Whole-brain fMRI using repetition suppression between action and perception reveals cortical areas with mirror neuron properties

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Erklärung

Hiermit versichere ich, dass ich die vorliegende Diplomarbeit selbständig und nur unter Verwendung der angegebenen Quellen verfasst habe. Die Diplomarbeit diente bisher keinen anderen Prüfungszwecken.

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List of Abbreviations

ANOVA	Analysis of variance
AD	Adaptation difference
aIPS	Anterior intraparietal sulcus
BOLD	Blood-oxygen-level-dependent
fMRI	Functional magnetic resonance imaging
fMRI-A	Functional magnetic resonance imaging adaptation
IBI	Inter block interval
IFG	Inferior frontal gyrus
IPL	Inferior parietal lobule
MI	Motor input
MNs	Mirror neuron(s)
NDM	Non-object-direct movement
ODM	Object-directed movement
PM	Premotor cortex
PSU	Post-stimulus undershoot
RS	Repetition suppression
RT	Reaction time
S1	Primary somatosensory cortex
SNR	Signal-to-noise ratio
STS	Superior temporal sulcus
TC	Time course
VI	Visual Input

Abstract

Mirror neurons (MNs), as originally described in the macaque monkey, respond to object-directed movements (ODMs) both when the action is executed and when the same action is observed. Theses neurons have been suggested to be the supporting neural mechanism for action recognition and understanding. However, there is a current debate about the localization of MNs in humans. Functional magnetic resonance imaging studies using adaptation paradigms (fMRI-A) for the identification of MNs provide mixed results. Studies supporting the existence of MNs restricted their analysis to a-priori candidate regions, whereas studies that failed to find evidence used non-object-directed movements (NDMs) as stimuli. In the present fMRI-A experiment we tackled these limitations by using ODMs and performing a whole-brain analysis. Participants observed and executed simple grasping movements differing only in their object-directness (grasping a button vs. grasping beside it). We reasoned that MN areas should be (1) more activated by ODMs than by NDMs and (2) exhibit cross-modal adaptation. The analysis revealed four significant clusters in the right anterior intraparietal sulcus (aIPS), right primary somatosensory cortex (S1), left premotor cortex (PM) and right cerebellum that showed these characteristics. While the aIPS and the PM have been reported before as a possible region for MNs, the S1 and the cerebellum have not been yet associated directly with MNs. We discuss the potential role of these regions in a human MN system. In the second experiment we addressed the question of whether the cross-modal adaptation as measured with fMRI has a behavioral correlate. Measuring the recognition speed of ODMs versus NDMs, depending on the previously executed movement type, revealed no significant differences.

1. Introduction

Social interactions play an exceedingly important role in the life of human beings. For any successful social interaction it is essential to predict and understand the actions of others in a time-efficient and accurate manner (Adolphs, 1999, 2003). The attribution of mental states to others, like goals, intentions and beliefs that can either predict or retrospectively explain their actions is assumed by many theories on folk psychology (Goldman, 1993). Indeed, it is a striking feature of our experience that we can understand the actions performed by others seemingly effortlessly and intuitively by only observing them. For instance, when we observe a person moving their hand towards a glass of water, we can predict that the person is going to grasp the glass and drink it well before the hand actually reaches the glass; and we might also infer that the person is doing so because he or she is thirsty.

Different theories have been proposed to explain how humans attribute mental states to others. One can broadly distinguish between so-called *theory theories* and *simulation theories*. The *theory theory* on action understanding assumes that humans generalize their own experience and develop a naïve theory - similar to an ordinary scientific explanation - in order to explain actions of third persons (Ravenscroft, 2008). Instead, the s*imulation theory* on action understanding also known as *motor simulation theory* states that actions of others are understood by adopting their perspective and using one's own motor system to model or simulate the action (Gordon, 2008). According to the *motor simulation theory*, brain areas that underlie the execution of a specific action are also involved in understanding the action carried out by others. The idea is that the simulation in one's own motor system is used to obtain predictions and possible lower-level intentions related to the action. To avoid overt movement by the observer the simulation in the motor system is finally taken off-line. As the supporting neural mechanism of *motor simulation* the so-called mirror neurons (MNs) have been suggested (Gallese & Goldman, 1998; Gallese, Keysers, & Rizzolatti, 2004). These neurons were first described in the brain of non-human primates: they are active when the animal executes an object-directed movement (ODM) and when it observes another monkey or the experimenter performing the same action (for introduction see Rizzolatti, 2005; for reviews see: Rizzolatti & Craighero, 2004; Rizzolatti & Sinigaglia, 2010). An ODM can be defined as a movement towards an object which can be manipulated or interact with the effector (e.g. a hand grasping a glass), while a non-object direct movement (NDM) does not lead to an interaction with an object (e.g. tapping a finger). Note that the terms 'object-

directed' and 'goal-directed' are sometimes used interchangeably. 'Action' is a rather generic term that can refer to any intentional motor behaviors including ODMs.

1.1. Mirror Neurons in Monkeys

MNs were originally discovered in area F5 of the macaque premotor cortex (di Pellegrino, Fadiga, Fogassi, Gallese, & Rizzolatti, 1992; Gallese, Fadiga, Fogassi, & Rizzolatti, 1996). This is a motor area which is known to control hand and mouth movements (Rizzolatti et al., 1988). Within area F5 both motor neurons and visuomotoric neurons have been described.

Motor neurons only respond to motor but not visual stimulation and can, in this area, be further subdivided into two classes. The first class of motor neurons fires when the monkey executes a specific action irrespective of the effector (e.g. the right or left hand or the mouth) that is used to accomplish the action goal (e.g. to pull an object). However, they do not fire when the monkey uses the same effector to achieve different action goals (e.g. pushing versus pulling). Accordingly, grasping, holding, tearing and manipulation neurons have been identified. Conversely, the second class of motor neurons codes the effector used to carry out an action independent of the action goal. For instance, some of these neurons discharge when an object is grasped depending on the type of prehension applied by the animal (e.g. precision grip versus power grip).

Visuomotoric neurons respond to both visual and motor stimulation and constitute approximately 20% of the neurons in area F5. These neurons can be further subdivided into two distinct groups. The so-called canonical neurons are active when an object is visually presented to the monkey (without an action) that is compatible with the type of action coded by the neurons. For example, the view of a graspable object elicits the firing of a neuron that is also active when the monkey executes the grasping movement. The other type of visuomotoric neurons fires when an ODM is observed performed by another individual and also when the monkey executes the movement itself. These neurons have been labeled as mirror neurons (MNs) (Gallese, et al., 1996). The observed movement is thereby equally effective in triggering these neurons when carried out by another monkey and when the movement is performed by the experimenter. The sight of the object alone or the same movement carried out without the object as target (pantomimed movement or NDM) is ineffective in triggering MNs. The firing rate of MNs increases well after the onset of the movement, just before the effector actually reaches the target (Fadiga, 2010, personal communication). A defining property of MNs is the relationship between their visual and

motor properties. According to Gallese and colleagues (1996) MNs can be divided into strictly congruent and broadly congruent. In the case of strictly congruent MNs (~30% of the MNs in area F5) the effective executed movement and the effective observed movement must be almost identical (Rizzolatti & Craighero, 2004). For broadly congruent MNs the correspondence between executed and observed movement is less strict and primarily confined to the action goal. These broadly congruent MNs are of particular interest for action understanding because they apparently generalize the goal of observed or executed movements irrespective of the means to achieve it.

How can these neurons serve as the underlying mechanism of action understanding? The idea is that MNs allow the activation of the observer's own motor system during the observation of an ODM. The observer can use her/his own motor system to simulate the observed action in order to predict and understand the observed action. Rizzolatti, Fogassi, & Gallese (2001) therefore suggest that "the mirror system transforms visual information into knowledge". Several electrophysiology studies have therefore attempted to find evidence for MNs encoding action goals.

In a study by Umilta and colleagues (2001) MNs in the macaque ventral premotor cortex were recorded in two conditions: in the first, the monkey could observe an ODM, in the second the final part of the movement was occluded by a screen and the monkey could not see the hand-object interaction. Before each trial the monkey could observe whether the experimenter placed an object behind the screen or not. More than half of the MNs that were active when the monkey observed the completely visible movement fired as well when the final part of the movement was occluded but only if an object had been placed behind the screen. Note that the two situations in the hidden condition were visually identical, the only difference being that the monkey could remember whether an object was actually present. Umilta et al. (2005) therefore suggested that MNs might encode the perceived action goal.

Kohler, et al. (2002) found that part of the recorded MNs in area F5 could be triggered by sounds associated with the specific action. These neurons responded to specific actions irrespective of whether these were heard, performed, or observed. For instance, some neurons were activated when the monkey was grasping a peanut or observing the experimenter grasping it and also when solely the sound of cracking a peanut was displayed. These findings support the idea that MNs code rather the abstract goal than the concrete instance of an action.

Recently, Fogassi and colleagues (2005) could demonstrate that MNs in the inferior parietal lobule (IPL) of macaques are activated differentially when the same motor act is

embedded in different actions. Monkeys either had to grasp a piece of food to eat it or to place it in a container. About two-third of the recorded neurons with mirror properties showed a preference for a specific action. This preference already occurred when the animal started to grasp the piece of food, which required the identical movement in both conditions. The authors argue that MNs enable the monkey to recognize the goal of the observed movement and also to discriminate identical movements according to the actions they are embedded in. They conclude that MNs provide a neuronal mechanism to infer the intentions of others.

Most recently, electrophysiological studies have investigated the role of MNs in attention to social cues (Shepherd, Klein, Deaner, & Platt, 2009), action suppression (Kraskov, Dancause, Quallo, Shepherd, & Lemon, 2009) and the coding of peripersonal and extrapersonal space (Caggiano, Fogassi, Rizzolatti, Thier, & Casile, 2009). MNs have also been demonstrated in birds (Prather, Peters, Nowicki, & Mooney, 2008) and most recently in the temporal cortex of humans using extracellular single-cell recording in patients suffering from pharmacologically intractable epilepsy (Mukamel, Ekstrom, Kaplan, Iacoboni, & Fried, 2010).

1.2. The Human Mirror Neuron System

Since the discovery of MNs in non-human primates the search for a homologous area in the human motor system began. Most studies have used functional magnetic resonance imaging (fMRI) to find evidence for MNs in humans. Early attempts were guided by the reasoning that a human MN system should be active during the observation and execution of ODMs (conventional paradigm). Accordingly, imitating an action should activate the human MN system even more since both processes observing and executing are engaged simultaneously. Hence an activation pattern [imitation > execution > observation] has been considered as indicative for MN activity (e.g. (Iacoboni et al., 1999). Based on this reasoning several regions have been claimed to be part of the human MN system, including the ventral PM, the aIPS and the superior temporal sulcus (STS) (for review see Dinstein, Hasson, Rubin, & Heeger, 2007; for a meta-analysis see Van Overwalle & Baetens, 2009). At the same time, other studies using fMRI have considered activation within putative MN areas as evidence for the functional contribution of MNs to the examined task. This has led to the formulation of various theories about the function of MNs in humans. However, the identification of a

human MNS based on a common activation during action observation and execution bears several fundamental problems.

1.3. Critique on the Human Mirror Neuron System

Several shortcomings have been put forward with the identification of a human MN system by using conventional fMRI and imitations paradigms. As a consequence the social functions associated with the human MN system as evidenced by fMRI are also under debate (for reviews see Hickok, 2009; Jacob & Jeannerod, 2005; Turella, Pierno, Tubaldi, & Castiello, 2009). Three major problems in previous studies have been outlined by Dinstein and colleagues (Dinstein, 2008; Dinstein, Thomas, Behrmann, & Heeger, 2008) that concern (1) the interpretation of activity in previous paradigms as evidence for MNs, (2) the vague and widespread associations of MNs with cognitive functions, (3) the usage of conventional fMRI as a method to reveal MNs in humans.

Identification of human MN system by means of common visuomotoric activation is problematic since observed visuomotoric commonalities might be attributable to other factors. For example, in an imitation task many other processes like visual recognition, motion perception, working memory and movement planning are integral components in both the visual and motor parts of the task. Therefore a cortical region that exhibits activation in both tasks might simply be associated with one of these processes rather than with MNs supporting action understanding. In line with this concern many cortical areas are activated during imitation tasks that are not thought to contain MNs, for instance early visual cortex. These areas are usually ignored in the analysis as 'non-candidate regions'. Ventral PM and the aIPS have been typically considered 'candidate MN regions' because these two areas are assumed to be homologous to monkey area F5 and PF/IPL. Following this circular reasoning these studies have failed to address the basic question that is whether and where there are MNs in the human cortex (Dinstein, et al., 2008). Given the evolutionary similarity of their brains (Petrides & Pandya, 1994) and first evidence from single-cell recordings in humans (Mukamel, et al., 2010), it seems likely that humans have MNs similar to the monkey - but the exact location is still unknown.

The lack of an unambiguous identification of MNs in humans turns out to be problematic for the examination of the cognitive-functional role of MNs with fMRI. fMRI studies investigating social cognition consider activation in putative MNs typically as evidence for the functional contribution of MNs to the examined task (e.g. Wheatley,

Milleville, & Martin, 2007). Based on this reasoning MNs in humans have been suggested to subserve various functions, including action understanding (Rizzolatti & Craighero, 2004), imitation (Iacoboni, et al., 1999), feeling empathy, theory of mind, and development of language (D'Ausilio et al., 2009) (for review see (Iacoboni, 2009; Iacoboni & Dapretto, 2006). Furthermore, a dysfunction of the simulation mechanism supported by MNs is supposed to be associated with clinical disorders such as apraxia (Goldenberg & Karnath, 2006), schizophrenia (Arbib & Mundhenk, 2005) and autism (Iacoboni & Dapretto, 2006). This raises the second criticism: if previous MNs identification paradigms are not a sound enough assumption to identify MNs circuits in humans what is the remaining evidence for the claim that MNs underlie these cognitive functions?

The electrophysiological experiments with monkeys cannot provide much support for the association of MNs with cognitive functions in humans. The functional role of MNs was not assessed directly in these studies and it seems difficult, if not impossible, to investigate higher-cognitive functions such as empathy, theory of mind or development of language in non-human primates. But also the idea that MNs are the supporting neural mechanism of imitation, which has been assumed by many human fMRI studies, cannot draw direct evidence from non-human primate studies. There is an ongoing debate on whether macaque monkeys have the capability to imitate others as this can be done by humans and apes (Turella, et al., 2009). Moreover, the proposal that MNs underlie action prediction and understanding has not yet been investigated systematically in monkeys. This would require assessing these functions with behavioral measurements together with single-cell recording. Although MNs fire during both action execution and observation, it remains unclear whether they have a causal role for action understanding.

This leads to the third, more methodological, concern: how can MNs be detected in humans using fMRI? In general, the BOLD-signal acquired with fMRI measures the change of blood flow in the brain, which has been shown to be related to neural activity (for reviews see Logothetis, 2008; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001). But compared to single cell recordings the spatial resolution of fMRI is limited. A voxel, the smallest information unit of MRI, has a size in the range of millimeters (in cognitive neuroimaging usually 3 mm^3, in this study 3.5 mm^3) and averages the cerebral blood flow due to the activity of up to hundred thousands of neurons (Bartels, Logothetis, & Moutoussis, 2008). However, MNs recorded directly in the monkey account only for a small portion of the total number of neurons in these areas. For instance in the study by di Pellegrino, et al. (1992) the activity of 532 neurons was recorded in area F5. Ninety-two of them (~17%)

discharged both when the animal executed a grasping movement and when it observed the experimenter doing it. The majority of the recorded neurons in this study were either motor neurons or other visuomotoric neurons without MN properties. From the 92 recorded MNs only 56 (~ 60%) were classified as broadly congruent. MNs, as found in the macaque's brain, do not form a uniform or even an exclusive brain area, and it is therefore unlikely that they do so in the human brain. Quite the contrary, they lie in close vicinity to other types of neurons in a small volume of tissue. Although the above reviewed fMRI studies have identified cortical areas that respond during both movement execution and observation or imitation, they fall short to isolate an MN response from the activity of other neural populations. The measured hemodynamic response could also be due to the activation of other motor or visuomotoric neurons which are intermingled with MNs in the same voxels.

1.4. fMRI Adaptation as a Method to Investigate Mirror Neurons

For the above mentioned reasons Dinstein, et al. (2008) proposes the usage of an fMRI adaptation protocol to study MNs in humans. fMRI adaptation (fMRI-A) also known as repetition suppression (RS) has been suggested as a method to assert the functional properties of neuronal subpopulations which are mixed within the resolution of a voxel (for reviews see Grill-Spector & Malach, 2001; Krekelberg, Boynton, & van Wezel, 2006). It is based on the well-known phenomenon that a cortical area exhibits a reduced BOLD response when its preferred stimulus is presented repeatedly. As a method fMRI-A is applied in two steps: (1) a neural population is adapted by repeatedly presenting the same stimulus, (2) the stimulus is modified on a specific dimension or a new stimulus is introduced and the recovery of the BOLD-signal from the adaptation is measured. If the signal remains adapted, i.e. stays on the same level, when the new or modified stimulus is introduced, it will be concluded that the underling neuronal population is invariant to the change. If the signal recovers, this indicates that different subpopulations code the two stimuli. Grill-Spector, Henson, & Martin (2006) discuss different models (fatigue, sharpening, facilitation) to explain fMRI-A. However, the exact underlying neural mechanism is yet unknown.

How can fMRI-A be used to reveal MNs in humans? The repeated observation or execution of an ODM should induce adaptation in the underlying neural population including MNs (within-modality adaptation). Note that MNs should receive, by theory, input from the visual stream when observing an ODM and from the motor system when the same movement is executed. Therefore MNs should keep their adapted state during the transition from

observing to executing the same ODM and vice versa (cross-modal adaptation) (see black line in Figure 1). On the other hand MNs should show a release from adaptation during the transition from observing an ODM to executing a different action (or vice versa) because the different action should activate a different set of MN (see red line in Figure 1). It is known that the observation of a movement can induce motor priming affecting the subsequent execution of a similar movement (Chong, Cunnington, Williams, Kanwisher, & Mattingley, 2008). Therefore it is more conclusive to demonstrate a cross-modal adaptation effect when an action is first executed and then observed than vice versa.
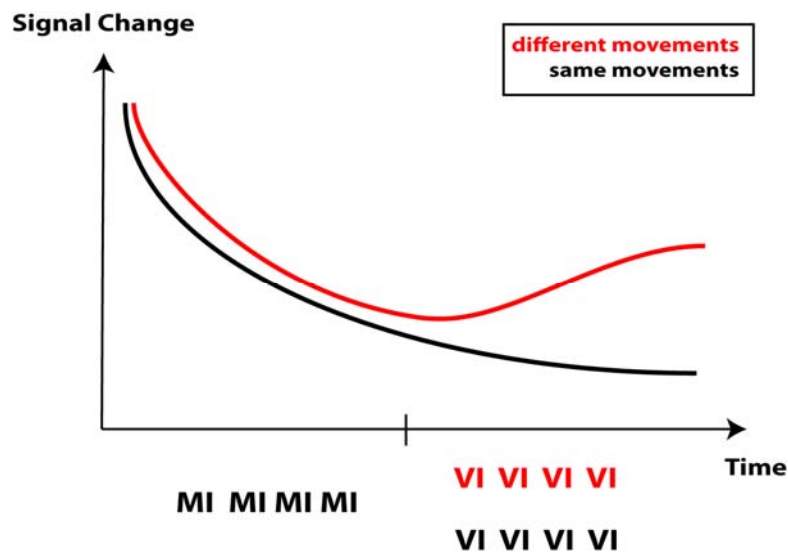
Figure 1. Schematic BOLD time course in brain areas exhibiting cross-modal adaptation. Subpopulation of MNs receives repeated motor input (MI) by the multiple execution of the same movement. In the transition to a visual task (marked by the vertical bin) the subpopulation should continue to receive visual input (VI) and remain adapted if the same movement is observed (black line). If a different movement is observed, the adapted signal should recover (red line).

To the best knowledge of the authors until now only four fMRI studies have used an adaptation protocol to investigate MNs in humans. (Chong, et al., 2008; Dinstein, et al., 2007; Kilner, Neal, Weiskopf, Friston, & Frith, 2009; Lingnau, Gesierich, & Caramazza, 2009).

The first study by Dinstein, et al. (2007) found five movement selective areas which showed within-modality adaptation when the same movement was observed and when it was executed repeatedly. The adaptation response was limited to the anterior inferior frontal sulcus, ventral premotor, anterior intraparietal, superior intraparietal and posterior intraparietal cortices. However, they could not find any cross-modal adaptation in these areas.

A possible explanation for the lack of cross-modal adaptation in that study could be that abstract-symbolic hand movements were used as stimuli. The participants were required to play the rock-paper-scissor game (http://en.wikipedia.org/wiki/Rock,_Paper,_Scissors) against a computer program. A movement was classified as 'repeated' when the decision for a hand gesture was the same for both 'players' and as 'novel' when the gestures were different. These hand actions are, however, no ODM as used in non-human primate studies and it is unclear why such stimuli should trigger MNs. Another drawback of this study is that the analysis was restricted using an imitation task as functional localizer in order to reduce multiple comparison.

Another fMRI-A study found cross-modal adaptation in the inferior parietal lobule (IPL) using abstract-symbolic stimuli (Chong, et al., 2008). In this study the task was to perform different hand actions, e.g. 'hammer hitting a nail' or 'pistol shooting', and subsequently observing the same or a novel hand action on video. However, a limitation of this study is that data was only acquired from IPL. This leaves open the question of whether other brain areas exhibit (even stronger) cross-modal adaptation and why Dinstein, et al. (2007) failed to find such an effect in the IPL.

A third study by Lingnau and colleagues (2009) did not find adaptation using newly learned meaningless hand movements as stimuli. The authors argue that the adaptation effect reported by previous studies could merely reflect semantic categorization. According to this objection the cross-modal adaptation found by Chong, et al. (2008) in the IPL could be due to a higher cognitive categorization of the hand actions. For instance, to pantomime a hammer hitting a nail and observing the same action both belong to the same semantic category. Although this is a sound caveat, the counter proposal by Lingnau, et al. (2009) to use newly learned, arbitrary hand movements is also problematic. An arbitrary, meaningless movement without a visible object as a target should not, according to motor simulation theory, activate the MN system since there is no goal or intention inferable from this movement.

Finally, the most recent study by Kilner and colleagues (2009) was designed to find evidence for MNs in the IFG of humans since this area corresponds to area F5 of the monkey. All analyses were restricted to a small volume within the IFG of both hemispheres based on the maximal extent of the peak activations reported in the meta-analysis by Dinstein et al. (2007)(table 1). Using a custom made manipulandum in the scanner, the participants either had to execute a precision grip or pulled with an index finger with their right hand. The same movements were recorded on video and presented in the observation trials. This task has two advantages in comparison to the tasks used by the three above reviewed studies: (1) both

hand actions are ODMs, which can reasonably be expected to elicit MN responses, (2) previous studies have shown that different types of grips modulate MNs in macaques area F5 (see Rizzolatti, et al., 2001). The study found significant cross-modal adaptation in the IFG, both when the ODMs are first observed and then executed and vice versa. However, due to the restriction of the analysis to a small volume within the IFG, the distribution and structural properties of the human MN system remains unknown. Furthermore, it is unclear why (Kilner, et al., 2009) found significant cross-modal adaptation in the IFG while two other studies failed to demonstrate such an effect in the IFG (Dinstein, et al., 2007; Lingnau, et al., 2009). It is possible that by restricting the analyses a priori to the IFG, and thus increasing the statistical power, an adaptation effect was found that did not surpass the significance threshold in the other studies. Another explanation would be that the particular ODM used in the study triggered a stronger MN response or that the stimuli employed by the other studies completely failed to activate the human MN system. We therefore designed a similar task as Kilner, et al. (2009) using ODM but did not restrict our analysis a priori to 'candidate regions'.

Summarizing, fMRI-A studies provide mixed evidence for the existence of MNs in humans. While two studies reported significant cross-modal adaptation, one in the IPL and the other in the IFG, two others failed to find significant effects. The studies used different types of tasks and restricted their analyses on different brain areas. While three of them used meaningless or abstract-symbolic hand actions as stimuli, only one of them employed ODMs as used by the studies originally describing MNs in monkeys. All of them restricted their analyses, either based on anatomical or functional defined regions of interest. Both the dissimilar tasks and the restriction to different brain areas make it difficult to reconcile these studies to form a coherent picture.

1.5. Aim of the Present Study

The aim of the present study was to examine human MNs with fMRI-A to gain more insights about their specific location within the human cortex (fMRI experiment) and to shed light on the functional properties of the human MN system by studying the behavioral consequences of induced cross-modal adaptation (psychophysical experiment).

In the fMRI experiment participants first executed and then observed one of two versions of a repeated precision grip towards a button box - either an object-directed movement (ODM) in which a button on the box was pressed or a non-object-direct movement

(NDM) in which the grasping movement terminated below the button box without touching it (see Figure 2). We reasoned that only the ODMs should activate the MN system both when observed and executed. We therefore considered cross-modal adaptation between the execution and the observation of the ODMs as evidence for MN activity. Cross-modal adaptation was determined by comparing the recovery of the BOLD-signal when participants either observed the ODM or the NDM after initial within-modality adaptation through repeated execution of one movement type. To avoid the circular reasoning that is caused by restricting the analysis to 'candidate regions', we conducted a whole-brain scan and analysis. This also allows for comparing the results of the present study to previous fMRI-A studies that have reported mixed evidence focusing on different brain regions.

Given that cortical regions exist which show cross-modal adaptation the question arises of whether behavioral changes go along with this physiological effect. In the psychophysical experiment we took a first step towards a better understanding of the functional properties of the human MN system by investigating the relation of cross-modal adaptation of MNs and its behavioral consequences. Specifically, we were interested in whether motor adaptation leads to changes in action prediction in a purely behavioral task. To do so participants carried out a motor action (ODM or NDM) repeatedly (adaptation) and immediately afterwards saw a video showing either the carried out action or the other action. Participants had to report as fast as possible which of the two actions they had seen (discrimination task). We reasoned that if the human MN system is used for action prediction, then participants' discrimination performance should depend on the previous motor adaptation.

Taking the above-mentioned criticisms into account, we decided to use simple ODMs, which closely resemble the tasks used in the monkey studies, originally describing MNs, for both experiments.

2. fMRI Experiment

2.1. Methods

2.1.1. *Participants*

Ten right-handed healthy volunteers (4 females) took part in the study (mean age, 22.5 years; range, 21–30 years). All subjects gave written informed consent before testing and were compensated for participating in the study. The study had been approved by the ethical committee of the University of Tübingen. All participants had normal or corrected-to-normal vision and were required to wear ear plugs to avoid hearing damage. They were asked to

minimize movements while in the scanner and their heads were stabilized with foam cushions.

2.1.2. *Visual Stimulation*

Videos clips were recorded using a digital video camera (Sony DCR-TRV900E) and were processed by video editing software (Virtual Dub 1.9.7) and MATLAB (The MathWorks Inc., Natick, Massachusetts, USA). The stimuli were projected onto a screen inside the MRI scanner at a frame rate of 60 Hz and a screen resolution of 1280 x 1024, and were visible to the participants by a mirror mounted on the head coil. All other stimuli were created in and displayed together with the video clips using MATLAB Psychtoolbox-3 (45) for Windows.
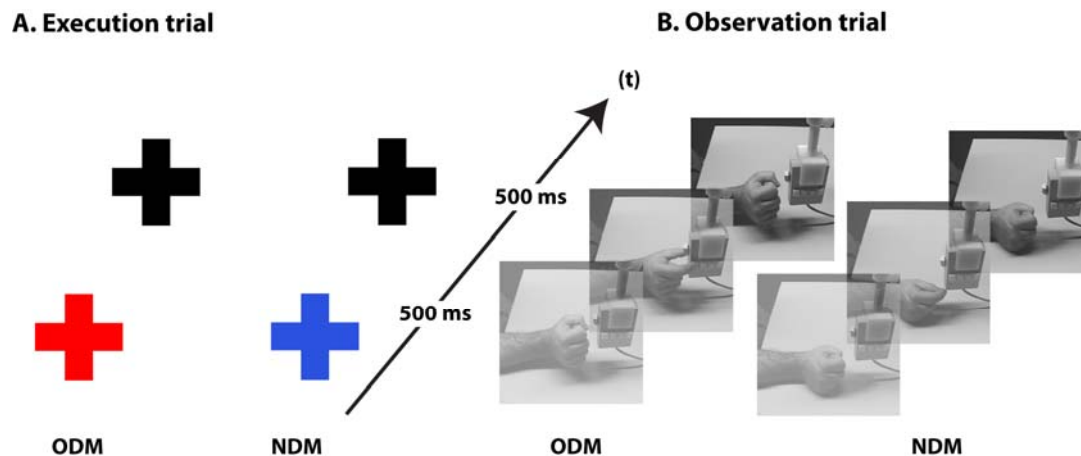
Figure 2. Schematic outline of an experimental trial. In the execution trials (A) the fixation cross turned from black to red or blue for 500 ms ten times in a row to instruct for a sequence of ten movements. A red fixation cross indicated the participants to execute an ODM and a blue fixation cross an NDM. During the observation trials (B) videos displaying either the ODM or the NDM were shown twenty times in repetition lasting for one second (for the experimental design see Figure 3). All stimuli were presented on a screen of medium grey (127 pixel value) which is not shown here.

2.1.3. *Stimuli*

The experiment consisted of two types of trials, namely execution and observation trials. Participants performed an ODM or an NDM in an execution trial and had to observe passively one of these movement types in an observation trial. Both movement types were precision grips towards a button box but in the ODM condition a button on the box was pressed and in the NDM condition the grasping movement terminated below the button box without touching it. The stimuli in the execution and observation trials are depicted in Figure

2. They were presented in front of a grey background and a black fixation cross was shown between trials.

The beginning of the execution trials was announced to the participant with the fixation cross turning grey for a second before the first execution trial in order to give the participants time to prepare for their first movement. During the execution trials the fixation cross changed its color for 500 ms. A red cross was an indication for the participants to execute an ODM while a blue cross instructed participants to carry out an NDM. The participants initiated the movement with the onset of the color change and reached the target position of the movement when the color of the fixation cross switched back to black. The fixation cross remained black for a period of 500 ms during which participants moved there hand back to the initial position. Participants carried out a movement (ODM or NDM depending on the condition) 10 times. On the last trial the fixation cross turned white instead of blue/red to indicate the last trial thereby minimizing the risk of an action execution overshoot into the IBI. Participants were instructed about this procedure prior to the experiment and received practice trials until being comfortable with the procedure.

For observation trials video clips of a right hand of a male person were taken. Two versions were created, showing the hand either grasping the button on the button box or grasping below it. The video clips were symmetric, i.e. the first 15 frames consisted of grasping towards and reaching the button and they were then played backwards. With a presentation speed of 30 frames per second the video clips (and thereby the ODM and NDM action) lasted a second. Each video was a normalized grey scale video (mean luminance of 127 pixel value and a root-mean-square of 30), and had a resolution of 400 x 400 pixels. A black fixation-cross was superimposed onto the center of the videos to help minimize eye movements. A random horizontal jitter of up to 10 pixels was applied to each video's position on every observation trial (the fixation-cross remained stationary) to prevent retinal adaptation. At the beginning of the video the hand of the actor formed a fist then reached out and grasped the button (ODM) or index finger and thumb touched each other below the button box (NDM) (see Figure 2). The viewing distance from the mirror to the projection plane was 86 cm, and the distance from the participant's eyes to the mirror approximately 10 cm, resulting in a visual angle of 8.63°x 8.63° for the videos and 0.72° x 0.72° for the fixation crosses.

2.1.4. *Procedure and Design*

The experiment consisted of two localizer runs and four experimental runs (tested always in this order). Localizer runs were designed to find brain regions that show an increased BOLD-signal for ODMs compared to NDMs both when observed (visual localizer) and executed (motor localizer). Hence, each localizer run probed the two movement types (ODM, NDM) within only one modality. Experimental runs were designed to measure cross-modal adaptation. To do so, each experimental run probed the execution-observation transition for a different movement type combination, namely ODM-ODM, ODM-NDM, NDM-ODM, NDM-NDM (the first movement type refers to the execution trials while the second movement type refers to observation trials).

Localizer runs

Each localizer run started with a 40 s baseline, which consisted of a gray blank screen during which the participant rested. This baseline was immediately followed by a block of ten observation or execution trials (depending on the localizer). Each trial lasted one second and all trials within a block probed always the same movement type (ODM or NDM). Eight ODM and eight NDM blocks alternated within a localizer run to a total of 16 blocks per localizer run. Blocks were separated by a 20 s inter block interval (IBI). Hence, the total run length was 8 min 40 s for each localizer. The testing order of the motor and visual localizer was counterbalanced across participants.

Experimental runs

Each of the four experimental runs probed one of the following execution-observation transitions, which differed with respect to their movement type combination: ODM-ODM, ODM-NDM, NDM-ODM, NDM-NDM. Testing order was randomized for each participant. The general design of the experimental runs is depicted in Figure 3. Each run started with a baseline (40 s) and consisted of 10 blocks separated by an IBI (60 s). A block combined 10 execution trials (10 s) immediately followed by 20 observation trials (20 s). Both a single execution and observation trial lasted for one second. In total this amounted to length of an experimental run of 15 min 40 s. Participants were informed about the upcoming condition by the investigator prior to the start of the condition. Between the second and the third experimental run an anatomical scan (6 min 40 s) was conducted allowing the participants to relax and close their eyes. The total scanning time was 1 h 26 min 40 s and the whole experiment with instructions (which were given outside the scanner) and informing took about 2 h 30 min.
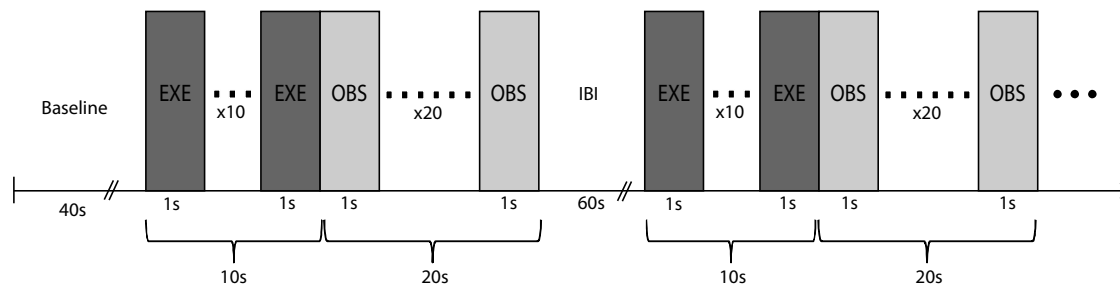
Figure 3. Experimental design of the fMRI adaptation paradigm. Participants executed (EXE) either the object-directed (ODM) or the non-object directed movement (NDM) for ten times immediately followed by the observation (OBS) of the ODM or the NDM for twenty times. Each condition started with a baseline (40 seconds) and consisted of 10 blocks interleaved with 60 seconds inter block interval (IBI).

2.1.5. *Data Acquisition*

Data were acquired on a 3 Tesla Siemens Trio Scanner (Siemens Medical Solutions, Erlangen, Germany) using a 12-channel birdcage head coil. Functional images were acquired with a T2*-weighted gradient-recalled echo-planar imaging (EPI) sequence with the following parameters: TR = 2500 ms; TE = 40 ms; Flip Angle = 60°; FOV = 240 x 240 x 36 mm^3; voxel size (resolution) = 3.5 x 3.5 x 3.5 *mm*. 34 slices covering the whole brain were acquired in all functional runs. The same parameters were used for localizer and experimental runs. The experiment began with two localizer scans of 208 volumes each (8 min 40 s). For the experimental runs each participant completed 4 scans of 276 volumes each (15 min 40 s). Stimulus presentation was automatically triggered by the fMRI sequence at the beginning of each scan. In order to co-register the low-resolution functional images to a high-resolution anatomical scan a T1 weighted anatomical scan with the following parameter was acquired: TR = 1900ms; Flip Angle = 9°; FOV = 256 x 256 x 36*mm*; voxel size (resolution) = 1 x 1 x 1 *mm*. The anatomical scan comprised 176 slices (6 min 40 s) covering the whole brain and was carried out in between the second and third experimental run.

2.1.6. *Data Analysis*

The data was analyzed using the FSL program package (Version 4.0, Analysis Group, FMRIB, Oxford, UK; Smith et al., 2004). All functional runs were motion-corrected to the middle volume of each individual run using the *mcflirt* feature of FSL (Jenkinson & Smith, 2001). Preprocessing of the data was completed with *Feat* (Woolrich, Ripley, Brady, & Smith, 2001) and included a high-pass filtering of 100 Hz to detrend the raw data, spatial

smoothing (5mm), and FILM prewhitening (Woolrich, et al., 2001). Image information belonging to brain tissue was extracted from the anatomical high resolution scans applying the *BET* routine of FSL (Smith, 2002). Functional runs of each participant were then registered to the corresponding brain-extracted image. Statistical maps for the two localizer and the four experimental runs were generated with *Feat,* performing a general linear model (GLM) analysis with an appropriate hemodynamic response function (gamma function).

The GLM contrast [ODM > NDM] was computed for the two localizer runs (motor and visual) and participant separately. On the resulting statistical maps a group analysis was run using *FLAME 1* (Beckmann, Jenkinson, & Smith, 2003) producing second-level z-statistic maps (Figure 4, upper and middle panel). Third level analysis using *FLAME 1+2* (Beckmann, et al., 2003) was performed on the two second level analyses to define regions that were more active during ODMs than during NDMs both when observed and executed (Figure 4, lower panel). We used the Jülich histological (cyto- and myelo-architectonic) atlas for FSL (Eickhoff et al., 2007) to describe patterns of activity anatomically.

For the experimental runs the first level GLM was computed with the contrast [task > baseline] for each participant individually. In the second GLM, the adaptation difference (AD) was obtained in the following way: We subtracted conditions in which the execution-observation transition occurred between the same movement types (ODM-ODM, NDM-NDM) (same conditions) from conditions in which the execution-observation transition occurred between different movement types (ODM-NDM, NDM-ODM) (different conditions):

$$AD = (\text{ODM-NDM} + \text{NDM-ODM}) - (\text{NDM-NDM} + \text{ODM-ODM}) \qquad (1.1)$$

This contrast was calculated for each participant separately. Note that 'same' and 'different conditions' contain the identical movement types but have different cross-modal transition types. Hence, if the type of transition has no effect, the movement types in equation 1.1 should cancel each other out and the AD should become zero. On the other hand, if cross-modal adaptation takes place, transition type should matter in the sense that adaptation should occur only for transitions between the same movement types (resulting in a smaller signal). In this case the sum of 'same conditions' is expected to be lower than the sum of 'different conditions' (see also Figure 1). Since we subtract 'same' from 'different' conditions, the AD would be expected to be larger than zero. In a third level analysis using *FLAME 1+2* the AD was determined across participants. No statistical threshold was imposed at any stage so far.

The localizer runs and the experimental runs were designed to test the two identification criteria for MNs, i.e. (1) higher activation during the execution and observation of ODMs compared to NDMs (localizer runs), and (2) cross-modal adaptation in the transition between execution and observation (experimental runs). To identify brain areas that adhere to both criteria, we conducted a cluster analysis in which the third level statistical maps of the experimental and localizer runs was combined using FLAME 1+2 (fourth level analysis). To protect against false positives we chose a voxel-wise threshold of $p < 0.001$ (uncorrected) and a cluster-threshold of $p < 0.05$. Parameter estimates for the cross-modal adaptation contrast [different conditions – same conditions] were extracted for all clusters revealed by the forth level analysis using the FSL toolbox *Featquery*.

We also were interested in the time course (TC) of the adaptation. Note that the many repetitions of execution and observation trials in the present fMRI experiment allow investigating the within-modality adaptation in the motor and the visual task and the cross-modal adaptation between tasks over time. We used the resulting clusters of the fourth level analysis (Fig. 6) as masks to extract the BOLD TCs from these clusters for each experimental run and participant separately using the FSL command *fslmaths*. The TCs were then averaged across subjects and blocks and normalized to the baseline of each experimental run using MATLAB. Finally, the scale of the TCs was converted to percentage of signal change.

Finally, we examined whether possible adaptation effects could merely reflect semantic categorization. Consider for a moment a worst-case confound scenario for this fMRI-A study. In this scenario we assume that the underlying semantic categorization for the used ODM and NDM is located in close vicinity in the same cortical area. Importantly, assume that the spatial distribution of the categorization units in the brain is below the resolution of a voxel. In general, the same semantic categorization unit should be triggered in the 'same conditions' and different units in the 'different conditions'. In this scenario, we would therefore expect that both categorization processes activate the same voxels but only the 'same conditions' could induce cross-modal adaptation. As a result the AD (equation 1.1) would become larger than zero. However, this is also predicted for cross-modal adaptation of MNs. To differentiate whether the AD rather reflects semantic categorization or the MN activity we computed the single adaptation differences for the ODM (oAD) and the NDM (nAD):

$$oAD = \text{NDM-ODM} - \text{ODM-ODM} \quad (1.2)$$
$$nAD = \text{ODM-NDM} - \text{NDM-NDM} \quad (1.3)$$

For semantic categorization it is reasonable to assume that executing ODMs and NDMs (first part of each subtrahend) as well as observing ODM and NDM activate the categorization mechanism to the same degree. Hence equation 1.2 and 1.3 should not differ from each other. MNs, however, are expected to be more activated during ODMs than during NDMs and should therefore exhibit stronger RS between ODMs than between NDMs. Hence, the oAD should become positive during the period of the visual task while the nAD should not deviate significantly from zero.

2.2.   Results

2.2.1.   *Localizer Runs*

The localizer runs were designed to find brain areas that show an increase in BOLD-signal during both the execution and observation of ODMs. Unlike previous studies (e.g. Kilner, et al., 2009) we did not compute the localizer contrasts against the baseline [task > baseline] but compared the activity of the two movement types directly [ODM > NDM].
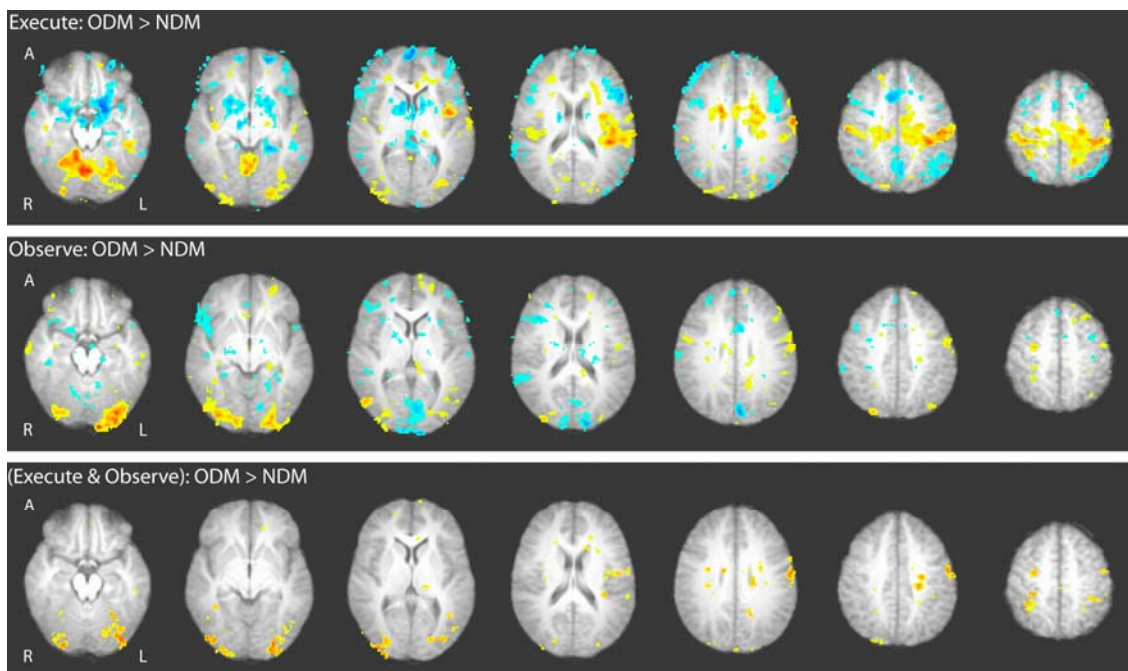
Figure 4. Result of the localizer runs outlining brain areas selective for ODMs. Statistical maps (threshold at z=1.522, N=10) displaying regions responding more to ODMs than to NDMs (warm colors) and vice versa (cold colors) during execution (upper panel) and observation (middle panel). The lower panel depicts the commonly activated areas between observation and execution. Axial view, left side of the image corresponds to the right hemisphere. A liberal threshold was chosen to demonstrate the general pattern of activity.

Note that previous studies often used the contrast [task > baseline] by which all areas are selected that are active in action observation and execution (e.g. Kilner, et al., 2009), while the present contrast only includes areas that show more activation for ODMs than for NDMs. Hence, the latter contrast can be considered as being more conservative.

The z-statistic map of the motor localizer run (Figure 4, upper panel) highlights regions that were more active when an ODM was executed compared to when an NDM was executed. There is a widespread activity difference, which is most pronounced in the dorsal parietal lobule. Note that part of the activity in visual areas might be due to the colored fixation crosses used as motion cues in the experiment. The middle panel of Figure 4 depicts regions that were more activated by the observation of ODMs than NDMs. The differentially activity is predominately located in the early visual cortex, especially V1. The combination of the two statistical maps resulted in a third-level contrast revealing brain areas that were more active when an ODM was observed and executed compared to an NDM. There is considerable overlap in both occipital and parietal areas including the bilateral area V2 and V4, bilateral primary somatosensory cortex (S1), left premotor cortex (PM) and left inferior parietal lobule (IPL).

2.2.2. *Experimental Runs*

We calculated a GLM for the adaptation difference (AD) (see equation 1.1.) on the second-level contrast [Task > Baseline, across all participants]. Several brain regions showed cross-modal adaptation (Figure 5.), most pronounced in the right orbito-frontal gyrus, bilateral PM, bilateral S1 and bilateral Broca's area. However, cross-modal adaptation can be in general due to diverse processes such as task representation, working memory, trajectories. We reasoned that only brain areas that exhibit cross-modal adaptation and are active when observing and executing ODMs can be considered as potential MNs areas. We therefore combined the analyses of the experimental runs and the localizer runs in a forth level analysis.
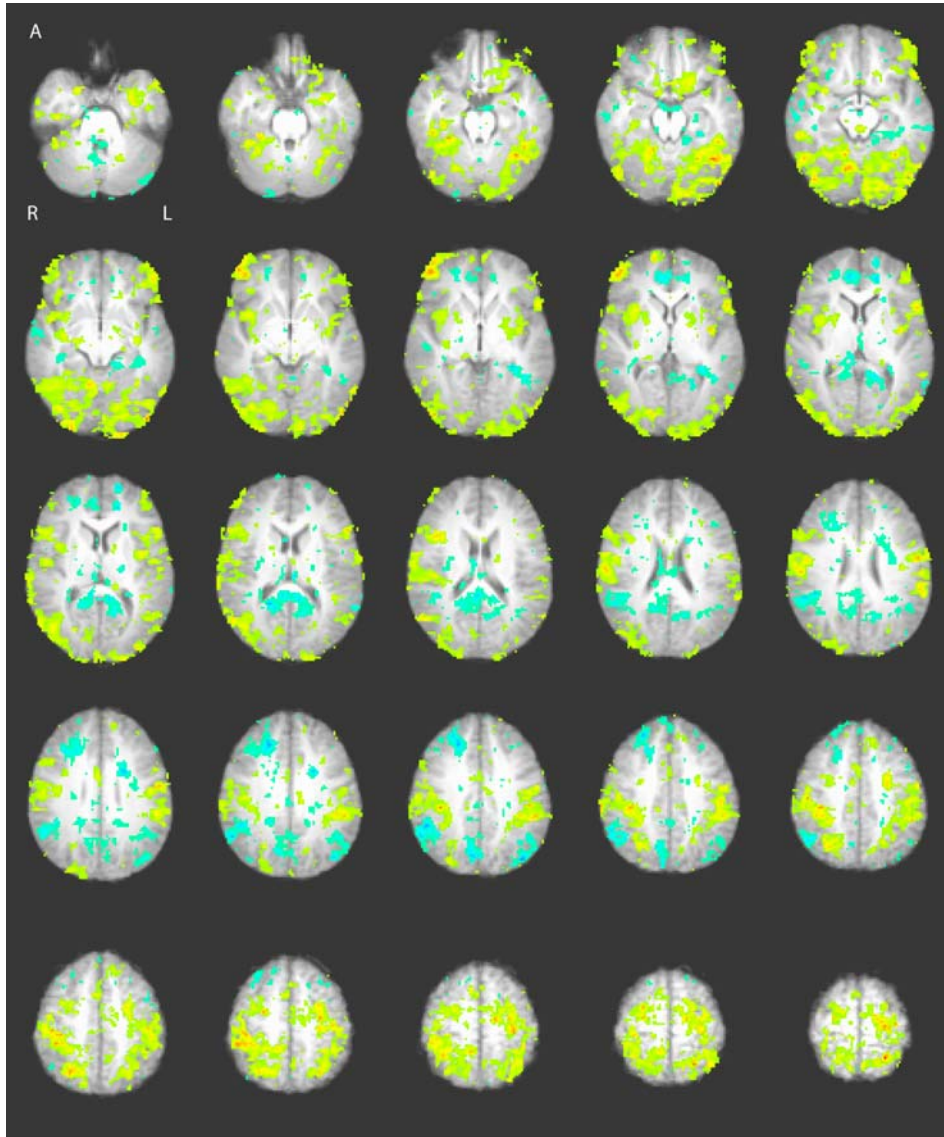
Figure 5. Result of the experimental runs outlining brain areas exhibiting cross-modal adaptation. Statistical maps (threshold at z=1.522, N=10) displaying regions showing positive cross-modal adaptation [different conditions – same conditions] in warm colors and negative adaptation in cold colors. Axial view, left side of the image corresponds to the right hemisphere. A liberal threshold was chosen to demonstrate the general pattern of activity.

2.2.3. *Cluster Analysis*

The cluster analysis combined the third order analyses of the localizer runs and the experimental runs to identify areas with overlapping activity. The aim was to reveal brain areas that meet both criteria for potential MN areas: (1) activation by observation and execution of ODMs, (2) cross-modal adaptation in the transition from the motor to the visual task. To protect against false positives we imposed a voxel-wise threshold of $p < 0.001$

(uncorrected) and cluster-threshold of p < 0.05. Four clusters turn out significantly in the right S1, right aIPS, left PM and the right cerebellum (Fig. 6). Table 1 contains position (MNI coordinates), maximal z-value, and size and p value for each cluster. The size of the clusters ranged from 37 to 52 voxels.

Table 1. Size (number of voxels), cluster p values, peak activations (z-scores), coordinates (MNI space), location (based on Jülich histological atlas) and side for the four areas that were significant in the cluster analysis combining the localizer contrasts and cross-modal adaptation.

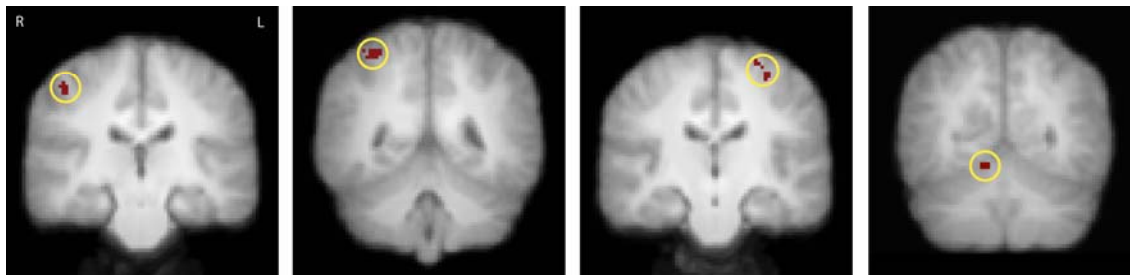| Size	P	Peaks	X	Y	Z	Location	Side
52	0.005	6.02	48	-24	52	Primary somatosensory cortex (S1)	r
45	0.011	5.4	36	-48	56	Anterior intraparietal sulcus (aIPS)	r
42	0.014	6.33	-24	-20	66	Premotor cortex (PM)	l
37	0.024	6.35	12	-58	-12	Cerebellum	r

Figure 6. Coronal view of significant clusters in the right primary somatosensory cortex (S1), right anterior intraparietal sulcus (aIPS), left premotor cortex (PM) and the right cerebellum. All voxels that compose the individual cluster are depicted in red (voxel volume: 3.5 mm^3). Left side of the image corresponds to the right hemisphere. (N=10)

2.2.4. *Analysis of Time Courses and Parameter Estimates*

Figure 7 depicts the trial-based-averaged TCs of the experimental runs (upper panel), the TCs of the trial-based-averaged AD (middle panel) and the estimated parameters of the cross-modal adaptation (lower panel) for the four clusters (A-D) (see section *data analysis* for details). The TC of the AD was computed by subtracting the upper graphs for the two 'different conditions' (ODM-NDM, NDM-ODM) from the two 'same conditions' (NDM-NDM, ODM-ODM).

The onset of the motor task, the offset of the motor task (which also marks the onset of the visual task) and the offset of the visual task are indicated by vertical, dotted lines in Figure 7. In order to assess a significant deviation of the AD from zero we computed the signal-to-noise ratio (SNR) by dividing the AD value of each time point by the standard deviation of the AD values. We consider scores that deviate more than two SNR from the mean AD (AD=0) (marked by the blue line) as substantial deviations. Note that the time courses are reshaped for display purposes so that there are only 8 TRs of the baseline/IBI at the beginning. Furthermore, the hemodynamic lag, i.e. the general delay from stimulus onset to the response of the BOLD-signal (about 3-6 s or two TRs in this figure) (Logothetis, 2008) is not taken into account in Figure 7. While the trial-based averages of the TC are model free, the estimated parameters are based on the general linear model (GLM) underlying the analysis done with FSL.

All TCs show a clear reflection of the motor task onset with different amplitudes and timings. The peak activation during the motor task is in general higher when the participants had to execute ODMs compared to NDMs as expected by the localizer contrast [ODM > NDM] that contributed to the cluster analysis. The decrease of BOLD-signal with ongoing repetition of the execution trials indicates within-modality adaptation in the motor task. In the transition to the visual task the clusters respond differently. While the TC of the cluster in the S1 does not seem to be very sensitive to the onset of the visual task, the other clusters exhibit a clear increase in BOLD-signal. In all clusters the peak activation during the visual task is below the peak in the motor task. This is in accordance with the differential activation pattern [execution > observation] reported by electrophysiological studies measuring MNs directly in non-human primates (Rizzolatti & Craighero, 2004). Within-modality adaptation for the visual task is visible especially in the PM (Figure 7C) and the Cerebellum (Figure 7D). In the other two clusters the signal drops onto or even below the baseline level during visual stimulation.

In order to estimate the effect of cross-modal adaptation, the experimental runs combing the same movement (solid lines) have to be compared to the runs combing different movements (dashed lines). If cross-modal adaption occurred, the signal level during the visual task should be lower when the execution-observation transition was between same movements than between different movements. Given that only the ODMs are expected to activate the MN system, it is crucial to analyze the difference between the NDM-ODM condition (red dotted line) and the ODM-ODM condition (red solid lines). At the beginning of the TC the ODM-ODM condition is higher in all clusters, as again expected by the

localizer contrast [ODM > NDM]. However, in the transition to the visual task the lines cross, and in the course of the visual stimulation the ODM-ODM line falls considerably below the NDM-ODM. Note that both conditions are physically identical after the onset of the visual task. However, somewhat surprisingly, the opposite is the case for the difference between ODM-NDM and NDM-NDM condition (black lines). In all four clusters the TC for the latter (black dashed lines) lies above the former condition (black solid line) after the onset of the visual task. This means that the NDM signal in the visual task is higher when it was preceded by an NDM execution than by an ODM execution.

A possible explanation for the apparently inverted cross-modal adaptation effect found with the NDMs visual tasks could be the post-stimulus undershoot (PSU) induced by the ODM in the motor task. The PSU described the phenomena that the BOLD-signal drops under the baseline level after an activation (Logothetis, 2008). The PSU is usually proportional to the amplitude of the activity induced by the stimulus. In area S1, for instance, the signal seems to be mostly unaffected by the visual stimulation and the difference after motor offset could reflect merely the difference of PSU induced by the motor task. However, this seems to be less likely in the other clusters since there the visual task caused a considerable reactivation reducing a PSU.

The TC of the AD depicted in the middle panel of Figure 7 is not affected by PSU since the difference is balanced. Note that the AD had been already used in the GLM together with the localizer contrast to define the clusters. It is therefore expected that the AD is in general positive. Surprisingly, the AD in the PM and the cerebellum did not deviate significantly from zero and seemed to fluctuate more or less randomly (the red line is always below the blue line). There are two significant peaks in the S1 and a long lasting deviation in the aIPS indicating cross-modal adaptation.

The parameter estimates depicted in the lower panel of Figure 7 indicate that the cross-modal adaptation varies between participants. For instance participant 4 exhibited almost no adaptation in all clusters. However, almost all participants have positive estimated parameters and the mean of the cross-modal adaptation is significant in all clusters. [S1: $t(9)$ = 4.29, $p < .005$; aIPS: $t(9) = 4.80$, $p < .001$; PM: $t(9) = 4.21$, $p < .005$; Cerebellum: $t(9) =$ 3.37, $p < .010$].

In summary, while all four clusters turn out significantly in the GLM for the cross-modal adaptation their respective trial-based averaged TCs did not show the adaptation effect as clearly. Only in the S1 and the aIPS a cross-modal adaptation effect is visible in the TCs.
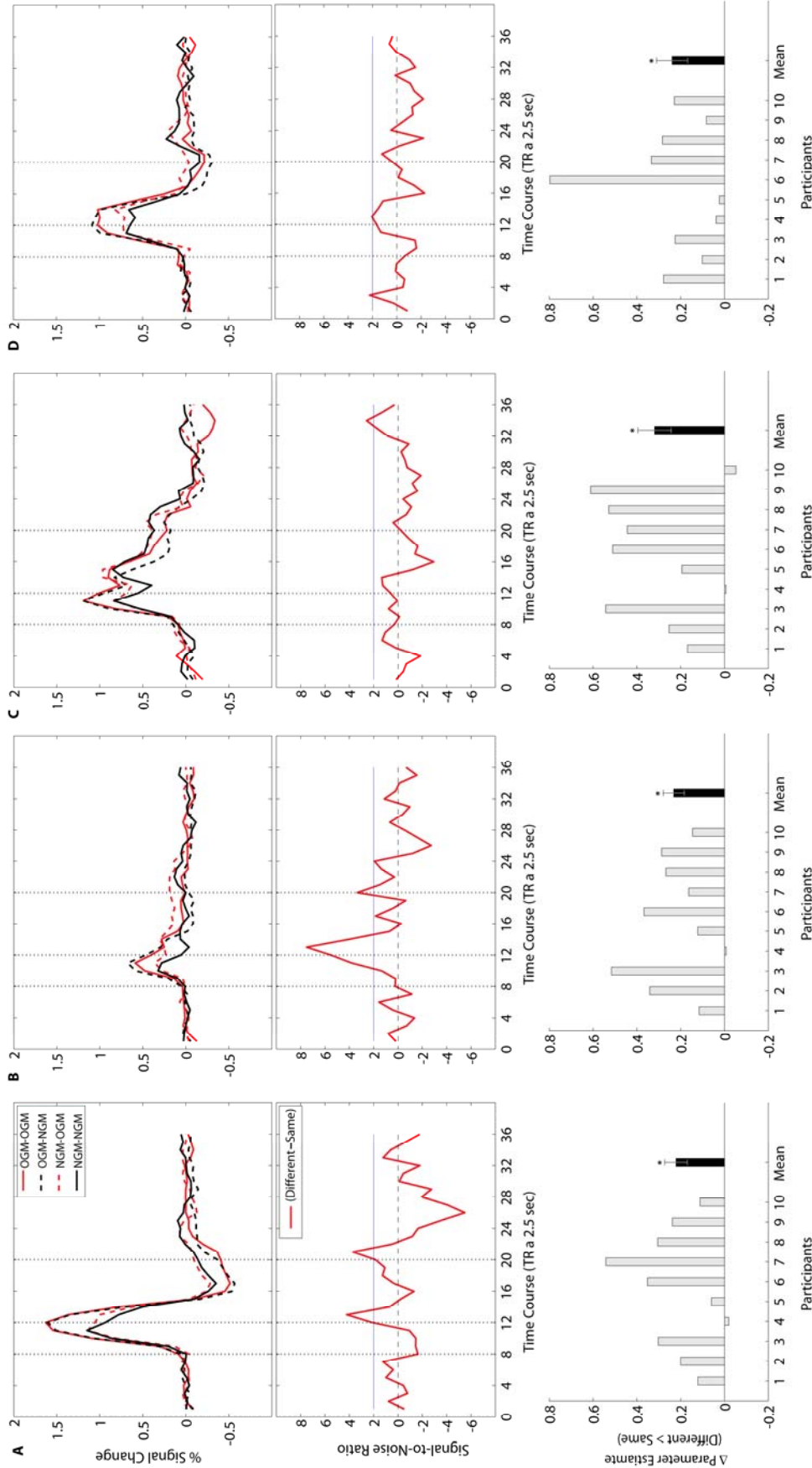
Figure 7. Trial-based-averaged and parameter estimates for the S1, aIPS, PM and cerebellum (A-D). Time courses (TC) of the experimental runs are depicted in the upper panels, the TC of the trial-based-averaged adaptation in the middle panels and the estimated parameters of the cross-modal adaptation in the lower panels (See chapter: *data analysis* for details). Vertical, dotted lines in the upper and middle panel indicate the onset of motor task, onset and offset of visual task. The blue horizontal line in the middle panel marks a SNR of two and is an estimation of significance. Parameter estimates were extracted for each participants, converted to percentage and mean and standard error were computed

Analyzing the TCs allows examining the linkage of cross-modal adaptation to experimental events. Moreover, conclusions can be drawn about whether the AD is rather due to RS in the transition of the ODM-ODM or the NDM-NDM condition. This is illustrated in Figure 8, which depicts the two single adaptation differences oAD (equation 1.2) and nAD (equation 1.3) for the S1. The relevant period to indicate whether the adaptation is rather due to ODM or NDM is after the offset of the motor task (marked as grey a rectangle in Fig.8, already taking the hemodynamic lack into account). The graphs clearly show that the adaptation effect is dominated by adapting to the ODM. Because semantic categorization predicts that both NDMs and ODMs result in similar adaptation effects, the observed activity is not in line with semantic categorization. However, as pointed out beforehand, the lack of an adaptation to the NDM might be due to the asymmetric PSU induced by executing the ODM.
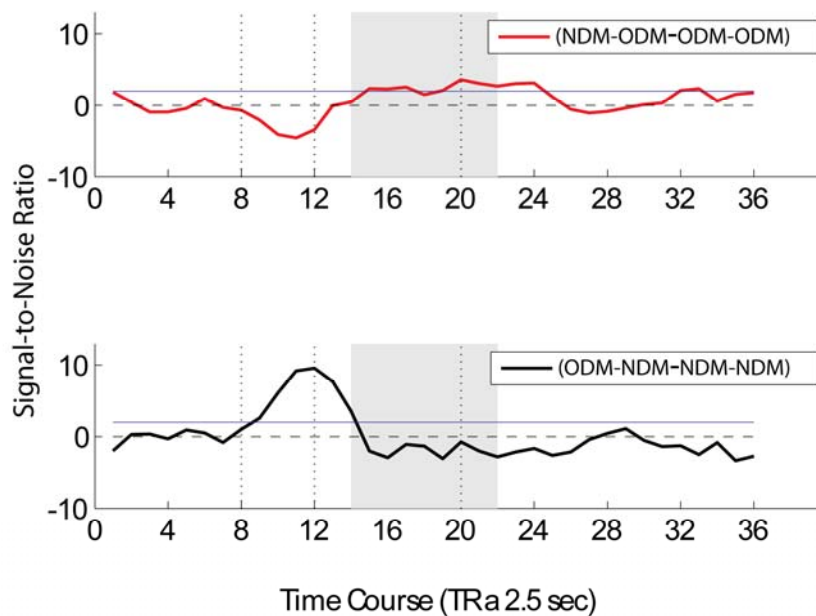
Figure 8. Single adaptation differences illustrated for the right anterior intraparietal sulcus (aIPS). In the upper panel the 'different' experimental run combining the NDM in the motor task and the ODM in the visual task is subtracted by the 'same' experimental run employing in both tasks the ODM. In the lower panel the 'same' and 'different' experimental runs with the NDM in the motor task are compared. The grey rectangle marks the area where both experimental runs are identical (already taking the hemodynamic lack into account). Vertical, dotted lines indicate the onset of motor task, onset and offset of visual task. The blue horizontal line marks a SNR of two and is an estimation of significance.

2.3. Discussion

The aim of the present fMRI experiment was to provide further evidence on the location and nature of MNs in humans. On the basis of the literature on MNs in non-human primates we reasoned that only the ODM but not the NDM should activate the MN system. We further expected that areas containing MNs would exhibit cross-modal adaptation between executing and observing ODMs since MNs should receive, by theory, input by both modalities.

In the first step of the analysis we identified brain regions that meet the minimal criterion of MNs, i.e. stronger activation by ODMs than by NDMs, both when observed and executed. Then we computed an adaptation difference to find regions that showed a reduced hemodynamic response when the same movement is executed and subsequently observed (ODM-ODM or NDM-NDM) compared to when a different movement (ODM-NDM or NDM-ODM) is executed and then observed. Finally, a cluster analysis was conducted combing these two analyses to reveal brain areas that exhibited both stronger activation by ODM and cross-modal adaptation.

The activity in the motor localizer was widespread and peaked in the motor areas of the parietal lobule. The activation map for the visual localizer was similarly extensive but the highest responses occurred in the early visual cortex. Combining both localizer runs to a second-order statistical maps showed peak activation in the visual cortex bilateral (V4, V3), in the S1 (bilateral), the left PM and the inferior and superior parietal lobule (bilateral). The GLM cross-modal adaptation contrast computed from the four experimental runs was highest in various frontal and parietal regions, including Broca's area (bilateral), the right orbito-frontal gyrus and the left PM. Finally, the cluster analysis revealed, with a voxel-wise threshold of $p < 0.001$ (uncorrected) and cluster-threshold of $p < 0.05$, four significant clusters in the right S1, the right aIPS, the left PM and the right cerebellum.

How are these clusters, which exhibited significant cross-modal adaptation, related to previously discussed MN areas? While the aIPS and the PM have been suggest before as putative MN areas, the clusters in the S1 and in the cerebellum have not yet been associated directly with MNs.

In the meta-analysis by Dinstein and colleagues (2007) six putative MN areas were summarized, based on previous conventional fMRI studies which had used imitation or observation protocols to identify MN areas. Among these areas were also the ventral PM (left) [-53, 8, 21] and the right aIPS [35, -46, 57] (average MNI coordinates across all studies). While the ventral PM is located in the IFG and is considered to be the homologue

area of macaque's area F5, the aIPS is part of the IPL and corresponds to area PF/IPL of the monkey. In both areas MNs have been reported using single cell recording in the non-human primate. Accordingly, Dinstein, et al. (2007) found significant within-modality adaptation for action observation and execution but no cross-modal adaptation in the ventral PM and the aIPS. However, using stimuli that resample those of the monkey studies we found significant cross-modal adaptation in the right aIPS and in the left PM, which is in line with Dinstein's review but in contrast to Dinstein's fMRI-A results.

The coordinates of the peak voxel of the right aIPS cluster [36, -48, 56] are almost identical to the average coordination reported in meta-analysis. This is also in accordance with the results of Chong, et al. (2008) who found cross-modal adaptation in the right aIPS although with a slightly different peak activation [58, -56, 34]. In summary, there is converging evidence from human fMRI studies that the IPL contains a neural subpopulation that is active both when an ODM is observed and when it is executed. Moreover, fMRI-A studies have demonstrated that this area exhibit cross-modal adaptation. In order to interpret these findings as evidence for a underlying MN circuit it is important that MNs have been demonstrated in the macaque's homologous area PF/IPL (Fogassi, et al., 2005). Therefore it is likely that the IPL contains MNs and that the cross-modal adaptation reported by the present study emerged from adapting MNs.

Activity in the left PM is also in agreement with previous studies. However, the coordinates of the peak activation [-24, -20, 66] revealed by the present study deviates considerably from the average coordinates reported in the meta-analysis and the study by Kilner, et al. (2009) [-50, -2, 12]. While these studies reported activity in the ventral PM, the cluster in the present study is located in the dorsal part of the PM. However, the scanning parameters in the study by Kilner, et al. (2009) did not cover the dorsal parts of the PM. Hence, one cannot rule out that the peak adaptation might have also occurred in the dorsal PM but Kilner, et al. (2009) might have not observed this effect due to spatial scanning restrictions. We therefore were interested in whether we observe cross-modal adaptation in the ventral PM. We reanalyzed the statistical maps of the cross-modal adaptation in the experimental runs. This contrast is depicted in Figure 5 (axial view) with a liberal threshold (z=1.5). Figure 9 (coronal view) shows the same contrast with a more conservative voxel-wise threshold of z=3.6 or p < 0.001 (uncorrected) for the PM (range y= [-8 8] in MNI coordinates; step size between brain slices: two volumes). The middle image in the lowest row of Figure 9 shows a distinct pattern of activity in the ventral PM (peak: [-56 -6 28]),

while the other slices demonstrate only scattered activity in the dorsal parts of the PM. We therefore find cross-modal adaptation in the ventral PM.
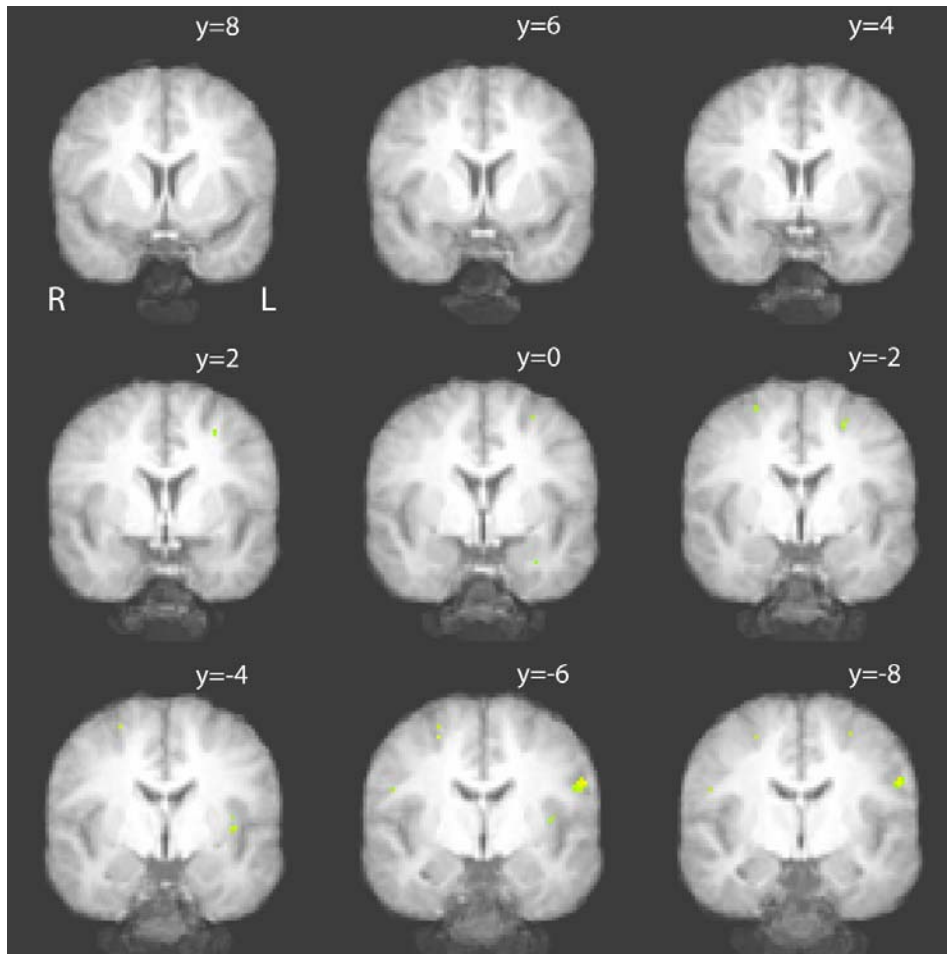

Figure 9. Cross-modal adaptation in the premotor cortex (PM). Statistical map (threshold at z=3.6, N=10) displaying regions showing positive cross-modal adaptation [different conditions – same conditions] in warm colors. Nine slices are depicted covering the PM (step size between slices: one volume as indicated by the y value above each slice). Axial view, left side of the image corresponds to the right hemisphere. A conservative threshold of z=3.6 or p < 0.001 (uncorrected) was chosen to demonstrate specificity of the adaptation effect in the ventral PM.

Why is the cluster revealed by the forth level analysis located in the dorsal parts of the PM when the peak activation in the cross-modal adaptation lies in the ventral parts? One explanation could be that the cluster analysis combines the third-level analysis of the experimental runs and the localizer analysis and therefore only areas that are active in both contrasts are taken into account. If the peaks for the cross-modal adaptation and the localizer contrast [ODM>NDM] do not completely overlap the cluster will be 'distorted' towards the

overlap of activity. Indeed, Kilner and colleagues (2009) pointed out, that the activation for observing and executing on the one side and cross-modal adaptation on the other side peaked in different locations within the PM.

The S1 has not been in the focus of neuroimaging studies investigating MNs. However, activation in this area during action observation and execution is in agreement with recent findings about the role of somatosensation in social perception. A recent review by Keysers, Kaas, & Gazzola (2010) summarizes evidence that parts of the somatosensory cortex can vicariously be recruited by observing another person being touched, executing an action or experiencing somatic pain. While area BA3 of S1 seems to primarily process own body states such as tactile and proprioceptive stimulation, the other two areas that are traditionally thought of as part of the S1 namely BA1 and BA2 show vicarious activations. The authors hypothesized that area BA2 could contain MNs since half of the neurons in ventral PM - the area with the best empirical evidence for containing MNs in humans - respond to somatosensory stimulation. Moreover, the fMRI-A study by Dinstein, et al. (2007) found within-modality adaptation in the S1, both when hand action were repeatedly observed and executed, but no cross-modal adaptation. The present study shows for the first time that area BA 2 and area BA 1 exhibit cross-modal adaptation and are therefore likely to contain MNs.

The cerebellum has not been associated with MNs yet, and we can therefore only speculate about the possible contribution of this area to the task used in the present study. The cerebellum is traditionally thought of as being important for motor control, especially for coding the details of a movement such as the trajectory, timing and applied force (Ghez & Fahn, 2000). It seems therefore likely that the cross-modal adaptation in the cerebellum found by the present study reflects some fine grading process of the difference in prehension between the used ODM and the NDM. The cluster was located in the medial, superior cerebellar cortex. A previous study demonstrated that there is a correlation between the regional cerebral blood flow in medial parts of the cerebellum and the force used by participants to flex their index finger (Dettmers et al., 1995). In the task used in the present fMRI experiment participants had to press a button on a box when performing an ODM and touch their index finger and thumb beside the button box when executing an NDM. However, only the pressing of the button requires a coordinate application of force. This could explain why areas in the cerebellum were more activate when the ODM was executed compared to the NDM. However, it is unclear whether such a differential activation also occurs when movements are observed passively. Further studies would be required to indicate if parts of

the cerebellar cortex code aspects of a movement irrespective of whether the movement is observed or executed and therefore could possibly contain MNs.

We analyzed the trial-based averaged TCs of the single experimental runs and the SNR of the AD. The SNR plot confirmed the results of the cluster analysis only for the clusters in the S1 and the aIPS but failed to show substantial cross-modal adaptation in the PM and the cerebellum. While the trial-based averaged TCs are not based on a model the cluster analysis is basically a higher-order GLM. In such a GLM the design matrix is convoluted with an appropriate hemodynamic response function and the parameters are estimated in order to predict the data. The design matrix is thereby determined by the experimental design but additional explanatory variables are included in the model as covariates, e.g. movement parameters. This can (at least partly) explain why trial-based averaging does not always correspond one to one to the results of a GLM.

We computed the measures oAD and nAD (Formula 1.2, 1.3) to rule out the confound scenario that semantic categorization of the two movement types takes place in the same voxels (see *data analysis*). In all clusters the cross-modal adaptation is clearly dominated by an adaptation to the ODMs, which is line with MN activity but not in line with semantic categorization.

Parameter estimates of the GLM for the cross-modal adaptation were extracted for all participants. The mean estimated parameters were for all clusters significantly positive confirming the cross-modal adaptation in these areas. The parameter varied between participants considerably although almost all estimated parameters were positive.

3. Psychophysical Experiment

3.1. Introduction

Although accumulative data about the anatomical location and physiological properties of MNs are available, still little is known about the functional properties of the human MN system. None of the four above-reviewed fMRI-A studies addressed this question directly providing behavioral data. It is therefore an open question what the behavioral consequences of an adaptation of the human MN system are. It is known from research on visual perception that a prolonged inspection of a visual stimulus can induce adaptation which affects the subsequent perception. For instance, adapting to a tilted line for few seconds causes a subsequently presented vertical line to appear tilted in the direction opposite of the adaptation line. (e.g. Muller, Schillinger, Do, & Leopold, 2009). This so-called aftereffect has been

linked to the neural tuning of orientation selective cells in the early visual cortex (Dragoi, Sharma, Miller, & Sur, 2002; Gutnisky & Dragoi, 2008). fMRI studies have recently demonstrated that these aftereffects can be measured as RS between the adaptation stimulus and the test stimulus (for review see Krekelberg, et al., 2006). Interestingly, fMRI-A studies have shown that higher areas in the visual processing stream seem to adapt more easily than lower areas (Soon, Venkatraman, & Chee, 2003). If the cross-modal adaptation of MN areas as measured in the present fMRI experiment is due to a change in neural firing, then the repeated execution of an action should affect the performance in a visual task involving this action. Since MNs have been discussed primarily in terms of action understanding and prediction we suggest that an aftereffect could be related to these processes.

It is well known that the observation of an action can influence the subsequent execution of the same action. Although interesting, this is a less critical test of the functional properties of the human MN system since these effects could predominantly reflect motor priming which is epiphenomenal to the understanding or predicting of the action (Chong, et al., 2008). It would be more compelling to measure an effect from executing an action on the observation of a similar action in order to tap into the functional properties of the human MN system. To the best knowledge of the author only a few studies have shown a general behavioral effect from a motor task to a visual task (e.g. Ernst, Banks, & Bulthoff, 2000). A recent study demonstrated the more specific effect from motor learning on biological motion perception (Casile & Giese, 2006). In this study participants had to learn a novel coordinated gait based only on verbal and proprioceptive feedback. Despite the absence of visual information during the motor training, the visual recognition of observed gait patterns from point-light stimuli improved selectively for gait patterns that had been learned before. However, it is unknown whether a similar effect could be measured with a highly familiar movement as used in the present study.

We therefore employed a new paradigm to test the effect of a repeatedly carried out movement on the subsequent observation of a movement that is either the same or different. As is in the fMRI experiment we compared ODMs with NDMs, but using a power grip movement as stimulus. This movement type is more like the grasping movement originally used in the monkey studies and seemed to be more natural. Participants had to repeatedly grasp a cylinder in front of them without visual feedback, and subsequently a single video was presented showing either the ODM or the NDM. Participants had to recognize the depicted movement type as fast and as accurate as possible giving verbal response. We reasoned that an adaptation of the MN system should have an influence on the recognition

speed of the movement types displayed in the videos. We hypothesized that the adaptation to ODMs could either improve or impair the recognition speed of the same movement type while leaving the recognition of NDMs unaffected.

3.2. Methods

3.2.1. *Participants*

Ten right-handed healthy volunteers (7 females) took part in the study (mean age, 24.6 years; range, 20–31 years). All participants gave written informed consent before being tested and were compensated for participating in the study.

3.2.2. *Visual Stimulation*

Videos clips were recorded using a digital video camera (Sony DCR-TRV900E) and were processed by video editing software (Virtual Dub 1.9.7) and MATLAB (The MathWorks Inc., Natick, Massachusetts, USA). The stimuli were displayed on a 17' monitor with a frame rate of 60 Hz and a screen resolution of 1152 x 864. The motor cues were created in and displayed together with the video clips using a custom made program based on MATLAB Psychtoolbox-3 (45) for Windows (Brainard, 1997; Pelli, 1997).

3.2.3. *Stimuli*

For the observation trials video clips were taken showing the frontal view of a male actor making one of two movements. In the object-direct movement (ODM) video the actor started with his hand positioned near his shoulder, reached out and grasped a cylinder in front of him with a power grip. Afterwards he released the object and brought his hand back to the initial position. The non-object directed movement (NDM) video was similar to the ODM but the cylinder was not grasped. In the NDM video the actor did a 'fake' grasp with a full closure of the grasping hand to the right of the cylinder (i.e. the actor did not grasp the cylinder). Each video was normalized to mean luminance level (127, intermediate gray with a standard deviation of 30) and the 18 frames until the turning point of the movement (when the hand started to move back) were cut and then played backwards. Thus, each video consisted of 36 frames and showed a symmetric movement. The videos were presented with a frame rate of 30 frames per second and a refresh rate of the computer monitor of 60Hz resulting in a video length of 1200 ms. A black fixation-cross was superimposed onto the center of the screen to

help minimizing eye movements. The viewing distance to the monitor was approximately 80 cm, resulting in a visual angle of 11.42°x 11.42° for the videos.

Participants were instructed to synchronize their movements (ODM or NDM) with an auditory signal consisting of a beep tone (50 Hz, 600 ms, presented through a headset) followed by a 600 ms silence period. In particular, participants reached their hand forward carrying out the ODM or the NDM during the presentation of the sound and moved their hand back to the initial position during the silence period. To avoid an overshoot after the last execution trial, the last repetition was indicated by a higher pitched tone. The participants were instructed to identify the movement type as fast and accurate as possible. They gave their responses verbally saying the syllables 'tab' for the ODM and 'cut' for the NDM. Participants were instructed about this procedure prior to the experiment and received practice trials until being comfortable with the procedure. The syllables 'tab' and 'cut' were chosen because it takes approximately the same time to pronounce them but they start with different sounds allowing a computer-based detection of the response onset. The responses were recorded using the microphone of the headset, which was calibrated for each participant individually. To ensure that the participant really executed the movement, we recorded the hand movement with a digital video camera.

Figure 10. Stimulus sequence of the psychophysical experiment. Each condition started with an adaptation period in which the participants had to execute either the ODM or the NDM 60 times or rest for the same amount of time (control condition). The movements were carried out in synchrony with an auditory signal. After a higher pitched warning tone a video either displaying the ODM or the NDM was presented for 600 ms followed by a masked response interval presented until response was given but maximally for 1400 ms. In the re-adaptation period the participants had to perform the same movement as in the adaption period (ODM or NDM) for 10 times or rest (control condition).

3.2.4. *Procedure and Design*

Figure 10 illustrates the stimulus sequence for the three motor conditions (ODM, NDM, control). At the beginning, participants executed either the ODM or the NDM 60 times (adaptation period) in the ODM and NDM condition respectively. In the control condition participants did not carry out any movements during the adaptation period but simply rested their arm while listening to the auditory signal. The control condition intended to measure visual discrimination performance of ODM vs. NDM videos in the absence of motor adaptation. After the warning signal (higher-pitched tone) either the video depicting the ODM or the NDM was displayed a single time. The videos lasted for 600 ms and were presented in randomized order. The presentation of the video was followed by a masked response interval until responses was given but maximally for 1400 ms. Participants gave their response verbally and were asked to do so as fast and accurate as possible. In the re-adaptation period the same movement as in the adaption period (ODM, NDM or nothing) was carried out ten times. After the re-adaptation period another observation trial started. The re-adaptation period was repeated 120 times allowing the collection of 120 responses for each condition. Every condition took approximately 30 min resulting in total experiment duration of 1h 30 min.

3.2.5. *Data Analysis*

Verbal responses were recorded and saved into audio files. To detect the onset of the response we used custom written software for MATLAB. The threshold to indicate a deviation from the baseline noise as the onset of a response was adjusted for each participant individually. RTs were calculated as time from the onset of the video presentation until response was given. A response was classified as 'incorrect' when the syllable did not match the presented movement type and as 'invalid' when the RT was faster than 200 ms or slower than 1400 ms. Only valid trials were considered for the analysis of the RT. The two independent variables 'reaction time' and 'accuracy' were analyzed using the software R for Windows (R Development Core Team, 2009) and the R package *aov* (Fit an analysis of variance model)(Chambers, Freeny, & Heiberger, 1992)

3.3. Results

The accuracy was in general high (mean across conditions $M = 96.6$ %) and did not vary significantly between conditions [two-way within-subjects ANOVA: Video Type, $F(1, 11) =$

1.03, $p = 0.33$, Motor Condition, $F(2, 22) = 0.09$, $p = 0.62$, Video Type x Motor Condition, $F(2, 22) = 0.85$, $p = 0.40$].

Figure 11 depicts the mean RT over all participants for the factor 'video type' (ODM, NDM) and 'motor condition' (ODM, NDM, Control). RT and accuracy were positively correlated [$r(70) = 0.59$, $p < .001$]. The factor 'accuracy' was therefore included as a covariate in the ANOVA to equate RTs for accuracy differences. A two-way ANOVA with 'video type' and 'motor condition' as the within-subject factors did not reveal any significant differences in RT between conditions [Video Type, $F(1, 11) = 0.74$, $p = 0.41$, Motor Condition, $F(2, 22) = 0.49$, $p = 0.62$, Video Type x Motor Condition, $F(2, 22) = 0.80$, $p = 0.46$].
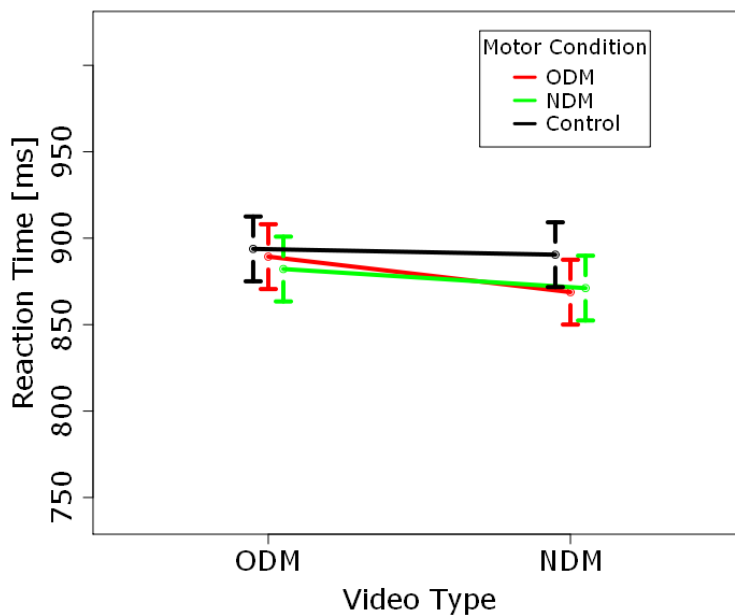

Figure 11. Effect of motor adaptation on the recognition speed of the movement type. Mean and standard error of RTs (in ms) displayed as function of the factor 'video type' (ODM, NDM) and 'motor condition' (ODM, NDM, Control) (N = 10).

3.4. Discussion

The aim of the present psychophysical experiment was to investigate possible behavioral correlates of an adaptation of the human MN system. Based on the results of the present fMRI experiment, we reasoned that MNs should adapt when an ODM is carried out repeatedly and that this effect transfers to the visual task. We therefore hypothesized that the cross-modal adaptation affects the recognition speed of ODMs. The task was designed to be compatible with an fMRI adaptation paradigm but was carried out as a pilot study separately

from the fMRI experiment. In general it would be interesting to relate the adaptation of MNs as measured with fMRI directly to behavioral performance.

The RT of recognizing the movement (ODM or NDM) displayed in the video was analyzed depending on the motor condition (ODM, NDM, Control). Accuracy was high across conditions, and the RT did no vary significantly between conditions.

The lack of evidence for a behavioral effect of repeatedly performing an ODM on the recognition of the same movement can have many possible reasons. Of course one explanation could be that MNs do not exist in humans and therefore no (true) adaptation can be induced by the task we used in the present study. Although we cannot rule out this possibility, there is converging evidence from fMRI studies and most recently from single-cell recordings in epilepsy patients (Mukamel, et al., 2010) for humans to have MNs similar to non-human primates. Another explanation would be that MNs exist but do not show adaptation. The evidence for cross-modal adaptation provided by this and other fMRI-A studies is based on an indirect measurement of neural activity and can in general be corrupted by other effects such as non-linear neurovascular coupling (Krekelberg, et al., 2006) or modulation through attention (Maier et al., 2008). The electrophysiology studies which recorded MNs directly in the non-human primate did not report adaptation on the neuronal level. However, this question was not addressed directly by these studies and it is unclear whether laboratory animals that have been exposed extensively to the stimuli still show adaptation (personal communication, Kilner, J., 2010). We reasoned that given MNs adapt when an ODM is repeatedly executed or observed there should be some behavioral correlate of this adaptation. But maybe the task we used in the present psychophysical experiment was not optimally fitted to reveal such behavioral effects. For instance, it could be that the effect is very subtle and did not survive statistical thresholds. Testing more participants would help to increase statistical power. It could also be that the task was too easy since the accuracy was very high. In this case, the RTs would reach ceiling performance, and not enough variance would be left in the data to find differences between conditions. Finally, it could be the case that the task is not suited for testing the functional properties of the human MN system. The used movement types might not require action predicting or understanding to be recognized. For example, the task could be accomplished by focusing solely on the different trajectories of the ODM and the NDM. If the trajectory deviates from the direction towards the object, the movement is categorized as NDM; otherwise as ODM. This would only require visual analysis and no action understanding. Moreover, if MNs are involved primarily in fast predictions of action goals, the outcome of the action might be predicted in both cases as

'grasping the cylinder' since the initial position is the same, and both movements are clearly directed towards the cylinder. It would require further studies to address these questions.

4. Conclusion

The fMRI experiment revealed four clusters in the right S1, the right aIPS, the left PM and the right cerebellum that exhibited the two criteria for MN activity: they were more active when an ODM was executed or observed compared to an NDM, and they exhibited cross-modal adaptation between execution and observation of an ODM. The present study demonstrates for the first time cross-modal adaptation in both areas which are homologous to the macaque's MN system, the ventral PM and the aIPS, using a simple grasping task and a whole-brain analysis. Furthermore, first evidence is provided that the S1 shows adaptation between action execution and observation and should therefore be considered as a putative part of the human MN system. Finally, the present study suggests that the cerebellum codes kinematic elements of a movement e.g. applied force not only when an action is executed but also when the same action is observed being performed by another individual. However, more studies are required to better understand the role of the cerebellum in action perception.

The psychophysical experiments failed to find differences in the recognition speed of ODM depending on the previously executed movement type. However, this lack of evidence does not mean that there is no behavioral correlate of the cross-modal adaptation as measured in the fMRI experiment. Further studies are required to investigate the relation of adaptation in putative MN areas and action recognition and understanding. In general, combing fMRI-A with behavioral measurements is a promising method to investigate the functional properties of the humans MN system.

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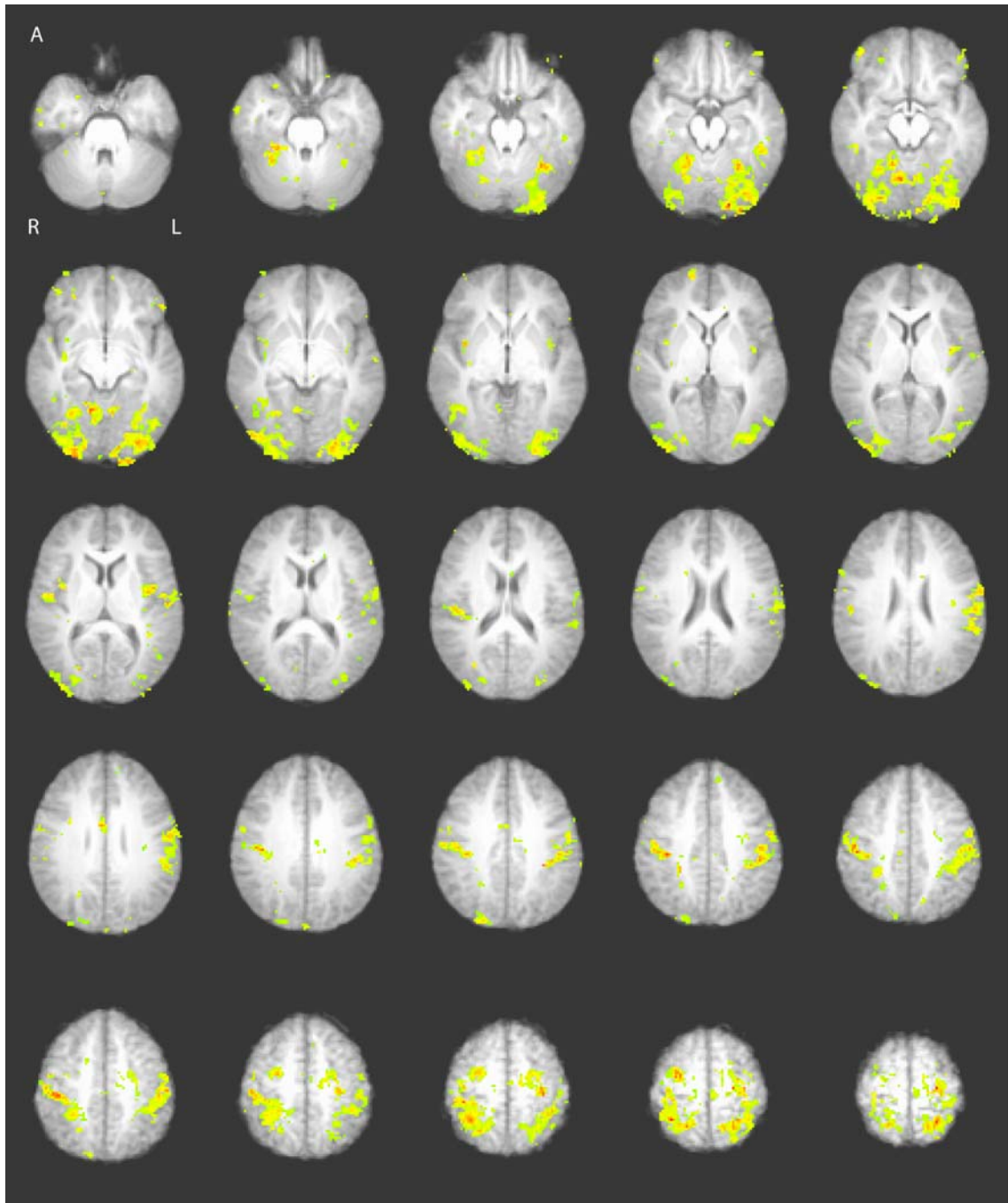
6. Supplementary Figures

Figure S1. Brain areas selective for ODMs and cross-modal adaptation. Statistical map (threshold at z=1.522, N=10) on which the cluster analysis was run combining the cross-modal adaptation contrast [different conditions – same conditions] in the experimental runs and the localizer contrast [(Execute & Observe):ODM > NDM]. Axial view, left side of the image corresponds to the right hemisphere. A liberal threshold was chosen to demonstrate the general pattern of activity.
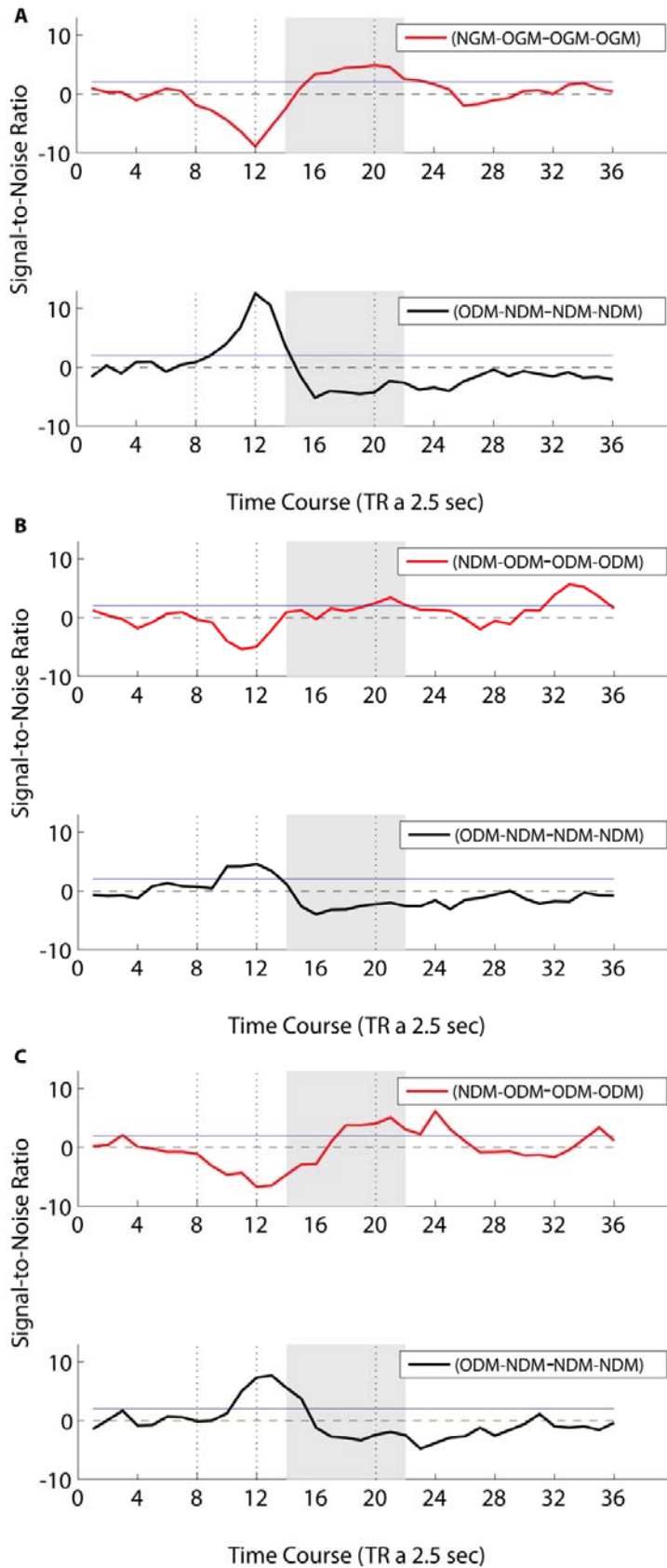
Figure S2. Single adaptation differences for the clusters in the right S1 (panel A), left PM (panel B) and right CER (panel C). In each upper panel the 'different' experimental run combining the NDM in the motor task and the ODM in the visual task is subtracted by the 'same' experimental run employing in both tasks the ODM. In the lower panel the 'same' and 'different' experimental runs with the NDM in the motor task are compared. The grey rectangle marks the area where both experimental runs are identical (already taking the hemodynamic lack into account). Vertical, dotted lines indicate the onset of motor task, onset and offset of visual task. The blue horizontal line marks a SNR of two and is an estimation of significance.