,24]. A study by Oram et al. [39] however reported that a tone-counting task did not significantly affect performance on a simple visual search task in patients with schizophrenia. In our study the impact of the visual task (STERN) on tone-counting was larger than the reciprocal effect of tone-counting on STERN performance. In Oram et al.'s study the effect of the visual search task on tone-counting performance was not reported. This does not exclude the possibility that visual search may have affected tone-counting performance as was the case in our study.

The drop in WM activity after practice predicted performance cost in controls. Thus subjects with a larger difference in WM activity after practice were better capable to utilize WM capacity to resolve interference between tasks when executed simultaneously [20]. The lack of such a relationship with performance cost in schizophrenia suggests that the dual-task was too difficult and that patients may have failed to recruit WM to accommodate concurrent task performance. Our data thus shows that patients were able to normalize initial inefficient WM function with practice. The question however remains why concurrent performance results in greater performance cost in schizophrenia.

SAT and STERN both activate WM, which is most likely involved in storage of verbal and auditory information and executive processes associated with response selection (STERN) or updating the count of target tones (SAT). Executive processes in a dual-task cannot be performed on more than one task at the time [43,44]. When SAT and STERN are performed concurrently processing conflicts

may thus arise as they compete for common WM resources [45,46,47]. This may induce errors as the interfering task temporarily disrupts and delays ongoing processing [43,44]. Excessive performance cost in schizophrenia may therefore reflect aggravated disruption of ongoing processing in a dual-task and may be associated with greater competition for WM to resolve processing conflicts when simultaneously performing STERN and SAT.

Performance cost was most pronounced for SAT. This task requires continuous adjustment of temporarily stored information each time an oddball is detected. Disproportionate SAT performance cost in schizophrenia further suggests that patients are most susceptible to the disruptive effect of a concurrent task when information needs to be frequently updated. Together with our finding of inefficient brain function during WM performance with continuously changing information, this may indicate that schizophrenia is associated with a failure to properly recruit WM when information changes frequently and thus requires continuous updating.

In spite of overall impaired dual-task performance, practice reduced performance cost in patients as well. This is in agreement with other behavioral studies reporting that patients are able to improve dual-task performance to some extent with practice on a single task [21,22]. The question may rise whether practicing the dual-task would have reduced performance cost in patients. Dual-task practice may reorganize two tasks into a single task, which may eliminate processing conflicts [48]. A recent study [48] compared the effects of single-task and dual-task practice on subsequent dual-task performance, but did not find evidence that dual-task practice induced more efficient task integration than single-task practice. Although it is likely that patients would improve dual-task performance with dual-task practice we do not expect that this would eliminate the difference in performance cost with controls.

The training session in the current study to induce automatization was relatively short. It has been argued that automaticity is not completed until performance has reached plateau level [49]. On the other hand it has been suggested that automaticity is induced after only a few trials of practice [50]. Although we cannot draw conclusions about potential long-term changes in brain function and performance associated with automatization, our current results suggests that during the early phase of practice patients were able to improve task performance and reduce demands on WM.

RT may be a more precise measure for performance cost [44]. In the current study however we used accuracy to calculate performance cost, as in our previous work this was found to be correlated with the difference in WM activity after practice in controls. In addition, in order to minimize interference at the level of response selection between the two tasks, only one task in this paradigm required a manual response, RT measures were therefore not available for both tasks.

All patients in the present study received pharmacological treatment with atypical antipsychotics. Studies have indicated that antipsychotics facilitate automatization in schizophrenia [21]. Particularly patients taking atypical antipsychotics became more efficient than patients on typical antipsychotics in performing a practiced task simultaneously with an additional cognitive task [22]. It is therefore possible that atypical antipsychotic medication may have positively affected automatization in schizophrenia. However this cannot explain the disproportional performance loss in the dual-task in our patients.

Several other potential limitations could be relevant to the interpretation and implications of the present study. For one, general intelligence may have been different for both groups. Although IQ was not assessed in the present study, we did find that both groups had equal numbers of years of education (Table 1). Moreover, elevated activity during NT is not likely to be the result of potentially lower IQ levels in patients: previous studies in healthy volunteers have reported a positive correlation between intelligence and magnitude of brain activity (eg [51]), which would predict patients to exhibit less rather than more activity in the WM network. As with other studies, the implications for schizophrenia as a whole should be considered with several limitations in mind. Given the fact that only 18 patients were included who were all capable of performing the task, that only a few females were assessed and that the duration of illness (i.e. 5 years) was relatively short, the results may not generalize across the schizophrenia spectrum. It may well be that more severe, chronic patients would perform more poorly and exhibit different brain activity levels. Inclusion of such patients, however, requires a different approach to data analysis and interpretation because poor task performance has complex effects on brain activity (e.g. [20]).

To conclude, the ability to reduce inefficient WM function with practice does not support the notion that automatization is impaired in schizophrenia. We also did not find a relationship between the difference in WM activity after practice and performance cost in schizophrenia. Together, this may suggest that inefficient WM

function and reduced capacity in schizophrenia are associated with a failure to properly engage WM when task demands are increased (i.e. during novel WM performance and when performing an additional task concurrently). In addition, this WM failure may be specifically related to an inability to process continuously changing information requiring frequent updating.

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Chapter 6
Summary and general discussion

Summary

The research in this thesis investigates the effect of automatization on brain function, to better understand the neurophysiology behind practice and its beneficial effect on our limited processing capacity. This thesis also aims to explain the neural basis of deficient processing capacity in schizophrenia. Patients suffering from this disease seem to have a more serious limitation in the amount of information they can process. Here we investigate whether a deficit in automatization can explain the severely limited processing capacity in schizophrenia.

This thesis builds on previous work suggesting an important role for working memory in the development of automatization and the ability to improve processing capacity with practice. The nature of activity changes in working memory regions and how they contribute to processing capacity are still poorly understood. Much remains unknown about the course of activity decreases for distinct behavioral components of automatization. In **chapter 2** we examined automatization of different components of performance. These components involve encoding and response selection. A rapid event-related fMRI design [1] was used to isolate effects of practice on brain activity related to encoding and response selection. Practice promptly reduced activity across the entire regional network involved in encoding, even before response selection activity and performance were affected. Changes in response selection activity emerged later and were not present in all regions. Our results indicate that practice can independently reduce working memory activation for different components of performance over the course of practice. These heterogeneous changes in activity do not fully support the view that practice induces domain-general (i.e. task-independent) effects on brain activity [2]. Our findings are more in line with current views on working memory [3,4] and cognitive control [5], by showing that over the course of practice, performance is supported by a dynamic allocation of working memory, depending on the level of control needed. Our findings may bear importance in understanding the role of automatization in human information processing [6], as increased efficiency of encoding sensory input in early stages of practice possibly improves processing capacity to otherwise interfering information.

Another question pertains to whether decreases in activity in brain regions important for working memory are accompanied by compensatory changes

elsewhere in the brain. In **chapter 3** we investigated three scenarios. First we tested whether practice shifts activity from one set of regions to another set. Second, we tested whether practice shifts activity within the initially active network(s), i.e. from cognitive control regions to perceptual and motor systems. Third we investigated whether practice induced a coherent decline in activity in the same networks that are involved from beginning to the end of training. In this study we investigated changes in brain activity following practice of a working memory task in a large sample of healthy subjects, and acquired whole-brain fMRI scans on a 3T scanner with an 8-channel head coil to obtain a high sensitivity for signal changes. In addition, we made use of the variability in practice-induced effects on brain activity among subjects, reflecting the rate or speed at which automaticity is achieved, to assess changes within and across networks. With practice, activity decreased in all regions associated with working memory. There was a set of regions where the BOLD signal increased with practice, but in all of these the signal was decreased relative to rest. Activity in motor regions remained mostly constant across tasks. The main finding hence supports the notion that practice does not lead to a shift from one network to another. To test whether practice induces shifts of involvement within the initially active network we assessed correlational structures within each of several sets of regions that emerged from the statistical image analyses (i.e. where signal decreased with practice, where signal was increased, and where signal was sustained during all tasks). Partial correlation and factor analyses revealed strong correlations between regions within the sets, in how they respond to practice. However, there were no significant negative correlations within sets, which should be present if activity shifts between regions within a set. Hence, our findings also do not support the notion that specific regions within the initially active network(s) gain in involvement. The data support the scenario where practice induces a general decline in the working memory system without compensatory changes elsewhere in the brain. We argue that working memory may engage in ‘chunking’ strategies that enables stimuli to acquire a new feature (e.g. target or non-target). Following practice the working memory network is no longer involved because stimuli have acquired a novel feature that allows for rapid classification.

Previous research [6,7] suggests an important role of working memory in establishing automatization. Yet the critical contributions of working memory

in the development of automatization remain undetermined. In **chapter 4** we used a combination of fMRI and transcranial magnetic stimulation to asses the critical importance of working memory in automatization. First, participants performed the Sternberg automatization paradigm while fMRI images were acquired. The fMRI maps were used to localize left dorsolateral prefrontal and parietal regions, where activity decreases with practice. Participants received rTMS stimulation of these regions during performance of the automatization task. As was expected novel task accuracy for target responses was more susceptible to interference than practiced task accuracy. This shows that automatization induces a gradual decline in cognitive control. Accuracy of the control task with overlearned associations was not affected by brief disruption of brain activity. This suggests that with sufficient practice, performance may become independent of cognitive control. We propose that working memory enables the development of automatization by restructuring of task-relevant information that allows more efficient implementation of internal task goals.

Automatization not only improves performance on the task that is practiced, it also allows processing of additional information. Automatization therefore seems an important mechanism by which we can overcome the limitations of our processing capacity. Patients with schizophrenia however, seem to have serious problems in processing as much informations as healthy individuals can [8]. When task load is within the boundaries of their capacity, brain regions are typically more activated than control subjects. This is thought to reflect inefficient brain function. In **chapter 5**, we investigated whether inefficient working memory function and reduced capacity in schizophrenia were associated with a failure in automatization. Both groups performed a modified version of the Sternberg task with novel and practiced material during fMRI. The difference in activity levels for performance of the novel and the practiced task was a measure of efficiency. It was expected that compared to healthy controls, patients would inefficiently activate working memory areas after practice and therefore exhibit only a small reduction in activity. It was predicted that as a consequence patients have decreased capacity to execute an additional cognitive task. After the fMRI session, both groups participated in a dual-task session. The difference in accuracy between dual task and single task performance was calculated and the drop in performance (performance cost) indicated how well subjects could process two tasks at the same time.

Performance cost was therefore a measure of capacity. Practice improved performance in patients to the same extent as in controls and normalized excessive levels of activity. Practice reduced performance cost in both groups, but patients exhibited overall disproportionate performance cost in the dual-task. The proportion of activity decreases in the working memory network after practice predicted performance cost in controls but not in patients. The findings do not support the idea that automatization is impaired in clinically stable patients with schizophrenia. In addition, there was no clear relationship between automatization and excessive performance cost in schizophrenia. Together, this suggests that inefficient working memory function and reduced capacity in schizophrenia are associated with a failure to properly engage working memory when task goals require frequent updating, for instance during novel performance and when performing an additional task concurrently.

The automatic brain

Efficiency and processing capacity

In **chapter 3** and **chapter 4** we postulate that working memory contributes to automatization by enabling a restructuring of information that allows for more efficient information processing. Possibly, sequentially presented information, such as the strings of letters that we used in the experiments, is pieced together in an information 'chunk' (e.g. pseudo-words), thereby optimizing its memorization and retrieval. The findings in **chapter 2** support this idea, where it was shown that automatization affects encoding very early in practice. This suggests that with repeated exposure of information, encoding strategies become less important as more efficient representations become established. This idea was confirmed by the findings in **chapter 4**, showing that working memory contributions diminish over the course of practice. More efficient representation of information may facilitate its retrieval when needed to guide task execution, resulting in faster and more accurate task performance. Also, if working memory is no longer needed to update or modify the internally represented task goals, this enables processing of otherwise interfering information at the same time. Automatization thus reflects the flexible and adaptive nature of human information processing that is often emphasized in theories on cognitive control [5].

Automatization and schizophrenia: reconciling discrepant findings

The present work strongly implicates working memory in the development of automatization. Patients with schizophrenia exhibit a profound working memory deficit, but contrary to what we expected, show intact automatization. At first glance, it seems difficult to reconcile these incompatible findings. If working memory enables automatization as we postulated in **chapter 4**, the question rises why patients with schizophrenia were not impaired on automatization as we initially expected.

In **chapter 5** we argued that inefficient brain function and reduced processing capacity in schizophrenia were best explained by a deficit in updating or modifying the contents of working memory when novel information was presented (e.g. a novel set of letters) or when information kept in mind required manipulation (updating the count of oddballs). This idea is supported by our findings discussed in **chapter 2**, where it was shown that automatization immediately reduced demands on encoding of sensory input. If processed information does not change over the course of performance, the internal representation with task information does not require modification or updating. Repeated exposure of consistent information may thus facilitate internally representing of task goals in working memory in patients with schizophrenia. Also, in **chapter 4** we created a 'virtual lesion' by temporarily disrupting prefrontal and parietal activity. We found that this predominantly affected novel processing and only moderately affected practiced performance. This supports the idea that prefrontal dysfunction in schizophrenia predominantly affects the ability to represent frequently changing information. If one is unable to represent novel information, this may lead to stereotypical responses that are often observed in schizophrenia and other psychiatric diseases such as autism. The cognitive deficit in schizophrenia is thus not explained by a deficit in automatization, where familiar information is inefficiently processed in a novel way. Rather the opposite may explain the cognitive impairments associated with schizophrenia. Due to an inability to process frequently changing information patients with schizophrenia may tend to engage in automatic behaviors in circumstances that ask for flexible and adaptive behavior.

Limitations and future directions

Although this work has elucidated some important contributions of working memory in the development of automatization, the neural mechanism that enables working memory to disengage from processing over the course of practice remains elusive. Remarkably, in spite of an evident working memory deficit, patients with schizophrenia show intact automatization. This suggests that the working memory system and in particular the prefrontal cortex, cannot account for the development of automatization all by itself. The focus in future research therefore should extend to regions interacting with the cognitive control network, that may jointly drive the transition from controlled to automatic processing.

Insights on this mechanism may come from theories on human perceptual category learning [9,10]. These theories emphasize the putative role of the basal ganglia in the establishment of automatic behaviors. The putamen, which is part of the basal ganglia, showed sustained activity during both novel and practiced performance in **chapter 3**. Although there was no clear evidence that this region gained in activity with practice, it does not exclude the possibility that this area contributes to automatization. The basal ganglia is well-known for its contributions to 'chunking' sequential motor actions into 'performance units' that allow for more efficient implementation of learned motor behaviors [11,12]. Evidence is increasing that the basal ganglia may play a similar role in learning cognitive behaviors [10,13]. In their reviews Ashby [9] and Seger [10] describe how pathways between the basal ganglia and the prefrontal cortex interact to allow for strengthening of corticocortical connections between visual and motor cortices over the course of practice. With sufficient practice this eventually enables performance that becomes independent on cognitive control. Also, interactions between the basal ganglia and prefrontal cortex are important for detecting and responding to unexpected or novel stimuli [5,14]. If this pathway does not function properly, this may induce a tendency to respond in a perseverative or stereotyped manner, which could be considered as an aggravated automatic response. This implicates a role of the frontostriatal pathway in controlled and automatic processing.

It is possible that the experimental task design used in the studies was not sensitive enough to elicit changes in other regions that may be important for automatization. The paradigm in the current studies is an example of

observational learning, where knowledge about categories (e.g. targets and distracters) is explicit, while parts of the basal ganglia important for learning such as the caudate nucleus are primarily sensitive to feedback-based learning, where knowledge is acquired implicitly [10]. Also, the number of items that are processed are well within the boundaries of normal human processing capacity (i.e. +/- 7 items), which may have attenuated the effort to engage in more efficient recoding strategies with practice. The findings in **chapter 2** show that activity related to encoding decreased very fast. With the limited temporal resolution of fMRI, changes in other regions relevant to automatization may therefore have occurred unnoticed. Novel task designs should be considered in future experiments to further elucidate the neural mechanism underlying automatization. These should include feedback learning and stimulus material that challenges subjects to engage in restructuring strategies.

A more speculative idea, and therefore a bit controversial, is that regions in the 'default mode' network may play a role in automatization. Of particular interest are the changes in activity in the default mode regions that occurred after practice, reported in **chapter 3**. Brain activity in the default mode network was decreased during novel processing and returned to similar levels compared to passive rest (i.e. 'baseline') after practice. The functional properties of this network in human behavior remain unclear. Research however suggests that it is implicated in internal processing of thoughts, emotions and awareness [15,16]. Our findings suggest that during novel performance the default mode network is suppressed (to inhibit interfering task-irrelevant internal thoughts and emotions) to enable controlled processing. With practice, activity levels in the default mode network may be restored as control diminishes and performance becomes resistant to interference of intrusive internal thoughts. This may suggest that the transition from controlled to automatic processing is linked to a switch in activation and 'deactivation' in these two distinct networks. The neural mechanisms underlying the switch between activation and deactivation of brain networks remain largely unknown. Recently however, a critical role has been suggested for the right frontoinsular cortex and the anterior cingulate cortex [17]. Both were present in the networks that we identified in **chapter 3**. It is possible that closer investigation of the interactions between 'task-positive' and 'task-negative' networks may reveal

how the transition between controlled and automatic processing may take place.

Conclusion

The research in this thesis shows that automatization involves a dynamic distribution of available processing resources that allows organizing and structuring of the large amounts of complex information present in our environment. Contrary to what was expected, the cognitive deficit in schizophrenia is not explained by a deficit in automatization. Here we postulate that due to an inability to process frequently changing information, patients with schizophrenia will tend to engage in automatic behaviors in circumstances that ask for flexible and adaptive behavior. The focus in future research on automatization should involve closer investigation of the neural mechanism that enables disengagement of working memory from processing. Putative candidate regions involve the basal ganglia and regions in the default mode network that interact with cognitive control under task conditions with high and low demands.

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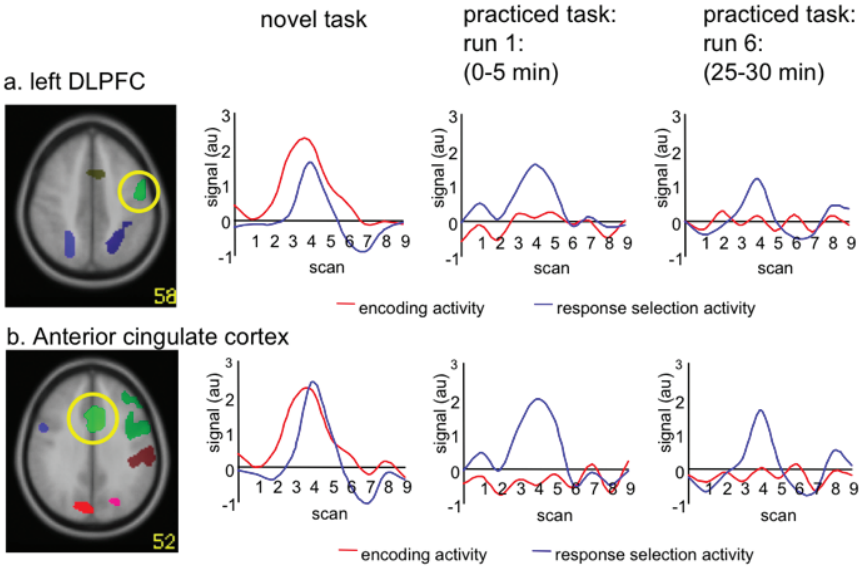
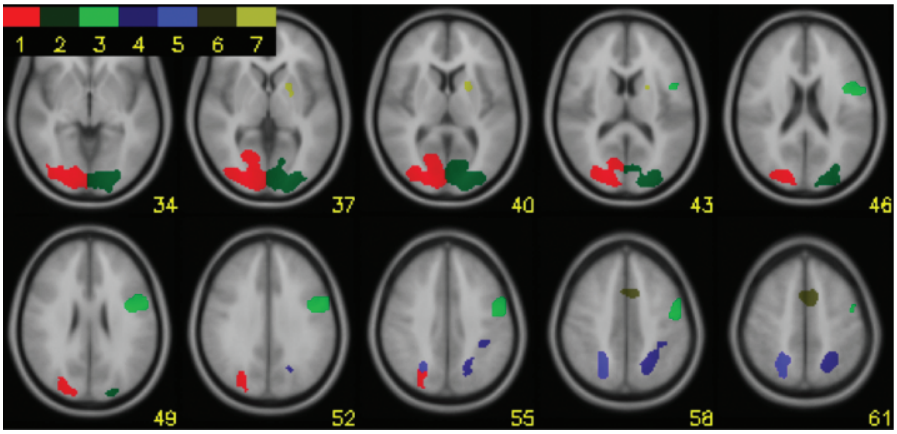
Color Figures



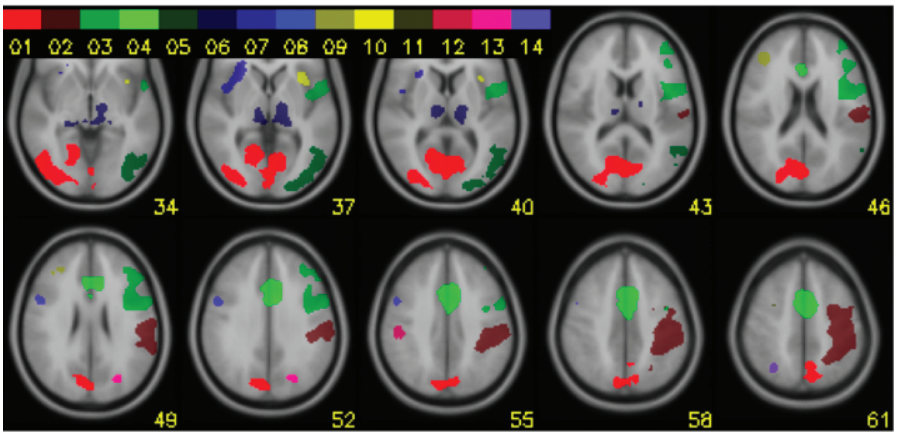
Chapter 2 / Figure 3. Heterogeneous effect of practice on regions activated by both encoding and response selection

Example of bold activity (arbitrary units) in regions activated by both phases: a. left DLPFC (top) and b. anterior cingulate cortex (bottom) during the novel task (left), after one practice run (middle) and six practice runs (right); showing the heterogeneous effects of practice for encoding and response selection.



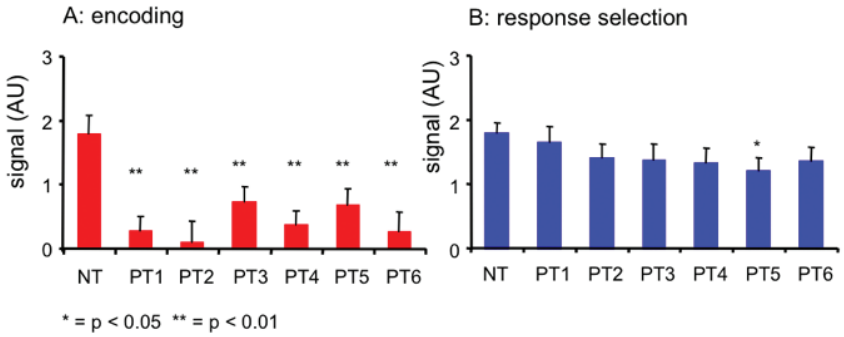
Chapter 2 / Figure 4. Encoding ROI's

ROIs showing activity related to encoding. The numbers in the color bar refer to the encoding phase ROIs (E1-E7) in table 2.



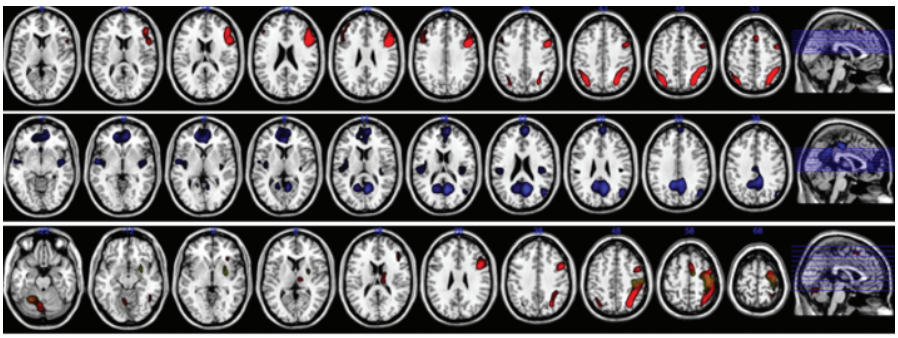
Chapter 2 / Figure 5. Response Selection ROI's

ROIs showing activity related to the response selection. The numbers in the color bar refer to the response selection ROIs (RS1-RS14) in table 3.



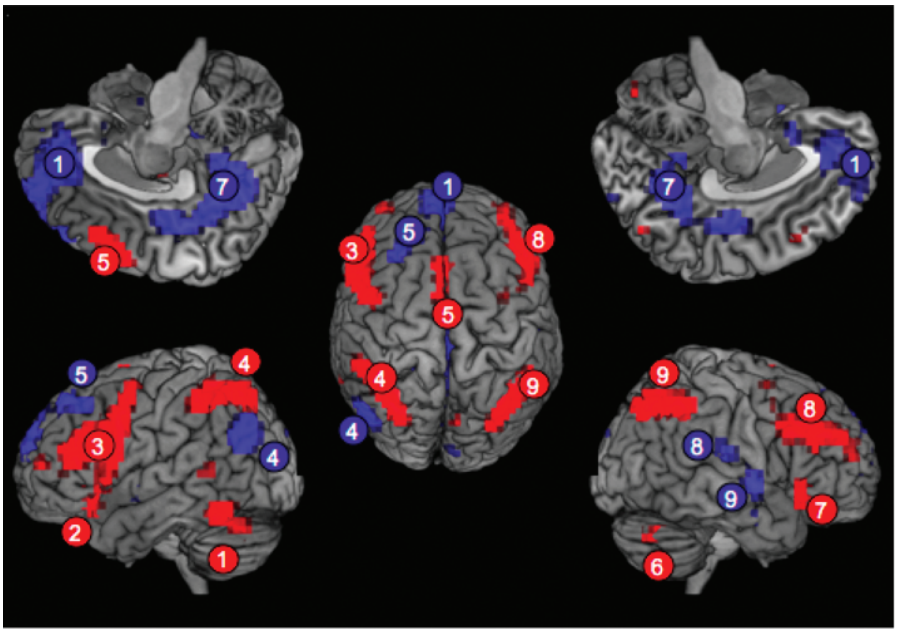
Chapter 2 / Figure 6. Practice and brain activity

a. activity in arbitrary units during encoding averaged over encoding phase ROIs (left) and b. activity during response selection averaged over response selection ROIs (right). Activity is displayed for novel task (NT) and each practice run (PT1-PT6).



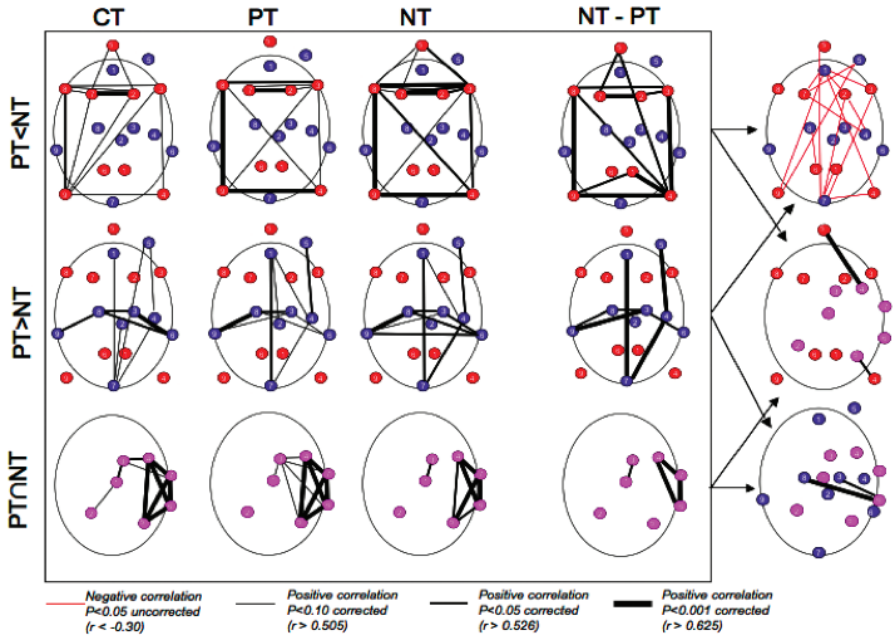
Chapter 3 / Figure 3. Regions of Interest

Group contrast images (average contrast map of all 46 subjects) showing the regions of interest. Contrasts were thresholded with t=4.5. The top panel shows the NT vs. PT contrast image. Red areas show the cortical network that was more active in NT (NT>PT). Blue areas show the cortical regions that were more active in PT (inverse contrast PT>NT). The bottom panel shows areas in red that were commonly active in the three task conditions: NT∩PT∩CT (i.e. the intersection of NT, PT and CT vs. rest). The numbers in the slices correspond to MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).



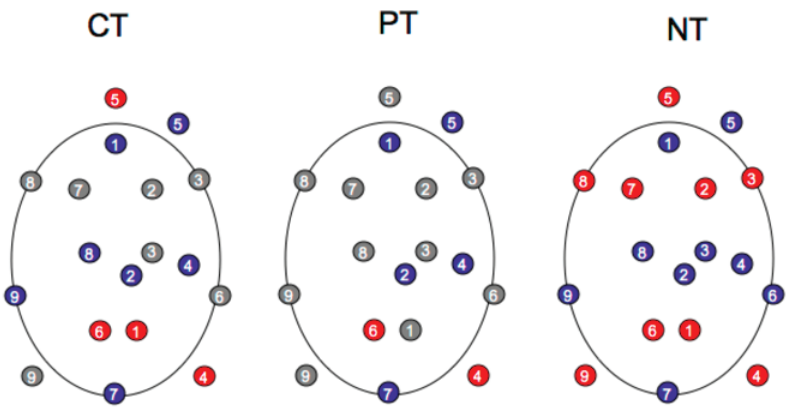
Chapter 3 / Figure 5. Positive and negative regions of interest

Surface representation of the positive (red) and negative (blue) regions of interest. Numbers correspond to those in Table 2. Negative regions 2, 3 and 6 are below the surface (see figure 3).



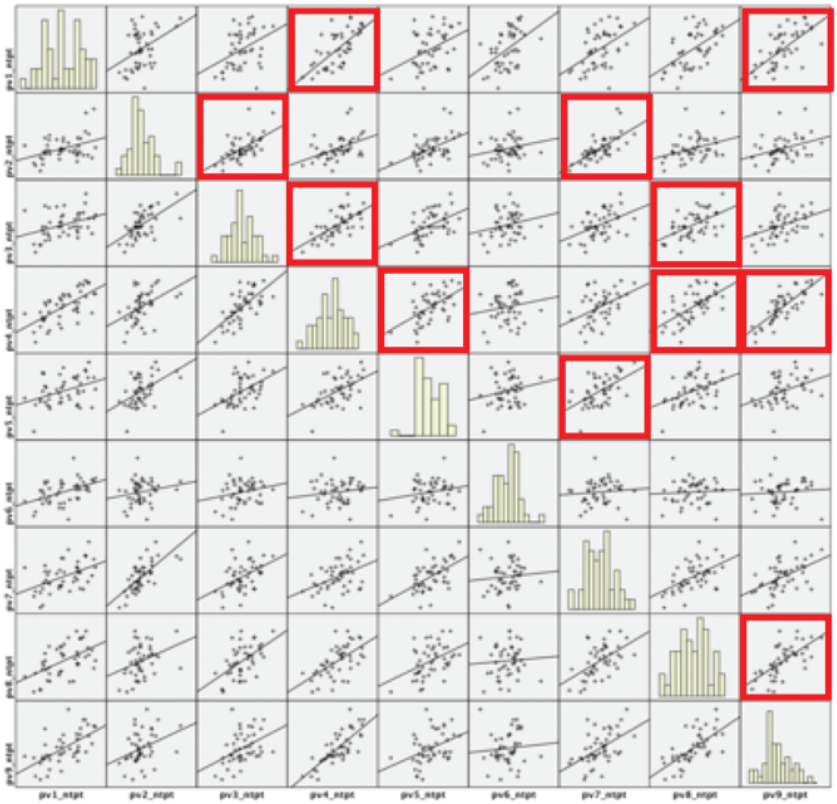
Chapter 3 / Figure 6. Interactions within and between regional networks

Correlations within regions (left 4 columns) and between (right column) regions. Left 3 columns display correlations of task versus rest values (not corrected for mean activity level, see text). Fourth column displays correlations of practice-induced signal change (NT - PT), corrected for mean activity of CT, PT and NT versus rest. Right column display correlations between regions across networks, corrected for mean activity of CT, PT and NT versus rest. Positive ROIs (NT > PT) are displayed in red, negative ROIs (NT < PT) in blue, and the common ROIs (NT> rest AND PT > rest) in purple. Numbers correspond to regions listed in Table 2. Right side is left hemisphere.



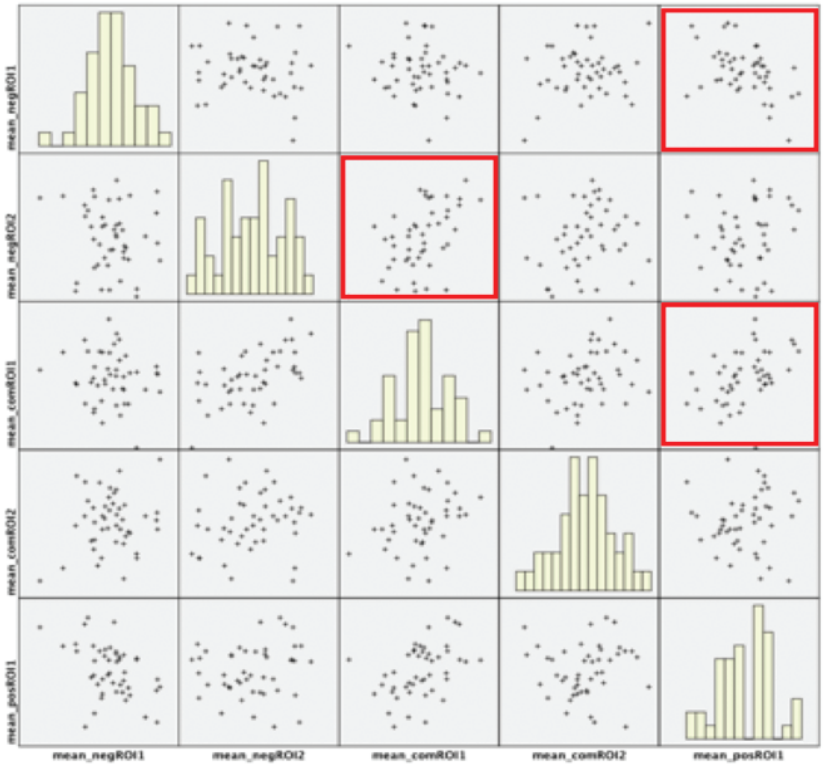
Chapter 3 / Figure S1

Color indication of significance of task versus rest. Positive ROIs (NT > PT) are displayed in red, negative ROIs (NT < PT) in blue. Colored regions are significantly (de)activated versus rest ($p<0.05$). Grey circles are not. Numbers correspond to regions listed in Table 2. Right side is left hemisphere. Common ROIs are not displayed, they are significant in all tasks.



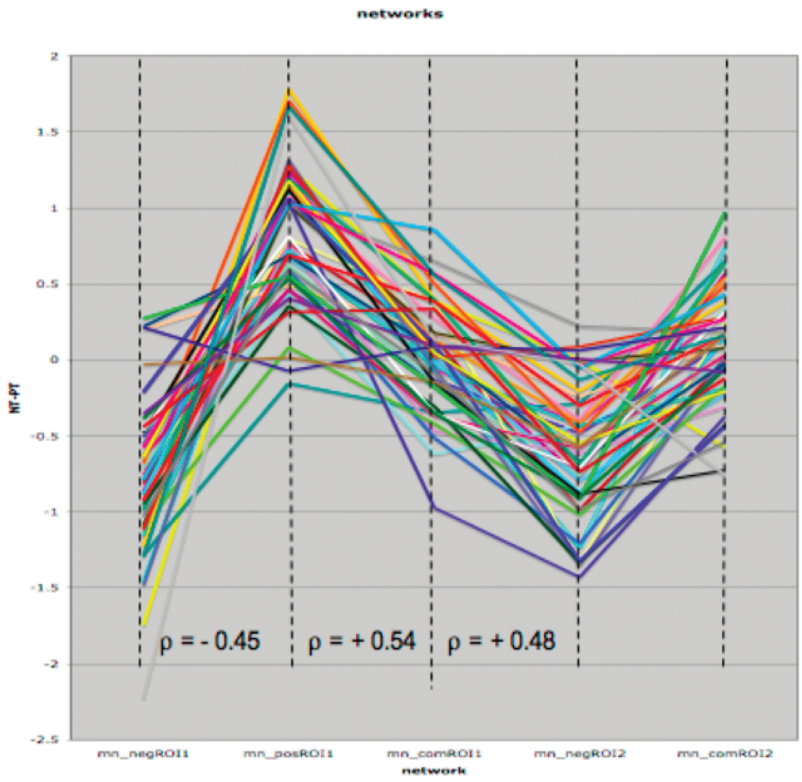
Chapter 3 / Figure S2

Scatterplots of positive ROI's versus each other, for the NT-PT change in brain activity. Red frames indicate significant correlations (see text for details). Diagonals display distributions of values across subjects, within ROI's.



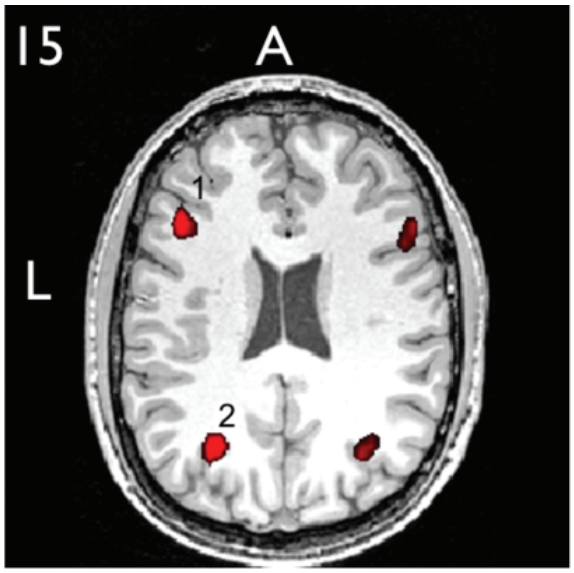
Chapter 3 / Figure S3

Scatterplots of networks versus each other, for the NT-PT change in brain activity. Red frames indicate significant correlations (see text for details). Diagonals display distributions of values across subjects, within networks.



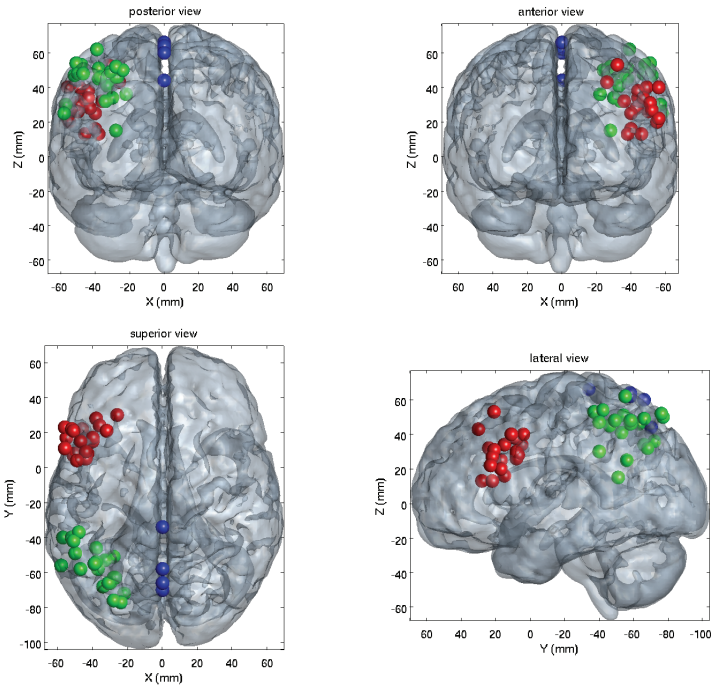
Chapter 3 / Figure S4

Mean levels of activity change (NT minus PT, displayed on y-axis) for all subjects. Values are shown for the 5 networks on the x-axis. Significant correlations between networks are displayed in the figure.



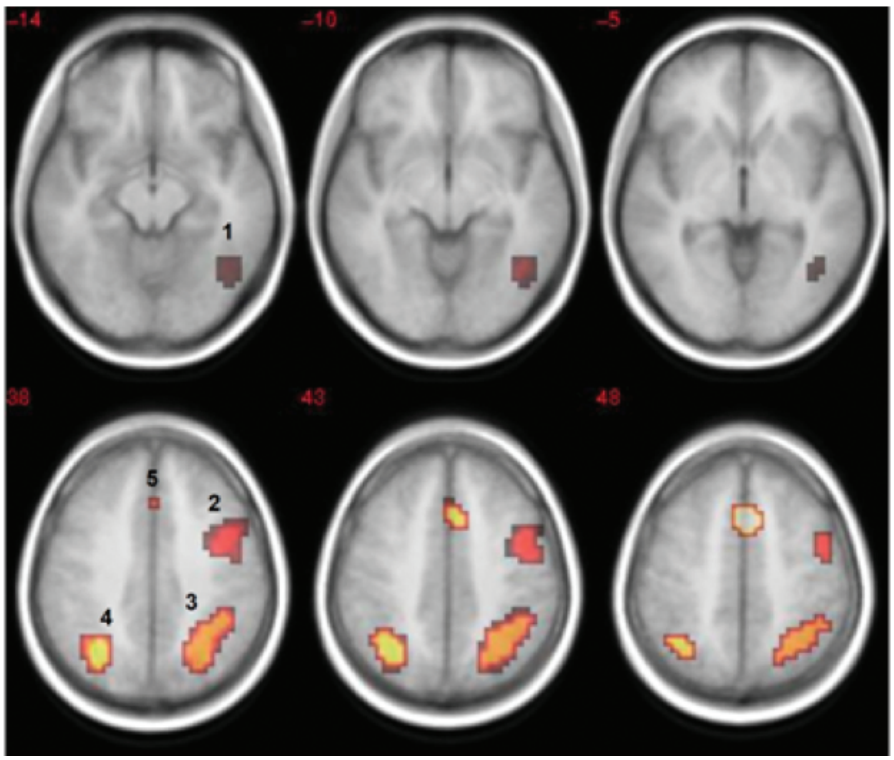
Chapter 4 / Figure 2.

An individual t-map in native space (single subject, t=3; p<0.05 corrected, L=left, A= anterior, z=15) is shown, displaying activation in the left prefrontal cortex (1) and left parietal cortex (2).



Chapter 4 / Figure 3.

The individual stimulation locations in the prefrontal (shown in red) and the parietal cortex (shown in green) are displayed in MNI space for all participants in the study. In blue the control region in the mid-saggital plane is displayed that was stimulated in five subjects. The top panel shows a posterior and an anterior view of the stimulation locations. The bottom panel displays a superior and lateral view of the individual stimulation locations.



Chapter 5 / Figure 3.

Combined group map of patients and controls showing WM regions: 1. left fusiform gyrus LFG, 2. left prefrontal cortex LPFC, 3. left superior parietal cortex LSPC, 4. right superior parietal cortex RSPC, 5. anterior cingulate cortex ACC. The numbers in the slices correspond to MNI z-coordinates. Slices are in radiological orientation (left side is right hemisphere and vice versa).

Nederlandse Samenvatting

Oefening baart kunst. Dit is met name het geval wanneer een handeling herhaaldelijk op dezelfde wijze uitgevoerd wordt. Deze vorm van leren wordt ook wel automatiseren genoemd. In tegenstelling tot het effect van automatiseren op ons gedrag, is over het effect van automatiseren op hersenfunctie nog weinig bekend. Het doel van dit proefschrift is om de neurale basis van automatiseren en het vermogen om onze beperkte informatieverwerkingscapaciteit te vergroten als gevolg van training, te onderzoeken. Patiënten met schizofrenie lijken een grotere beperking te hebben in de hoeveelheid informatie die zij kunnen verwerken. In dit proefschrift onderzoeken we tevens of een stoornis in automatiseren de verminderde cognitieve capaciteit bij schizofrenie kan verklaren.

Het onderzoek bouwt voort op eerdere studies die hebben aangetoond dat automatiseren de activatie beïnvloedt van hersengebieden die belangrijk zijn voor het werkgeheugen. De aard van deze veranderingen en hoe deze bijdragen aan het verbeteren van de informatieverwerkingscapaciteit zijn nog grotendeels onbekend. In hoofdstuk 2 onderzochten we de veranderingen in hersenactiviteit als gevolg van training voor verschillende informatieverwerkingsprocessen binnen een cognitieve taak; het encoderen van informatie en responsselectie. De resultaten lieten een snelle daling in hersenactiviteit zien in gebieden die betrokken zijn bij het encoderen van visuele informatie. De activiteitsdaling in het netwerk van gebieden betrokken bij responsselectie vond later plaats en beperkte zich tot een selectie van gebieden. Deze resultaten laten zien dat de mate waarin het werkgeheugen de informatieverwerking ondersteunt over het verloop van training, verschillend is voor afzonderlijke informatieverwerkingsprocessen binnen een cognitieve taak. De snelle daling in activiteit voor encoderen suggereert dat automatiseren met name het vermogen verbetert om meerdere bronnen van informatie tegelijkertijd te kunnen verwerken en te onthouden.

In hoofdstuk 3 onderzochten we of de daling in hersenactiviteit in werkgeheugengebieden na training gepaard gaat met een compenserende toename in activiteit in andere hersengebieden. We hebben gekeken naar drie scenario's. Ten eerste onderzochten we of er een verschuiving is van activiteit in het werkgeheugennetwerk naar een netwerk van andere gebieden. Ten tweede, of er sprake is van een verschuiving van activiteit binnen het netwerk van gebieden dat actief is voorafgaand aan de training.

Ten derde, of activiteit in zijn geheel daalde in de hersengebieden die van het begin tot het einde van de training bij de cognitieve taak betrokken zijn. We identificeerden drie netwerken van gebieden die een zelfde effect van training op hersenactiviteit lieten zien. Buiten het netwerk van werkgeheugengebieden die een daling in activiteit lieten zien, steeg het BOLD signaal steeg als gevolg van training in een aantal gebieden. Echter in deze gebieden was het signaal voorafgaand aan training lager dan tijdens rust. Daarnaast was er een netwerk van motorische gebieden waar het signaal constant bleef na training. Er is dus geen verschuiving in activiteit van het werkgeheugen netwerk naar een ander netwerk van gebieden waargenomen. Tevens waren er geen negatieve correlaties tussen gebieden in de geactiveerde netwerken als gevolg van training. Er was dus geen sprake van een verschuiving in activiteit binnen het netwerk dat actief was voorafgaand aan training. De bevindingen in deze studie ondersteunen het idee van een algehele daling in activiteit van het werkgeheugen als gevolg van training, zonder compensatie van toegenomen activatie elders in het brein. Deze bevindingen suggereren dat het werkgeheugen mogelijk belangrijk is voor 'chunking' tijdens training, waardoor informatie mogelijk opnieuw gecodeerd of gestructureerd wordt, voor een efficiëntere verwerking.

Eerder onderzoek suggereert de betrokkenheid van het werkgeheugen bij de ontwikkeling van automatiseren. De kritische bijdrage van het werkgeheugen voor automatiseren is echter nog niet vastgesteld. In hoofdstuk 4 gebruikten we een combinatie van fMRI en transcraniële magnetische stimulatie (TMS) om de functionele betrokkenheid van het werkgeheugen bij automatiseren vast te stellen. De fMRI beelden die tijdens het uitvoeren van een werkgeheugentaak waren gemaakt m.b.v. de MRI scanner werden gebruikt om op individuele basis de gebieden te lokaliseren die betrokken zijn bij het werkgeheugen en waar activiteit daalt als gevolg van training. Vervolgens werden deze gebieden gestimuleerd met TMS tijdens het uitvoeren van een nieuwe en een geoefende werkgeheugentaak. De resultaten laten zien dat interferentie met activiteit in de dorsolaterale prefrontaal cortex en de parietaal cortex, de taakprestatie verslechtert, voor zowel nieuwe als (deels) geautomatiseerde taken. Dit effect is het sterkst bij nieuwe taken. Kortdurende interferentie met werkgeheugenactiviteit had geen invloed op de prestatie op de controle taak. De associaties tussen stimuli en responsen in de controle taak kan als

(volledig) geautomatiseerd worden beschouwd. Dit suggereert dat wanneer een taak volledig geautomatiseerd is na voldoende training, deze zonder betrokkenheid van het werkgeheugen uitgevoerd kan worden. Deze bevindingen ondersteunen het idee dat het werkgeheugen automatische informatieverwerking mogelijk maakt door informatie te herstructureren, waardoor het efficiënter verwerkt kan worden.

Automatiseren verbetert niet alleen de prestatie na training, maar verbetert tevens het vermogen om meerdere bronnen van informatie tegelijkertijd te verwerken. Patiënten met schizofrenie hebben een beperktere informatie-verwerkingscapaciteit dan gezonde mensen. Schizofrenie kenmerkt zich bovendien door een inefficiënte hersenfunctie. Wanneer de hoeveelheid te verwerken informatie binnen de grenzen is van hun capaciteit, zijn hersengebieden vaak actiever vergeleken met controle deelnemers. In hoofdstuk 5 hebben we onderzocht of inefficiënte werkgeheugenfunctie en verminderde informatieverwerkingscapaciteit bij schizofrenie gerelateerd zijn aan een stoornis in automatiseren. We verwachtten dat werkgeheugen-gebieden bij patiënten met schizofrenie inefficiënter zouden functioneren na training, omdat hersenactiviteit in deze gebieden minder zou dalen. We verwachtten dat daardoor dat minder capaciteit vrijgemaakt kan worden voor het simultaan uitvoeren van een tweede cognitieve taak. De resultaten laten zien dat training de prestatie bij patiënten verbeterde en tevens excessieve hersenactiviteit normaliseerde. Training verbeterde het vermogen om twee taken tegelijkertijd uit te voeren in beide groepen, maar desondanks waren patiënten minder goed in staat dan controle deelnemers om simultaan twee taken uit te voeren. De daling in werkgeheugenactiviteit als gevolg van training voorspelde hoe goed iemand in staat was om twee taken tegelijkertijd uit te voeren bij de controle deelnemers, maar dit was niet het geval bij de patiënten. Deze bevindingen laten zien dat automatiseren intact lijkt te zijn bij klinisch stabiele patiënten. Bovendien was er geen relatie tussen automatiseren en beperkte informatie-verwerkingscapaciteit bij patiënten met schizofrenie. De resultaten suggereren dat inefficiënte hersenfunctie en beperkte verwerkingscapaciteit bij schizofrenie gerelateerd zijn aan een onvermogen het om het werkgeheugen adequaat te activeren wanneer het gedrag regelmatig aangepast moeten worden; bijvoorbeeld wanneer nieuwe informatie zich aandient of wanneer snel geschakeld moet worden tussen meerdere opdrachten.

Het automatische brein: efficiëntie en verwerkingscapaciteit

Op basis van de bevindingen in hoofdstuk 3 en 4 concluderen we dat het werkgeheugen belangrijk is voor automatiseren door efficiëntere verwerking van (complexe) informatie door middel van herstructurering mogelijk te maken. Wanneer informatie in een bepaalde structuur aangeboden wordt, kunnen deze gegevens samengevoegd worden in een 'chunk' (een serie van letters kan bijvoorbeeld samen een pseudo-woord vormen), wat het makkelijker maakt om de afzonderlijke stukjes informatie op te slaan of terug te halen uit het (werk)geheugen. Dit idee wordt ondersteund door de bevindingen in hoofdstuk 2 die laten zien dat automatiseren werkgeheugen-functie beïnvloedt, voornamelijk tijdens het encoderen van informatie. Dit zou kunnen betekenen dat encoderingsstrategieën van het werkgeheugen na training vervangen worden door efficiëntere verwerking van informatie. Dit wordt ondersteund door de bevindingen in hoofdstuk 4 die aantonen dat de prestatie minder afhankelijk wordt van het werkgeheugen als gevolg van training. Efficiëntere informatieverwerking in het brein faciliteert mogelijk het terughalen van tijdelijk opgeslagen informatie waardoor de prestatie sneller en nauwkeuriger wordt. De vrijgekomen werkgeheugencapaciteit maakt het tevens mogelijk om nieuwe informatie tegelijkertijd te verwerken. Automatiseren weerspiegelt daarmee het flexibele en adaptieve cognitieve vermogen van de mens dat centraal staat in huidige cognitieve informatieverwerkingstheorieën.

Het automatische brein bij schizofrenie

Het onderzoek in dit proefschrift toont de belangrijke rol aan van het werkgeheugen bij automatiseren. Het onderzoek bevestigt tevens dat er sprake is van een werkgeheugenstoornis bij patiënten met schizofrenie. Desondanks lijkt automatiseren bij deze patiënten intact te zijn. In eerste instantie lijken deze bevindingen in tegenspraak met elkaar; als het werkgeheugen cruciaal voor automatiseren is, waarom laten patiënten met schizofrenie dan geen afwijking in automatiseren zien?

Het werkgeheugen is met name ontoereikend bij patiënten wanneer informatie snel verandert of het gedrag aan nieuwe informatie aangepast dient te worden. In hoofdstuk 2 lieten we zien dat de betrokkenheid van het werkgeheugen snel afneemt als gevolg van training bij het encoderen van herhaaldelijk aangeboden consistente informatie. Dit wekt de suggestie dat

patiënten goed in staat zijn gestructureerde informatie te verwerken omdat het werkgeheugen dan minimaal belast wordt. Dit wordt ondersteund door de bevindingen in hoofdstuk 4, waar met behulp van een 'virtuele lesie' werd aangetoond dat interferentie met werkgeheugenactiviteit (in de dorsolaterale prefrontale en parietale cortex) met name de prestatie verslechterd wanneer nieuwe informatie verwerkt wordt. De prestatie-verslechtering was minder wanneer tijdens stimulatie de getrainde taak werd uitgevoerd. Dit ondersteunt het idee dat de werkgeheugen-stoornis bij schizofrenie voornamelijk het vermogen beïnvloedt om informatie te verwerken die regelmatig verandert of vernieuwd wordt. Een onvermogen om nieuwe informatie adequaat te verwerken, kan leiden tot stereotype gedragingen, wat een belangrijk kenmerk is van psychiatrische aandoeningen als schizofrenie en autisme. De cognitieve problemen bij schizofrenie en mogelijk ook bij aanverwante psychiatrische aandoeningen kunnen mogelijk verklaard worden door een onvermogen om onverwachte en nieuwe informatie te verwerken. Door een onvermogen om adequaat te reageren op nieuwe of onverwachte prikkels zijn psychiatrische patiënten meer geneigd om automatische strategieën te hanteren in omstandigheden die vragen om flexibel en adaptief gedrag.

Aanknopingspunten voor toekomstig onderzoek naar automatiseren

Dit proefschrift levert een belangrijke bijdrage in de verduidelijking van de rol van het werkgeheugen bij automatiseren. Er blijven een aantal vragen onbeantwoord. Opvallend is dat ondanks een werkgeheugenstoornis, het automatiseren bij (stabiele) patiënten met schizofrenie intact lijkt te zijn. Dit suggereert dat het werkgeheugen niet alleen verantwoordelijk kan zijn voor automatiseren. Toekomstig onderzoek zou zich moeten richten op mogelijke andere systemen in het brein die in interactie met het werkgeheugen automatiseren tot stand brengen. Mogelijke kandidaten zijn de basale ganglia. Deze subcorticale kernen in het brein spelen een belangrijke rol bij leren en bij de totstandkoming van automatismen. Samen met de prefrontale cortex versterken de basale ganglia de verbindingen tussen sensorische en motor gebieden in het brein, waardoor na voldoende training bepaalde gedragingen direct uitgelokt kunnen worden door de juiste sensorische prikkel. Het frontostriatale circuit speelt tevens een belangrijke rol bij het vermogen om adequaat te reageren in onverwachte en nieuwe situaties. Wanneer het frontostriatale circuit niet optimaal functioneert, kan de neiging groter zijn om op stereotype of perseveratieve

wijze te handelen. Samen met de prefrontale cortex spelen de basale ganglia dus mogelijk een belangrijke rol in de overgang van gecontroleerde naar automatische informatieverwerking als gevolg van training.

Het is mogelijk dat de experimentele opzet in het huidige onderzoek niet sensitief genoeg was om veranderingen in hersenactiviteit als gevolg van training teweeg te brengen in andere gebieden die mogelijk betrokken zijn bij automatiseren. De nadruk in de cognitieve taak lag op het expliciet leren van associaties tussen verbale stimuli en responsen. Het is mogelijk dat de basale ganglia hierdoor niet voldoende geactiveerd werden, omdat zij gevoeliger zijn voor het impliciet leren van relaties en patronen. Het is tevens mogelijk dat de hoeveelheid informatie (i.e. vijf letters) binnen de grenzen van de informatieverwerkingscapaciteit liggen en daardoor het vermogen om efficiëntere hercoderingsstrategieën te gebruiken onvoldoende prikkelde.

Een meer speculatief idee, ingegeven door de resultaten van hoofdstuk 3, is dat gebieden in het 'default mode' netwerk een rol zouden kunnen spelen bij automatiseren. Activiteit in de 'default mode' gebieden is verlaagd tijdens cognitieve inspanning en herstelt zich tot het basis niveau tijdens rust na training. Het onderzoek naar de functionele eigenschappen van het default mode netwerk bevindt zich in een pril stadium. Recente publicaties suggereren dat dit netwerk mogelijk een rol speelt bij introspectieve gedachten en emoties, en bij het bewustzijn. Tijdens cognitieve activiteit kunnen deze processen interfereren met de prestatie. Voor optimale prestatie is het daarom van belang om deze storende processen te onderdrukken. Na training neemt de cognitieve controle af en vormen introspectieve processen geen bedreiging meer voor de prestatie en wordt activiteit in dit netwerk weer hersteld. Het is daarom mogelijk dat de overgang van gecontroleerde naar automatische informatieverwerking gerelateerd is aan de balans tussen deze twee netwerken.

Conclusie

Het onderzoek in dit proefschrift laat zien dat automatiseren ons in staat stelt de grote hoeveelheid informatie die het brein binnenkomt, op efficiënte wijze te organiseren en te structureren, en daarmee een gunstig effect heeft op de van nature beperkte verwerkingscapaciteit van het brein. In tegenstelling tot wat was verwacht, konden we de verminderde

verwerkingscapaciteit bij schizofrenie niet verklaren door een stoornis in het automatiseren. We concluderen dat de cognitieve beperking bij patiënten met schizofrenie voortkomt uit een onvermogen om veranderlijke en inconsistente informatie adequaat te verwerken. Om de verwerkings-capaciteit bij mensen met en zonder psychiatrische aandoening beter te kunnen begrijpen zou de focus van toekomstig onderzoek naar automatiseren zich moeten richten op het neurale mechanisme dat het mogelijk maakt om cognitief functioneren onafhankelijk te maken van het werkgeheugen door training. De basale ganglia en het ‘default mode‘ netwerk lijken twee kandidaten die, in interactie met het werkgeheugen, hierbij betrokken zijn.

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van Raalten TR, Ramsey NF, Duyn J, Jansma JM (2008) Practice induces function-specific changes in brain activity. PLoS ONE 3: e3270.

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van Raalten TR, Boessen R, Neggers SFW, Kahn RS, Ramsey NF (2009). Practice induces a gradual decline in cognitive control. 15th Annual Meeting of the Organization for Human Brain Mapping, San Francisco CA USA. *(oral presentation)*

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van Raalten TR, Ramsey NF, Jansma JM, Jager G, Kahn RS (2003). On the neurophysiology of information processing in schizophrenia. fMRI Experience V, London UK. *(oral presentation)*

Dankwoord

Oefening baart kunst, maar promoveren blijft een lastig kunstje. Met hulp en steun van velen is het dan toch gelukt! Op deze pagina wil ik graag een aantal mensen bedanken. Allereerst wil ik graag alle patiënten op de afdeling Volwassenen Psychiatrie en gezonde vrijwilligers bedanken die deelgenomen hebben aan de studies. Zonder jullie deelname was er geen data om op te schrijven, laat staan een proefschrift.

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Curriculum Vitae

Tamar van Raalten was born on January 6th 1976 in Terneuzen, the Netherlands. She graduated from the Goese Lyceum in 1994. In 2000 she obtained a Master Degree in Biological Psychology and a Master Degree in Neuropsychology at University Utrecht. Tamar van Raalten started working at the Rudolf Magnus Institute of Neuroscience, Department of Psychiatry at the University Medical Centre Utrecht as a Research Assistent contributing to structural MRI studies in schizophrenia in March 2000. In 2001 she obtained a position as data flow coordinator in the former functional MRI lab of the Department of Psychiatry. In 2003 she started a PhD program in neuroscience, which she combined with the position of data flow coordinator in the first year. In 2004 she spent six months at the National Institutes of Health in Bethesda MD, USA. After defending her thesis on June 4th 2009, she will continue the post-doc position she started in January 2008 at the NICHE lab at the Rudolf Magnus Institute of Neuroscience Department of Child- and Adolescent Psychiatry, investigating the neural basis of rigid behavior in autism.

Tamar van Raalten werd op 6 januari 1976 geboren te Terneuzen. In 1994 behaalde ze in 1994 het VWO eindexamen aan het Goese Lyceum in Goes. In 2000 studeerde ze af in de Biologische Psychologie en in de Neuropsychologie aan de Universiteit Utrecht. In maart 2000 was Tamar van Raalten als onderzoeksassistent verbonden aan het Rudolf Magnus Instituut voor Neurowetenschappen, Afdeling Psychiatrie van het Universitair Medisch Centrum Utrecht. In 2001 werkte ze als data flow coördinator in het voormalige functionele neuroimaging lab van de afdeling Psychiatrie. In 2003 begon ze aan een promotieonderzoek in de Neurowetenschappen. In het eerste jaar combineerde zij haar onderzoek met de functie van data flow coördinator. In 2004 heeft zij zes maanden doorgebracht aan de National Institutes of Health in Bethesda MD, USA. Na de verdediging van dit proefschrift op 4 juni 2009 continueert zij als post-doc haar in 2008 gestartte onderzoek bij de Afdeling Kinder- en Jeugdpsychiatrie van het UMC Utrecht naar de neurale basis van rigide gedrag bij autisme.