The Simon Effect in Rats:
A Comparative Study on Conflict and Error Processing Using Electrophysiology and Functional µPET Imaging

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"One thing I've learned:
you can know anything,
it's all there,
you just have to find it."

-Neil Gaiman, Sandman

Dedicated to Anita Veit
(* 13.02.1931 - † 6.03.2012)

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III List of Abbreviations

ACC	anterior cingulate cortex
ADHD	attention deficit hyperactivity disorder
aM1	anterior motor cortex
aM2	anterior premotor cortex
Amy	amygdala
ANOVA	analysis of variance
C	compatible condition
Cg1	rostral anterior cingulate cortex area 1
Cg2	rostral anterior cingulate cortex area 2
Cz	central electrode
dlPFC	dorsolateral prefrontal cortex
DMN	default mode network
D_n	large negative deflection
D_p	late positive deflection
EEG	electroencephalography
EOG	electrooculogram
ER	error rate
ERN/Ne	error-related negativity
ERPs	event-related potentials
FCz	frontocentral electrode
FDG	[^{18}F]fluorodeoxyglucose
FDR	false discovery rate
fMRI	functional magnet resonance Imaging
FrA	frontal association cortex
hEOG	horizontal EOG
Hip	hippocampus
I	incompatible condition
ICA	independent component analysis
IL	infralimbic cortex
LFP	local field potentials
LPC	late positive component
LS	lateral septum
lStr	dorsolateral striatum
MT	movement time
M1	rat motor cortex
M2	rat premotor cortex
MicroPET/µPET	animal positron emission tomography
MMN	mismatch negativity
MPFC	medial prefrontal cortex
MRI	magnet resonance imaging
MS/DB	medial septum and diagonal band of Broca
mStr:	mediodorsal striatum.
MUA	multiunit activity
N	neutral trials
N2	negativity with a latency around 200 ms. Associated to conflict
OCD	obsessive compulsive disorder
OFC	orbito-frontal cortex
P3	positivity 300
Pe	(error-related) positivity
PET	positron emission tomography

PFC	prefrontal cortex
pM1	posterior motor cortex
pM2	posterior premotor cortex
PPT	posterior parietal cortex
PrL	prelimbic cortex
RARE	fourier rapid acquisition with relaxation enhancement
RCZ	rostral cingulate zone
R_{fast}	fast response
R_S	resting state control , with naive rats before operant conditioning
R_{slow}	slow response
RT	reaction time
SD	standard deviation
SEM	standard error of means
$T_{20\%I}$	simon task with 80% compatible and 20% incompatible trials
$T_{50\%I}$	simon task with 50 % incompatible and 50 % compatible trials in randomized order
$T_{60\%N}$	simon task with 20% compatible, 20% incompatible and 60% neutral trials
$T_{80\%I}$	simon task with 20% compatible and 80% incompatible trials
T_C	basic Simon task with compatible trials only
Te3V	caudoventral auditory cortex
T_I	basic Simon task with incompatible trials only
T_N	no-conflict control with neutral trials only
T_R	basic Simon task with 50 % incompatible and 50 % compatible trials in randomized order
vEOG	vertical electrooculogram
VINCI	"Volume Imaging in Neurological Research, Co-Registration and ROIs included"
VOI	volumes of interest

IV Kurzzusammenfassung

Sowohl Menschen als auch Tiere besitzen die Fähigkeit, aus vergangenen Erfahrungen zu lernen und ihr Verhalten an diese anzupassen, um so Konfliktsituationen vorzubeugen, oder diese ganz zu vermeiden. Konflikte in räumlichen Stimulus-Antwort-Aufgaben erfolgen, wenn sich Stimulus-Eigenschaften von den Eigenschaften der Antwort räumlich unterscheiden. Diese kognitiven Konflikte führen zu erhöhten Fehlerraten (ER), Reaktionszeiten (RT) und Bewegungszeiten (MT), was als Simon-Effekt bezeichnet wird. Ein Modell, das vorgeschlagen wurde, um diese Art von Effekten zu erklären, geht von einer Zwei-Wege-Verarbeitung von Stimulus-Eigenschaften (automatisch und intentional) aus, die – sollten beide Routen inkongruent zueinander sein – einen Konflikt vorhersagt. Auch wenn es verschieden Theorien zu den zugrunde liegenden neuronalen Mechanismen gibt, wird gemeinhin angenommen, dass der anteriore cinguläre Cortex (ACC) eine tragende Rolle in der Konflikt- und Fehlerverarbeitung spielt. Die Simon-Aufgabe ist eine neuropsychologische Interferenz-Aufgabe, die allgemein zur Untersuchung der Prozessüberwachung dient. Interessanterweise ist der resultierende Interferenz-Konflikt nicht nur bei Menschen zu finden, sondern konnte auch in Tauben, Ratten und Affen gezeigt werden. Auf neuronaler Ebene kann die andauernde Überwachung von richtigem und falschem Verhalten in Form von ereigniskorrelierten Potentialen gemessen werden. Man nimmt demnach an, dass die fehlerbezogene Negativität (ERN) – eine Komponente des resultierenden ereigniskorrelierten Potentials, die vermutlich im ACC generiert wird – Konflikt- und Fehlerüberwachung widerspiegelt. Unter Verwendung von Positronen-Emissions-Tomographie (PET) in Kombination mit dem metabolischen Tracer [^{18}F] Fluorodesoxyglukose, der während der Verhaltensaufgabe in metabolisch aktiven Zellen akkumuliert wird, wollen wir zunächst relevante Gehirnareale in einem Rattenmodell der Simon-Aufgabe identifizieren. Nach dem Zwei-Wege-Modell werden Gehirnareale, die an der Konfliktverarbeitung beteiligt sind, aktiviert, wenn die automatische und die intentionale Route zu unterschiedlichen Antworten führen. Unsere Ergebnisse zeigen spezifische Aktivierungsmuster, die darauf hindeuten, dass der Motorcortex der Ratte (M1) möglicherweise ein Bestandteil der automatischen Route ist oder diese zumindest unterstützt, während der Prämotorcortex (M2), die prälimbischen Areale sowie der ACC anscheinend wichtig für die Inhibierung der falschen, automatischen Antwort sind, was seinerseits auf eine Überwachungsfunktion hinweist. Interessanterweise stimmen unsere Ergebnisse

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bemerkenswert genau mit den bei Menschen beobachteten aktivierten Regionen überein.

Um unsere Ergebnisse weiter zu stützen, wurden desweiteren Messungen lokaler Feldpotentiale (LFP) von Elektroden im ACC der Ratte durchgeführt. Diese LFPs zeigten eine langsame negative Welle, die für Fehler im Messbereich von 250-400 ms nach einer Reaktion geringer ausgeprägt war. Stimulus-bezogene Daten offenbaren einen Kompatibilitätseffekt mit einer ausgeprägten negativen Welle innerhalb der Latenzzeit der Reaktion. Um letztendlich diese Ergebnisse mit dem eines Humanexperiments vergleichen zu können, entwickelten wir in dieser Studie weiterhin eine übertragbare Humanaufgabe. Mit diesem Ansatz fanden wir in beiden Spezies vergleichbare Verhaltenseffekte einschließlich einer erhöhten Fehlerrate, RT und MT. Das Human-EEG wies zwar keine Unterschiede der Amplitude für Fehler und Korrekte im Zeitbereich der ERN auf, jedoch zeigte sich eine deutliche Fehlerpositivität 250 bis 350 ms nach der Reaktion. Überraschenderweise zeigten Menschen weiterhin eine ausgeprägte negative Potentialwelle in kompatiblen im Vergleich zu inkompatiblen Versuchsdurchgängen. Ähnlich wie bei Ratten setzt dieser Effekt während der Reaktionszeit ein. Daraus folgt, dass beide Spezies i) ähnliche elektrophysiologische Antworten in Unterscheidung zwischen falschen und korrekten Antworten innerhalb eines ähnlichen Zeitbereiches zeigen, (ii) ein valides Auftreten des Simon-Effekts mit ähnlichen Antwortstrategien in Bezug auf RT und MT aufweisen und (iii) lang anhaltende Unterschiede in den ERP – abhängig von korrekten und inkorrekten Antworten – ab dem Zeitpunkt der Reaktion und vor dem Auftreten der Belohnung zeigen. Daher ist es verlockend anzunehmen, dass beiden Spezies ähnlicher kognitiver Prozesse zugrunde liegen.

Abschließend kann festgestellt werden, dass wir bemerkenswerte behaviorale, elektrophysiologische und funktionelle Ähnlichkeiten in der Fehler und Konfliktverarbeitung bei Ratten und Menschen finden konnten. Unser Paradigma eröffnet neue Möglichkeiten für eine integrative, speziesübergreifende Forschung und liefert ein brauchbares Nager-Modell zur Untersuchung von Leistungs-Überwachung.

V Abstract

Both humans and animals have the ability to learn from past experience and to adapt their behavior to resolve future conflicts faster or avoid them entirely. Conflicts in spatial stimulus–response tasks occur when the origin of the stimulus and the response area differ in location. Those conflicts lead to increased error rates, reaction times (RT) and movement time (MT) which has been termed Simon effect. A model of dual route processing (automatic and intentional) of stimulus features has been proposed, predicting response conflicts if the two routes are incongruent. Although there are various theories related to underlying neuronal mechanisms, it is commonly assumed that the anterior cingulate cortex (ACC) plays a crucial role in conflict and error processing. The Simon task is a neuropsychological interference task commonly used to study performance monitoring. Interestingly, the resulting conflict is far from uniquely human, as it has also been observed in pigeons, rats, and monkeys. On a neural level, the on-going monitoring of correct and incorrect behavior appears in the form of event-related potentials (ERPs). More precisely, the error-related negativity (ERN/Ne) component of the resulting ERP, assumed to be generated in the ACC, is suggested to reflect conflict and error monitoring. Unfortunately, there is often little correspondence between human and animal studies. On this account the present study uses a modified auditory Simon task to investigate a) the anatomical basis, b) the conflict- and error-related electrophysiological correlates and c) the performance monitoring from a cross-species point of view.

By using positron emission tomography (PET) in combination with the metabolic tracer [^{18}F]fluorodeoxyglucose, which accumulates in metabolically active brain cells during the behavioral task, we first aim at identifying relevant brain areas in a rat model of the Simon task. According to the dual route model, brain areas involved in conflict processing are supposed to be activated when automatic and intentional route lead to different responses (dual route model). Results show specific activation patterns for different task settings coherent with the dual route model. Our data suggest that the rat motor cortex (M1) may be part of the automatic route or involved in its facilitation, while premotor (M2) and prelimbic areas, as well as the ACC appear to be essential for inhibiting the incorrect, automatic response, indicating conflict monitoring functions. Interestingly, our findings remarkably fit the pattern of activated regions reported during conflict processing in humans. To further support our findings, we measured local field potentials (LFP) from electrodes centered in the rat ACC. LFPs showed a negative slow

wave less pronounced for errors at about 250-400 ms after reaction. Stimulus-locked data revealed a compatibility effect in rats, with a negative slow wave with onset in the latency range of the reaction. To finally compare these results with a human setup, we also developed a translational task for humans. In both species, similar behavioral effects were found, including an increase in error rate, RT and MT. In humans, although no difference in EEG amplitude between errors and hits in the ERN latency range was found, a pronounced error positivity between 250 and 350 ms after reaction was seen. Humans surprisingly demonstrated a stronger negativity for compatible compared to incompatible trials. Similarly to rats, this effect started at about the time of reaction time. Thus, both species (i) showed electrophysiological responses differentiating between errors and correct in a similar latency range, (ii) demonstrated a valid occurrence of the Simon effect and seem to pursue similar response strategies, both in terms of RT and MT and (iii) displayed sustained differences in the modulation of the ERP depending on correct or incorrect responses starting at the time of response and prior to reward/no reward. It is thus tempting to speculate that the underlying cognitive error processing mechanisms are highly similar across species.

In conclusion, we found remarkable behavioral, electrophysiological and functional similarities between rat and human conflict and error processing. Our paradigm offers a new approach in integrative, cross-species research and provides a useful rodent model for investigating performance monitoring.

1. General Introduction

Everyday occurrences require flexible and ongoing adjustments of behavior in response to different situations. Unfortunately, cognitive control has only limited capacity. This becomes evident in situations where one is subjected to waves of information and choices, which have to be integrated and monitored at the same time, requiring switches between different choices, actions and distractions. Imagine sitting in your office, writing an email, answering the phone and thinking about your next presentation simultaneously. We know from experience that it is possible to deal with all these processes at the same time, but often not to a satisfactory degree. Each process will slow down and will be prone to errors, such as spelling mistakes or losing track of the conversation. Although it seems that this processing requires high-order cognitive control processes, the ability to juggle mutable cognitive demands is not a recent phenomenon brought about by the challenges of today's rapidly changing society, but a fundamental ability which leads to goal directed behavior, that was established early in evolution.

"It is a law of nature we overlook, that intellectual versatility is the compensation for change, danger, and trouble. An animal perfectly in harmony with its environment is a perfect mechanism. Nature never appeals to intelligence until habit and instinct are useless. There is no intelligence where there is no change and no need of change. Only those animals partake of intelligence that have a huge variety of needs and dangers."
— H.G. Wells, The Time Machine

10,000 years ago our ancestors were hunters and gatherers. They had to forage or hunt for food and look out for predators at the same time. Conflicts arising from the processing of several competing demands could have led to slow responses resulting in missing or becoming the prey. This phenomenon is not restricted to humans or primates, but can be found in other species as well. Cotton rats, for example, simultaneously assess resource patchiness, scan for predators and listen for possible alarm calls of close birds to predators (Felts 2010). The execution of different concurrent processes gets even more difficult if these are very similar or share common features. Bats, for example, process incoming signals that allow them to orient and navigate and simultaneously detect and understand incoming signals from other communicating bats (Kanwal, 2010). What this means for cognition, is that humans and animals need the

ability to continuously monitor dynamic information such as environmental cues, their own behavior as well as the behavior of others and adapt to reach a certain goal. Erroneous responses have to be inhibited, or if committed, remembered to help avoiding them in future situations.

All these abilities are defined as cognitive control, fittingly described by Folstein & van Petten:

1.1. Cognitive control

"Cognitive control is partly defined as the monitoring or regulation of strategy

("How fast am I responding?" "How fast should I be responding?") and the processing

of feedback that is informative for strategy regulation ("Another mistake"; "That reward

was worse than I expected"; etc.). Additionally, the concept of cognitive control covers

immediate control of action, such as canceling a prepared response."

(Folstein & Van Petten, 2008)

In general, humans and animals have the ability to learn from past conflicts and to adapt their behavior to solve future problems faster or avoid them entirely to improve outcomes. This means that there needs to be a cognitive control loop in the brain which covers immediate control of responses and allows behavioral adaptation. A schematic representation of a possible regulatory circuit of cognitive control is shown in **Figure 1**. The action monitoring system integrates internal and external information and compares the possible outcome of the behavior with the desired goal. If the behavior reduces the possibility that the outcome reaches the goal, the system signals the need for adaptation. This leads to compensatory actions and optimization of behavior.

Figure 1: Schematic representation of a regulatory circuit of cognitive control. The action monitoring system compares the possible outcome of the behavior with the desired goal. If the behavior reduces the possibility that the outcome reaches the goal the system signals the need for adaptation. This leads to compensatory actions and optimization of behavior (Ullsperger & Derrfuß, 2012).

Although a plethora of research has been carried out during the past decades which has demonstrated that impaired cognitive control leads to neuropsychiatric disorders such as obsessive compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD) and schizophrenia, little is known about its underlying neuronal processes. In general, frontal medial and orbital activity in imaging studies has often been associated with internally driven and goal oriented decisions, emotions, and the selection of appropriate actions.

Typical psychological models of action control describe cognitive processes which are directly involved in planning, initiating and executing actions.

1.1.1. Action control

In 1868 Donders was the first to break down the process that takes place between the appearance of a stimulus and the conduction of a response (e.g. pushing a button) into partial subprocesses, as shown in **Figure 2**.

Figure 2: Cognitive sub processes of action preparation (Elsner & Prinz 2006)

After processing a stimulus, a selection is conducted between motor programs representing different competing reactions. This requires more time, as demonstrated by higher reaction times, than when there is only one possible response. The more different options are considered, the higher this reaction time will become (Karnath and Tier, 2006). After the selection of a response, premotor programming of reactions follows. This programming determines the characteristics of the upcoming movement and is set as a coordinated plan. Subsequently this plan is passed on to the motor system to be translated into muscle activity. This system is capable of interacting with the process by delaying or even suppressing its execution. This interaction further increases the reaction time. Another factor that influences reaction time is the extent of similarity between the stimulus and the reaction, described as "stimulus-reaction-compatibility".

In experiments such as the Stroop- and Simon task discussed below it has been shown that a greater stimulus-reaction-compatibility facilitates the choice of the right response and speeds up reaction time.

Frequent repetition of a certain stimulus-reaction-relation can also lower reaction time, even when the stimulus and the reaction differ significantly. Responding to a stimulus with a certain plan of action can be learned. In such a case, parts of the movement do not have to be implemented separately, but the action plan is activated as a whole. Consequently, a lesser amount of cognitive preparation is needed to plan the response.

If the stimulus-reaction-compatibility is very low, the multiplicity of the provided behavioral patterns can be variably motivational favoured, or even compete with each other. This leads to conflict in the execution of an action.

1.1.2. Conflict in the cognitive System

Cognitive control has only limited capacity. This is observable during simple daily situations in processes like multitasking. When trying to carry out multiple activities at once, for example composing an e-mail and making a telephone call, this mostly leads to conflicts in the cognitive system, resulting in a deceleration of activity or, in the worst case, in errors such as losing track of a conversation or making typing mistakes.

The sequence of such a conflict situation can be broken down into three parts:

1) emergence of an error, 2) conflict monitoring and 3) conflict resolution. This effect can be simply illustrated with the help of the Stroop Test, developed by John Ridley Stroop (1897-1973). Within this experiment coloured words are shown to the subjects (BLUE, GREEN, etc,). The colouring is different for each word and not necessarily in compliance with the semantic meaning of the word. Subjects are required to name the printed colour. This experiment highlights that the reaction time is shorter when there is a match between the colour and the word, than when there is a mismatch (Stroop, 1935). These conflicts get especially challenging when a spatial component is added. This can be well illustrated with the example of attending a dancing class. The teacher stands facing you and instructs you to perform a step to your right, while demonstrating the movement. As he is facing you, from your perspective this movement is carried out towards the left. This tends to result in you taking a step towards your left, although you were requested to move to the right. In the optimal case the error is detected before the erroneous movement has been performed, and the step is carried out to the right. In the worst case you step onto your dancing partner's right foot. A similar effect occurs in the Simon task.

1.2. Simon Task

During an experiment in 1969, Simon discovered that subjects showed a tendency to align their reaction towards a stimulus. Tones of two different carrier frequencies were presented to the subjects. They were asked to respond by pushing a left or a right button, depending on whether a high pitched or a low pitched tone was heard. The task was carried out without difficulty when the tones were presented binaurally. However, when

the tone was presented monaurally, delay occurred in the reaction time when the location of the stimulus and that of the reaction did not match.

Responses in compatible trials (C), where the requested answer had to be given in a location corresponding to the position of the stimulus were faster than in incompatible trials (I), where these positions did not correspond. Simon himself called this effect "reaction toward the source". It was later renamed after him. In subsequent works by Simon and Rudell, a multitude of analyses relating to the Simon Effect were conducted, proving its validity in the visual modality as well (Simon 1990).

Here stimuli were presented, which appeared to the left or right of the midline of the screen, whereby the side of the stimulus presentation was irrelevant for the expected response (pushing one of two buttons, left or right). Task-relevant were other features of the stimulus, such as shape, color, etc. In spatially compatible conditions i.e. situations where irrelevant stimulus positions corresponded to the location of the response, reactions were faster and contained less errors, than in spatially incompatible conditions, where the position of the stimulus and place of the response were opposite.

One could argue that this effect occurs solely due to the involvement of different hemispheres (i.e. compatible processing uses a single hemisphere, while incompatible conditions incorporate both). However, measurements of so called "crossdesigns" where subjects could give responses with crossed arms were conducted and showed that the Simon effect was not significantly affected by this (Wallace, 1971).

Additionally the Simon effect is not only noticeable in trials where answers are designed to involve either one or both hemispheres, but also in vertical designs, where answers are given using a top or bottom response button (Valle-Inclán, 1996, Christ et al., 1999).

It is still an open issue how the brain manages interference during the Simon task. To explain such compatibility effects several suggestions have been presented:

1.2.1. Simon effect theories

One of the first theories put forward to account for the Simon effect is the stimulus-stimulus-congruence by Hasbroucq and Guiard (1991), which states that the incongruence of stimulus dimensions is responsible for the effect. According to this theory the identification of the stimulus is delayed when the irrelevant dimension of the stimulus (position of stimulus presentation) does not match the relevant stimulus dimensions (level of tones).

This theory has proven to be rather unlikely. The currently accepted theory is that of response selection (Umiltà and Nicoletti, 1990). According to this the conflict that is present between relevant and irrelevant responses must be resolved before an answer can be given. According to Lu and Proctor (1995) three assumptions of response selection should be emphasized:

1. Alignment of attention

The "reaction towards the source", observed by Simon and Small (1996) was explained by the alignment of attention. Through the occurrence of the stimulus, attention is allocated towards its location, and a reaction will be evoked in its direction (Simon and Small 1969). Later on this assumption was supplemented by the theory of a "temporary response buffer memory/store" by Merwaldt et al. (1980), according to which a temporary buffer memory/store exists in which every possible answer, including all stimuli and the corresponding relevant response are stored.

These memories are processed one after another. As a result of the spatial appearance of the stimulus, the memory corresponding to the position of the stimulus is processed at first. This also holds relevance for another assumption related to spatial coordination.

2. Spatial coordination

The assumption that spatial coordination is the cause of the Simon Effect is based upon the works of Umiltà (Umiltà & Nicoletti, 1985) and Wallace (Wallace, 1971), asserting that in addition to the relevant response code, a spatial response code is set up, even though the position of the stimulus is irrelevant for the task. The selection of a response is slowed down if two codes induce different responses. For example, if the colour red indicates "push the button on the right", but the red stimulus is presented left, the response code for the colour information would build up on the right side and the response code for the appearance of the stimulus on the left. Given that both response codes contain contradictory information, the answer is slowed down.

3. Dimensional overlapping

The emphases of the "dimensional overlapping model", by Kornblum et al. (1990), are the dimensions of stimuli. A stimulus activates its corresponding response automatically, if the stimulus and the dimension of the response overlap (place of the stimulus – place of response; colour of the stimulus – colour of the response). In the

Simon task, the irrelevant stimulus dimension (place of stimulus) and response dimension (response place) overlap.

When the place of the stimulus and the place of response are congruent, the response time is accelerated. In contrast, if they are incongruent, the wrong answer is triggered and the response is slowed down.

In the last assumption the compatibility conflict is seen as a mechanism, which is processed closer in time to the actual reaction, rather than to the stimulus. In this case, the conflict originates from the fact that both reactions are prepared and they compete against each other in terms of compatibility (Kornblum et al., 1990; De Jong et al., 1994; Eimer et al., 1995). Theories that emerge from this assumption are discussed in the literature under the term "dual route model".

There are other alternative approaches in addition to the dual route hypothesis aimed at explaining the Simon effect. For example, the binding hypothesis proposes the formation of event files (Hommel et al., 2004) which are temporary associations of cognitive representations ("codes") containing features of stimuli and response. The speed of event file formation is thought to account for variations in reaction times. Another approach, the tectonic theory (Melara et al., 2008), suggests that inappropriate attention to the irrelevant spatial stimulus dimension disrupts selective attention to the relevant non-spatial stimulus dimension. As the dual route hypothesis appears to have more supporting evidence in contemporary studies we will primarily focus on this theory.

1.2.2. Dual route model

The generally accepted assumption is that of a "dual route" first put forward by De Jong et al. (1994). According to this hypothesis, the spatial S-R-compatibility (stimulus response) affects response efficiency in two different, independent routes. In one of the routes the right response is triggered after identification of the task instruction and conversion of the stimuli into parameters. This is intentionally controlled.

In contrast, the other route is automated and runs unintentionally. An example is shown in illustration 3. In this task response has to be given using the left button when sound one is heard (compatible response) and using the right button when sound two is presented. In case of a compatible response the correct reaction (push left button) is directly triggered. This is because the same response code is activated over both routes.

If however sound pitch one is presented on the right side, the correct left response code is triggered by the intentional route, yet the automated route activates the false right response code. This results in conflict and delayed execution.

Figure 3 Principle of the dual route model (modified version of Hommel et al., 2004). The automatic processing is indicated by dashed lines. Indirect intentional processing is indicated by broken lines with dots. The task in this example is: If pitch one occurs press the left button, if pitch two occurs, press the right button. The processing of the spatial features of the stimulus is carried out through the automatic, the stimulus information through the intentional route

However, only recent works deal with the possible base of the Simon effect, respectively the involved brain mechanisms and areas.

1.3. Neuronal correlates of performance monitoring

It is thought that the orbito-frontal cortex (OFC) consists of two different networks (Carmichael & Price, 1996). One network consists of areas of the central OFC and another network consists of areas in the medial orbitofrontal and medial frontal cortex. This second network seems to be the one responsible for executive functions, as network one has only weak connections with the motor system (Carmichael & Price, 1995). One crucial part of this network is the anterior cingulate cortex (ACC).

The ACC is believed to be involved in the monitoring of actions, relating actions to their outcomes, including positive as well as negative consequences and thus helps to

guide decisions in challenging situations where cognitive conflict and errors arise. These functions enable an organism to plot its behavior through partial aims, concentration of perception and suppression of inappropriate actions.

While the neuronal bases of the automatic and intentional pathways are not yet known, human fMRI and EEG studies have suggested that the dorsal ACC monitors conflicts arising during incompatible dual route processing and signals to the dorsomedial prefrontal cortex to improve performance in subsequent conflict trials (conflict resolution; Botvinick et al., 1999; Botvinick et al., 2001; Botvinick et al., 2004; Kerns, 2004). Particularly, the right inferior frontal cortex is thought to participate in response inhibition as one mechanism of conflict resolution (Forstmann et al., 2008). This leads to the extended dual route model shown in Figure 4, containing the suggested relevant brain areas.

Figure 4: Dual route model, after Botvinick et al. (2001), Hommel et al. (2004), Kerns et al. (2004) & Zhang et al. (1999). Abbr.: dlPFC-dorsolateral prefrontal cortex

With the application of imaging techniques (PET, MRI) and electrophysiological derivation (EEG), increased activity in the ACC and PFC has been identified using conflict trials (Botvinick et al. 1999; Falkenstein et al. 1991). This has led to two theories which grant a special role to the ACC in cognitive control, namely the "conflict monitoring" and "error detection" hypotheses.

Conflict monitoring

fMRI measurements in humans during the solution of conflict tasks have demonstrated increased activity in the ACC throughout the entire experiment (Botvinick et. al., 1999). Furthermore, an increased activation in the ACC has been shown in trials where conflict potential was high. These and further results have led to the "conflict monitoring" hypothesis (Botvinick et al., 2001, 2004) which assumes that two brain regions, the ACC and the PFC are especially involved in the adaptation of the system after a conflict. The dorsal ACC is activated when a conflict in potential responses occurs (Carter et al., 1998, Botvinick et al., 1999), is also involved in conflict monitoring and it has a role in passing information on to other brain regions such as the PFC (Botvinick et al., 2001; Cohen et al., 2000; Kerns et al., 2004). Evidence has also emerged showing that the PFC is involved in resolving conflicts after being recruited by the ACC, lowering the conflict in the system to enable better coping in further conflict situations (Botvinick et al., 2000).

Error detection

The "error detection" theory can be traced back to Falkenstein and colleagues. They showed that with an incorrect response, an error-related negativity (ERN) occurs in the EEG recordings (Falkenstein et al., 1991; will be discussed in paragraph *error negativity*). Further experiments demonstrated that the ERN is generated by the ACC (Deheane, 1994; Debener et al., 2005) underlining the significance of the ACC-PFC-interaction in the detection of errors (Gehring & Knight, 2000).

Although there are several indications for the particular roles of the ACC and the PFC in conflict and the error detection, the detailed function of the ACC and the participation of the PFC in this mechanism are still not fully understood. To investigate this interference, experiments like the Stroop or the Simon task are especially well suited, because they highlight the close relationship between conflict and reaction time. Furthermore, they demonstrate high stability and reproducibility compared to other paradigms (Peterson et al., 2002). Therefore, a Simon paradigm was chosen for this study.

However, there are currently no comparable results to demonstrate the activation of brain areas in a Simon task in rodents.

A closer look at rodent conflict processing could be worthwhile due to the huge analogies in functionality and connectivity between human and rodent prefrontal (PFC) and anterior cingulate (ACC) cortices. In addition, it has been shown that the PFC and the ACC play an important role in rodents in action selection, inhibition of inappropriate behavior (Chudasama et al., 2003) and reward learning (Gabriel et al., 1990; Bussey et al., 1997).

In order to demonstrate the excellent comparability to human studies, the general homology of the anatomy, connectivity and function of brain areas will be discussed in the following paragraphs.

1.3.1. Comparison of human and rat PFC anatomy

It is difficult to identify the rat prefrontal cortex on the basis of cytoarchitectonic characteristics. This is because rats have no layer IV which contains small granular neurons (**Figure 5**). The human prefrontal cortex possesses a gradient of granular neurons from agranular (no layer IV) to dysgranular, (rudimentary layer IV) to granular cortex (contains layer IV). This gradient is present in humans and primates, but is lacking in the rat OFC which solely consist of agranular cortex. Although the rat PFC is not as differentiated as the human PFC, both share crucial parallels in terms of cytoarchitectonics, topography and functionality (Divac et al., 1978, Uylings et al., 2003; Preuss, 1995., Wise 2008).

From a cytarchitectonic point of view the rat agranular cortex is homologous to the primate agranular cortex, and is similarly subdivided into regions like the infralimbic (IL), prelimbic (PrL), agranular insular, granular orbital, and ACC (Wise, 2008).

It is still under discussion whether the rat medial PFC is functionally equivalent to the primate dorsolateral cortex, or whether it is more similar to the medial frontal cortex, more specifically the ACC(for reviews see: Kolb, 1984; Brown and Bowman, 2002; Uylings et al, 2003), even though the dlPFC of primates contains a Layer IV.

Connectivity studies provide further evidence that the rat PFC has a similar organization as primate PFC. For instance, there appear to be similar connections from the PFC to premotor and somatosensory cortices, sensory cortices and limbic areas (Ongur & Price, 200; Heidbreder & Groenewegen, 2003; Uylings et al. 2003). In conclusion one could assume that the cerebral cortical organization of the rat brain bears a solid resemblance to the human brain.

Figure 5: Comparative anatomy of the human and rat medial frontal cortex. *right*: sagittal plane of the rat brain by the atlas and definition of the prefrontal cortex after Paxinos und Watson (2005) *left:* sagittal plane of the human brain after Carmichael and Price (1994) The granular areas appear in dark grey; agranular areas in light blue; allocortical areas in light grey, distribution adapted from Wise 2008. ACC appears in dark blue. Abbr.: AC- anterior cingulate cortex; Cg1,2- cingulate cortex (area 1,2); IL- infralimbic cortex; M1-primary motor cortex; M2- secondary motor cortex; PrL- prelimbic cortex.

1.3.2 Function of the rat medial prefrontal cortex (MPFC)

Most of the findings related to the functionality of the rat ACC are based on lesion studies.

In general, these findings correspond well to findings from human and/or primate studies. Similarly to humans there are many studies with rats which have found evidence for the contribution of the ACC to the evaluation of reward magnitude and effort as well as to the inhibition of incorrect competing responses.

For example, lesion studies by Bussey et al. (1997) and Cardinal et al. (2003) demonstrated that the rat ACC is crucial for the discrimination between different stimuli and the establishment of a relationship between stimuli and reward. Schweimer and

Hauber (Schweimer & Hauber, 2005) showed that rats had dramatic deficits in making decisions regarding the investment of effort to gain a high reward after lesions of the ACC. Later electrophysiological studies specified this finding more precisely by demonstrating that the ACC "encodes a relative, integrated cost-benefit representation of available choice options that is biased toward the "better" option in terms of effort/outcome ratio" (Hillman & Bilkey, 2010).

Findings which demonstrate that lesions of the rat prefrontal cortex have a crucial effect on the contextual control of response conflict are also in favor of the conflict processing hypothesis (Haddon & Killcross, 2006). Furthermore, inactivation of the dorsomedial prefrontal cortex by muscimol infusion leads to the inhibition of incorrect responses when there are competing responses (Wit et al., 2006). More precisely the prelimbic cortex, together with the anterior part of the cingulate cortex, seems to be essential for inhibiting incorrect, competing reactions (Chudasama et al., 2003) and therefore may be involved in conflict resolution.

1.4. Electrophysiological correlates of conflict and error processing

If a cognitive process is executed by a certain set of neurons which are activated at the same time point, there will be a correlation in total electrical activity. By averaging over several events the high spontaneous activity is mathematically eliminated and the event-related potentials (ERP) can be detected. ERPs are defined as electrocortical potentials, which are initiated before, during or after a sensory, motoric, cognitive or emotional event. They appear to be associated with cognitive control processes which can be distinguished on the basis of several distinct components reflecting different control subprocesses. Three of such components are the ERN, N2 and Pe, which will be further discussed, as they seem to reflect conflict or error monitoring processes.

1.4.1. *Error-related negativity (ERN)*

The error negativity (Ne; Falkenstein et al., 1990) or error related negativity (ERN; Gehring et al. 1993) is an event related potential (ERP) which was first described in 1990. It arises around the time of an incorrect response, sometimes even slightly before and has its maximum peak at around 50 to 100 ms after incorrect responses over frontocentral electrode sites. There are several versions of incorrect responses in which the ERN arises: overt response errors where the ERN arises immediately after the response (Falkenstein et al.,1990, 1991; Gehring et al., 1993), following response

feedback (Holroyd & Coles, 2002; Miltner, Braun, & Coles, 1997), and following late responses in deadline RT tasks (Johnson et al., 1997; Luu et al., 2000;). As we are interested in the direct monitoring of performance, we will observe the first version. The ERN is elicited after incorrect response regardless of the modality in which the stimulus is presented (acoustic or visual) and regardless of the modality of the response (saccade, button press; Falkenstein et al. 2000, Holroyed, 1998). EEG and fMRI studies give evidence that it is generated in the rostral cingulate zone (RCZ) on the posterior frontomedial wall (Debener et al. 2005; Ridderinkhof et al, 2004). As mentioned before there are several theories concerning what the ERN actually reflects. Some of these provide further support for the conflict monitoring hypothesis, others for the error detection theory. On the one hand it is assumed that the ERN reflects a monitoring process which signals errors if it detects mismatches between the intended response and the proper response (Coles et al.2001; Falkenstein et al.,1990, 1991, 2000; Gehring, 2000; Scheffers et al.,1996).

On the other hand the ERN has been proposed to reflect post-response conflict in error trials, that is, the conflict between the executed, erroneous response tendency and the still-evolving correct response tendency (Yeung & Cohen, 2006; Yeung, Cohen, & Botvinick, 2004). The findings of Danielmeier et al. (Danielmeier et al., 2009) support the above theory by demonstrating that in a Flanker task, ERN increases in error trials with a low-conflict condition compared to error trials with a high conflict condition. While, according to the conflict monitoring model, the ERN reflects post-response conflict, the N2 is thought to reflect pre-response conflict.

1.4.2. *N2*

The N2 is a negative deflection, emerging around 250 ms after stimulus in conflict related tasks like the Flanker, Stroop or Simon task. It seems to reflect very similar processes to the ERN, is largest on frontocentral electrodes and seems to be generated in the ACC (for reviews, see: Folstein & Van Petten, 2008; van Veen & Carter, 2002). In contrast to the ERN however, which follows a response the N2 precedes a response (Yeung, 2004). It is assumed that the N2 reflects the cognitive demands of situations involving a high level of conflict between competing potential responses (Yeung & Nieuwenhuis, 2009). This theory receives support from studies which demonstrate an increase in N2 amplitude in trials with an incongruent condition compared to trials with a congruent condition, possibly reflecting the inhibition of automatically but

erroneously primed responses (Heil et al., 2000; Liotti, et al., 2000). In further support of this hypothesis are the findings of Kopp et al. who have shown that the N2 amplitude increases with the degree of motoric activation related to the incorrect response (Kopp et al., 1996,). However, the monitoring account does not dismiss the error detection account completely, as the monitoring of conflict may provide a simple mechanism for detecting errors (Yeung et al., 2004). Furthermore the N2 is found in a variety of experiments often in the context of a positive deflection of the P3. One example is the auditory oddball task, were the N2 is elicited after the occurrence of a deviant stimulus, another is the no-go task were a response has to be inhibited (Pfefferbaum et al., 1985; Jodo & Kayma,1991).

One explanation for this is that the N2 can be subdivided into several subcomponents namely the N2a, N2b and N2c. The N2a mismatch negativity (MMN) is only found in auditory tasks. It is elicited in response to a deviant stimulus in sequence of standard stimuli. The N2c is related to visual attention and is sometimes referred to as the visual MMN. The N2b is related to cognitive control encompassing response inhibition, response conflict and error monitoring which is the primary focus of the present paper. It is this component, or rather its characteristics, that will be referred to as N2 in the remainder of this thesis.

In addition to the two aforementioned negative deflections which occur in association with errors, a further error related deflection in the positive direction (Pe) is also observable.

1.4.3. *Pe*

The error positivity (Pe, Hohnsbein et al., 1989; Falkenstein et al, 1991) typically follows the ERN. It is a slow positive deflection with a maximum amplitude over centro parietal electrodes between 200-400 ms after errors. Like the ERN it is unrelated to the stimulus modality but seems to reflect additional processing of errors. Traditionally, the Pe has been associated with the evaluation or active processing of errors (Falkenstein et al., 2000; Nieuwenhuis, 2000). Falkenstein and colleagues (2000) were able to demonstrate that the Pe is elicited in uncorrected trials and even false alarm trials. They argue that the Pe is not directly related to error correction but rather to error monitoring, albeit with neural and cognitive roots that differ from the error-related processing reflected in the ERN.

It is often related to a more "aware processing" of errors (Ford, 1999; Nieuwenhuis, 2000; Band & Kok, 2000; Larson & Perlstein, 2009; Wessel, Danielmeier & Ullsperger, 2011). However, some studies relate the Pe to error detection (Vidal et al., 2000). Unfortunately, as the Pe is less studied than the ERN or the N2 it is difficult to pinpoint its actual functionality.

2. Techniques overview

2.1. Imaging

2.1.1. Positron Emission Tomography (PET)

Positron Emission Tomography (PET) is a molecular, functional imaging technique for living organisms. Instead of structural anatomy, biological functions are mirrored and measured.

PET generates cross sectional, three-dimensional images of tissues, by imaging and displaying the distribution of a radioactive marker within the organism. The general principle of PET comprises the ß+-decay of radionuclides and the resulting emission of positrons (e+/ß+). The positrons move tortuously some millimeters through the tissue (the linear distance for an 18F positron flight in soft tissue is approx. 0.54 mm; Sánchez-Crespo et al., 2003), are decelerated and thereby lose kinetic energy until they are able to interact with electrons. If a positron encounters an electron, the two particles annihilate and two gamma rays (511 keV) are emitted at a 180 degree angle from each other. These emitted gamma rays are collected by the detector ring and the simultaneous arrival of the signals on two opposite detectors (coincidence) is registered. The distribution of radionuclides in the organism can be inferred from the physical distribution of these coincidences. The most common radionuclide in human research is Isotope Fluor-18 (18F, half-life 109.77 min). It can be produced using cyclotrons and is injected into the organism as a tracer, mostly in the form of 18F-fluorodeoxyglucose (FDG). In 18F-fluorodeoxyglucose the radionuclide replaces the hydroxyl group of the second carbon of a D-glucose forming a 2-fluorine-2-desoxy-D-glucose. FDG follows normal metabolisation until the time point when it is catalyzed by the glucose-6-phosphate-isomerase. At this point the isomerase needs the hydroxyl group, which had been replaced by the fluorine, for the catalysis. As this is not available, it cannot be metabolized. After the first phosphorylation, glucose cannot leave the cell again. Consequently, the FDG-6-phosphate in the cell accumulates and can be detected until

the 18F decays completely. The dispersion of the FDG in the organism allows for conclusions about the metabolism of glucose and thus indirectly provides information about the activity of distinct tissues. Active tissues need more glucose and therefore display a higher activity, less active tissues need less glucose and thus show low activity. PET has a spatial resolution of 5-10 mm. Using µPET a resolution of about 2up to 0.7 mm can be achieved. Comparing µPET and µMRI, µPET has a higher temporal signal sensitivity (µPET 10^{-11}-10^{-12} mol/l; µMRI 10^{-3}-10^{-5} mol/l), but a lower spatial resolution (µPET 0.7-2.0 mm; µMRI 100 µm).

2.1.2. Magnet resonance imaging (MRI)

Magnet resonance imaging (MRI) is a non-invasive technique designed to image structures of tissues and organs of organisms. The magnetic properties of unpaired protons, which can be found in hydrogen for example, are used to image structural properties. The idea is, that regions with lower proportions of hydrogen (e.g. bones) release less signals. MRI measures the total number of spins of unpaired protons (intrinsic angular momentum of the protons) per voxel of interest. For the measurement, a static magnetic field aligns the spins of the protons into one direction (z-plane), while they precess around the axis of the magnetic field. Another high frequency alternating field (it equals the frequency of the precession and is called lamorfrequency) is then applied with a 90 degree angle to the first field, which deflects the precession in a way that the spins now only rotate on the xy-plane. The increase in the xy-component of the magnetic field's vector is called transverse magnetization. An increase in this transverse magnetization thus diminishes the longitudinal magnetization in the z-direction. Following this a potential is induced in the coils, arranged around the organism, which is proportional to the transverse magnetic field of the magnetic moment. The transverse magnetization is different for different kinds of tissues. MRI produces layered images of this transverse magnetization.

The reduction in signal indicates relaxation of the spins, which then align back to their unexcited default. This results in increased longitudinal magnetization and reduced transverse magnetization. Longitudinal magnetization is, similarly to transverse magnetization, tissue specific and dependent on the magnetic field strength. Because the spins loose energy through interacting with their environment (spin-lattice relaxation, T1) and interacting with each other (spin-spin relaxation, T2) and the relaxation times are different for distinct tissues, these differences indicate contrasts in the subsequent

images. Additionally, the amount of hydrogen atoms in the different tissues contributes to the contrast in the images. In the T1 images, tissues having short relaxation-times appear light, while those with long relaxation-times are dark. T2 contrasts have to be interpreted vica versa. Therefore, T2 images have the advantage that liquid filled cavities can be identified better, because water has a relatively long relaxation time.

2.2. Electrophysiology

2.2.1. Electroencephalography (EEG) and Event-related Potentials (ERP)

EEG is a non-invasive technique to measure summed electrical potentials, typically in the range of 5 to 100µV, from the surface of the skull.

The recording is obtained by placing electrodes, mostly attached to an elastic cap, on the scalp. The elastic cap assures that the electrodes are placed and named after the commonly used and internationally approved 10-20 System. The electrodes are connected to a differential amplifier, which amplifies the voltage between the active electrode and the reference. The analog EEG is then filtered, digitized via an analog-to-digital converter and stored electronically.

The normal, spontaneously measured EEG potentials always reflect the summation of the synchronous activity of thousands of neurons that have similar spatial orientation (i.e. cortical potentials derived from the pyramid cells of the neocortex). To measure event related potentials of cortical and subcortical regions, results over several events must be averaged. If a cognitive process is executed by a certain set of neurons which are activated at the same time point, this will result in correlated total electrical activity. The normal, spontaneous EEG has higher amplitude, but differs across instances where a particular cognitive process is repeated, whereas the event-related activity, although smaller, is assumed to stay constant. By averaging over several trials the high spontaneous activity is mathematically eliminated and the event ERPs can be detected. ERPs are defined as electrocortical potentials, which are initiated before, during or after a sensory, motor, cognitive or emotional event. It is detectable with EEG under the following conditions: there has to be a sufficiently large set of neighboring neurons which are a) all active at the same time point b) with the same type of activity (either inhibitory or excitatory) and c) the same geometric structure (i.e. parallel), so that the electrical potentials add up to a summation activity which is transmitted through the

scalp. The source of the ERP cannot be unequivocally derived from the measured activity as the spatial resolution lies at a depth of several cm. In special, mostly clinical cases, where it is possible to measure EEG directly from the cortex surface (Electrocorticogramm) higher spatial resolutions of down to 1 cm are possible. However, the strength of this technique lies in its nearly unlimited temporal resolution, which stands in sharp contrast to its relatively low spatial resolution.

2.2.2. Local field Potentials (LFP)

The EEG signal mainly consists of slower (<250Hz) local field potentials. These LFPs are derived from the large excitatory pyramidal cells of the cortex and their apical dendrites (Logothetis and Wandell, 2004). To measure the potentials associated with smaller, equally aligned cell assemblies intracortical electrodes have to be inserted. Potentials derived from these electrode tips are the high frequency multiunit activity (MUA; 1000Hz) and the low-frequeny local field potentials (500Hz).

Both represent extracellular recorded signals from local networks of neurons, but the MUA appears to reflect the spiking of local neurons and the LFP shows dendritic membrane currents of neurons in the close vicinity (as reviewed by Logothetis, 2003, 2008; Berens et al., 2008). These low frequency membrane currents are of greater interest as they are thought to be related to excitatory or inhibitory postsynaptic potentials (Mitzdorf, 1985, 1987), index processes which are causal to action potentials and therefore provide information about the networked activity of groups of nerve cells related to local processing and neuronal synchrony. Furthermore, they seem to be correlated to hemodynamic signals (fMRI; Logothetis et al., 2001) which makes them a possible candidate for providing a link between neurophysiological and functional imaging studies. Admittedly, the biophysical origin of the LFPs and their spatial resolution are still under discussion. Early studies estimate the range of the local field potentials to be between 600-1000µm (Berens et al., 2008), to 2–3 mm (Nauhaus et al., 2009; Wang et al., 2005) or even 5 mm (Kreiman et al.,2006). Later studies assume that the LFPs are more local in the range of 200–400 µm (Katzneret al., 2009; Xing et al., 2009). A recent study, however, determined that LFPs spread over more than one centimeter (Kajikawa & Schroeder, 2011). One advantage of the LFPs is their distance to myopic artifact sources. EEG, in contrast is prone to be influenced by sources of interference. Especially in the high frequency range there are many disturbances which

could lead to artifacts in brain specific potentials. Most of these artifacts come from muscle activity of the neck, eye or head.

3. Objectives and structure

The correct functioning of the brain is based on ensuring a smooth cooperation of different neuronal networks. Inhibition, excitation, feed forward and feedback processes are the basic mechanisms of interaction between different network modules (Bulliere et al, 2001). To localize some of these networks and participating brain regions, it is common to use neuroimaging studies. However, neuroimaging, due to its low temporal resolution, leaves the open question of time points at which the different modules participate in the process, especially the involvement of sequential or parallel activation, feed forward or feedback processes. EEG offers an opportunity to measure real time neuronal activity, but without the ability to localize the active neurons (Michel, 2004). Therefore, a combination of several techniques is needed. The greater goal of this study was to understand the general processes that underlie performance monitoring which is subdivided into conflict and error monitoring or detection and to get an understanding of cognitive control and to comprehend or maybe even cure diseases like obsessive compulsive disorder, ADHD and schizophrenia.

As an initial step to approach this goal, we propose the establishment of a rat model to investigate these processes. The proposed model of conflict and error monitoring allows us to take advantage of the capabilities of both imaging and electrophysiology techniques. The imaging model provides an excellent opportunity for repeated scanning of the subjects which would not be possible with humans due to the repetitive exposure to radioactivity. Measuring LFP intracortically in a rat model has a great potential to enrich findings over and above what would be possible with human EEG analyses. Furthermore, a rat model allows us to study the processes in less complicated system. However, a rat model is only valuable if the findings can be translated to what we find in humans. This fact is often neglected in animal studies. Therefore, the current thesis aims to complete the model with a human study to ensure comparability, which is required to reach the greater goal of being able to translate observations from animal studies to human clinical applications.

Finding both metabolic involvement and emitted field potentials in the rat ACC in relation to performance monitoring would not only establish a bridge between ERPsand functional brain-imaging studies in rodents but would pave the way for similar studies

in humans and non-human primates as well. The opportunity to carry out invasive studies in animals can contribute to a resolution among alternative hypotheses of conflict monitoring, error detection and error monitoring.

The aim of this study is to establish a rat model, which can be used to investigate whether rats:

i)   have a functional network of MPFC, PFC and motor areas that are involved in conflict processing, comparable to the human brain.

ii)   show a similar temporal processing of conflict and the resulting errors in the MPFC.

In order to achieve a satisfying result the following issues have to be discussed:

1)   which brain areas contribute to the processing of conflicts in the rat

2)   at which time point between the onset of a stimulus and the response reaction is the processing of the conflict done.

3)   how is conflict processed by the rat brain?

In order to investigate these questions, we utilize several techniques such as behavioral measurements of reaction time, movement time and error rates, functional imaging and electrophysiological measurements of event-related-potentials. With the help of behavioral methods we expect to get a closer look into incompatibility effects resulting from the Simon effect (**Experiment 1**), such as analyzing strategies of response adaptation, error avoidance, and adaptation to higher and lower error probabilities (experiments 2 and 3). Functional imaging is used to detect brain areas which are involved in conflict processing and to point out the functional connectivity between them. Attention is primarily directed towards the involvement of the ACC (experiment 1). As a further step, event-related-potentials derived from local field potentials will be analyzed to get a view into the temporal resolution of conflict- and error processing and its adaptation (**Experiment 2**). Finally, a comparative study with humans will be discussed as a first step towards bridging human electrophysiology and rat neurophysiology (**Experiment 3**).

4. Experiments

4.1. Experiment 1: Metabolic imaging of a rat Simon task

4.1.1 Purpose

A model of dual route processing (automatic and intentional) of stimulus features has been proposed, predicting response conflicts if the two routes are incongruent. Although there is evidence that the prefrontal cortex, notably the anterior cingulate cortex, plays a crucial role in action monitoring, especially in conflict and error processing, the neuronal basis of this is still unknown. In this study, we pursue a novel approach using positron emission tomography (PET) to identify relevant brain areas in a rat model of conflict processing comprising an auditory Simon task in an operant chamber. We focus on the proposed dual route theory and the underling brain networks especially. In contrast to previous experiments, using an imaging technique (PET) is still a rare approach in animal studies, even though these studies represent a valuable addition to lesion experiments. Lesion studies can only review behavioral deficits after complete shutdown of brain regions such as the ACC but cannot evaluate the functional activation of the area. In contrast, using an imaging technique has the advantage that the activation of the area can be captured under natural conditions. Another benefit is that no effect of accidental lesions on adjacent brain areas can occur. Therefore, in the present study we sought to use $[^{18}F]$fluorodeoxyglucose in order to identify the pattern of metabolic activation in the brain of rats concomitant with performance on a Simon task. The complex manner of the Simon task requires multiple testing and imaging. Conducting this study in humans would lead to an unhealthy accumulation of radioactivity. Furthermore, the huge amount of human studies lead to different even contradicting assumptions about the anatomical basis of performance monitoring. An evolutionarily more simple system could provide better access to the dual route architecture. By way of conclusion, an appropriate animal model is needed. The Simon effect has already been described in rats (Courtière et al., 2007), and it was shown that metabolic behavioral positron emission tomography (PET) in animal models is a suitable method to detect activation in focal brain regions (Jang et al., 2009; Sung et al., 2009; Endepols et al., 2010). Therefore the purpose of the current study was to develop an animal model

that links behavior with metabolic brain activity to investigate the anatomical and functional basis of conflict processing.

Experiment 1 is taken over with a slight redrafting from the paper: Marx C, Lex B, Calaminus C, Hauber W, Backes H, Neumaier B, Mies G, Graf R, Endepols H (2012) Conflict Processing in the Rat Brain: Behavioral Analysis and Functional µPET Imaging Using [18F]Fluorodeoxyglucose Front Behav Neurosci.;6:4

Authors' contribution:
<u>*C.M*</u> *designed and performed experiments, analyzed behavioral and PET data and wrote the paper*
B.L. and C.C. helped to construct the Skinner box running programs, commented on the manuscript. W.H. discussed the results and implications and commented on the manuscript. B.N. gave technical support on Radiotracer chemistry, allocate the FDG. H.B map reconstruction of the PET Images, gave technical support on PET Physics. G.M. and R.G. commented on the manuscript. H.E. designed the study, analyzed PET data, and edited the manuscript.

4.1.2. Materials and Methods

Animals

All animal procedures adhered to German Welfare Act and were approved by the local animal care committee and regional government authorities.

Eleven male Lister hooded rats (Harlan-Winkelmann, Borchen, Germany) were used, weighing 250 g at the start of the training. Animals were housed in pairs under an inverted 12:12 h light-dark cycle (lights out at 8 am) in a temperature- and humidity-controlled facility room (20±2 °C, 50-60 %) and restricted to 15 g food per animal per day. Water was available ad libitum.

Apparatus for behavioral testing

Animals were tested in an operant chamber (30.5 cm x 24.1 cm x 21.0 cm; Med Associates Inc. Georgia, VM, USA) with a central nose poke unit and two trough-like food receptacles on either side, equipped with light barriers for measuring reaction and movement times (Robbins et al., 1993). Food receptacles were connected to a motor-driven pellet dispenser, delivering 45-mg precision pellets (Bioserv) as reward. Two loudspeakers (Med Associates "cage tweeter", range: 5-15 kHz) were placed above the pellet receptacles (**Figure 6**). The acoustic stimuli consisted of two 300 ms (rise/fall

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time 5 ms) pure tones with carrier frequencies of 10 kHz and 15 kHz, and a sound pressure level of 60 dB. All experiments took place during the animals' dark phase under red light.

Figure 6: **General Setup of the auditory Simon task and schematic illustration of the dual route model**. (**A**): The rat starts each trial with a nose poke of t>1.5, which results in playback of an acoustic stimulus from one of the speakers. (**B**): Reaction time (RT) is measured from start of the stimulus to withdrawal from the nose poke unit. (**C**): According to stimulus pitch (10 kHz / 15 kHz), the rat moves to one of the pellet feeders (left/right). The time from withdrawal from the nose poke unit to

Operant conditioning of the basic Simon task

The auto shaping procedure of the operant conditioning behavior was conducted through three steps (**Figure 7**):

   (1) habituation,
   (2) nose poke training (phases a and b),
   (3) sound discrimination training (phases a, b and c).

On the first day of training (habituation; **Figure 7. A**) rats were allowed to become accustomed to the operant chamber, the auditory stimuli and the food reward (45-mg precision pellets, Bioserv). The two auditory stimuli alternated in a pseudo randomized fashion every 10 s, and were associated with a food reward from the pellet trough on the side associated with the stimulus. From the second day on, the rats had to learn to

initiate a trial by themselves with a nose poke (nose poke training; **Figure 7. B**) which should last at least 1.5 s (phase a). In phase b the occurrence of too short nose pokes is reduced by introducing a punishing time out. This means nose pokes under 1.5 s were indicated by diode illumination in the nose poke unit and punished with 2 s in which the rat could not start a new trial. In both phases a correct nose poke resulted in a bilateral tone presentation (i.e., one of the two stimuli was played back simultaneously from the two speakers) and immediate delivery of a pellet at the side associated with the stimulus. In the last training step (sound discrimination training; **Figure 7. C**), the reward was no longer delivered automatically after stimulus presentation. Instead, the rat had to choose one side according to the auditory stimulus and enter the pellet trough. Five of the eleven rats were trained to go to the left food receptacle after a 10 kHz stimulus and to the right food receptacle after a 15 kHz stimulus. The other six rats were trained to make the opposite association between frequency and side of reward. If the choice was correct, a pellet was delivered. If the rat chose the wrong side, the nose poke diode was illuminated for 2 s during which the rat could not start a new trial.

To consolidate the association between stimulus and response side, each stimulus was repeated for five consecutive trials, starting with 15kHz during the first phase of the sound discrimination training, and in phase two starting with 10kHz (**Figure 7. C** green arrows). During the third phase both stimuli were alternated randomly. The procedure of this training step was identical with the control task consisting of 100 % neutral trials (T_N) in behavioral PET (see below).

In all other tests following the training stage, the acoustic stimulus was delivered unilaterally (i.e., one of the two stimuli was played back from one of the speakers, either left or right). Rats were trained for several weeks, always on five consecutive days and then rested for two days. Each training session lasted 15 min or was terminated if the rat accomplished 60 correct responses in less than 15 min. Rats advanced to the next training step after reaching a performance level of 85 % correct responses.

Figure 7: Shaping of the simon task. Example for group 1 with 10 kHz reward right and 15 kHz reward left. Group 2 had a similar shaping procedure but with exchanged frequency information (10 kHz reward left 15 kHz reward right. **A)** Habituation phase **B)** Nose poke training phase 1 (black) and Phase 2 (plus green part). **C)** Sound discrimination training phase 1/2 (plus green part) and 3 (without green part).

Basic Simon task

The basic Simon task resembled the last training step (**Figure 7 C**), only with unilateral stimulus presentation, and a total of 120 trials. During the basic Simon task, rats had to initialize every trial with a nose poke >1.5 s in the central nose poke unit, which lead to playback of one single auditory stimulus (300 ms; tone pitch 10 or 15 kHz in pseudorandomized order). According to pitch, rats had to choose the left or right food receptacle, and were rewarded after correct choice. Five of the eleven rats were trained to go to the left food receptacle after a 10 kHz stimulus and to the right food receptacle after a 15 kHz stimulus. The other six rats were trained to make the opposite association between frequency and side of reward. If the sides of stimulus presentation and correct response concurred, this was recorded as a compatible condition (C). During incompatible conditions (I), stimulus and response occurred on different sides (see **Figure 8**). Only in neutral trials (N) tone stimuli were emitted from both speakers simultaneously. Conditions were presented in a pseudo-randomized sequence. The reaction time (RT; **Figure 6**) was taken as the time between start of the auditory stimulus and withdrawal from the nose poke unit, while movement time (MT) was taken as the time from nose withdrawal until entrance of the food receptacle. Because MT was similar under all conditions, it will not be mentioned further in this study. The trial was terminated if the nose was withdrawn before the end of the required nose poke-time of 1.5 s, while trials with RT >1 s or <130 ms were discarded off-line. Error rate (ER) was taken as the percentage of wrong choices, and was arc-sine square root transformed before statistical analysis.

Figure 8: Conditions of the auditory Simon task.
    (1) Compatible condition: stimulus and response
        are on the same side.
    (2) Incompatible condition: stimulus and
        response are on opposite sides. A response
        conflict occurs.

Test schedule

Two experimental blocks were conducted: one behavioral PET imaging block with five different tests, followed by one purely behavioral block with three different tests. All tests, except the resting state control, were variants of the basic Simon task (see below). Each rat had to perform all tests within a block.

Behavioral PET imaging block

In the behavioral PET block we wanted to see focal metabolic brain activation associated with Simon-like conflicts. Each rat underwent five behavioral PET sessions. We took the task with 50 % compatible and 50 % incompatible trials as a basis, because a balanced number of compatible and incompatible trials avoids biasing the metabolic response by one type of trials.

(1) Basic Simon task with 50 % incompatible and 50 % compatible trials in randomized order (T_R).

The Simon task had to be compared to several control conditions:

(2) No-conflict control with neutral trials only (T_N). Here, the rats had to do exactly the same as during T_R. However, conflicts did not occur because bilateral stimulus presentation prevented spatial information.
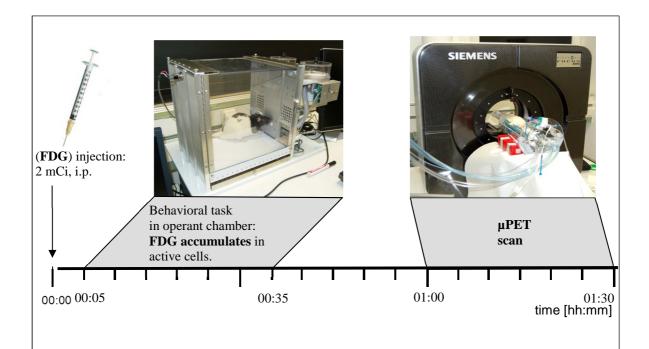
Because of the cumulative nature of PET it is not possible to separate metabolic responses to compatible and incompatible trials. We therefore conducted two additional controls with 100 % compatible and 100 % incompatible trials, respectively:

(3) Basic Simon task with compatible trials only (T_C; side of stimulus presentation and required response side always matched);

(4) Basic Simon task with incompatible trials only (T_I; stimulus and required response were always on opposite sides);

Finally, we wanted to compare the Simon task with the naive situation before training:

(5) Resting state control (R_S), with naive rats before operant conditioning. In R_S there were no cognitive requirements, the rats only heard the sound stimuli in random order with 10 s interstimulus intervals and food pellets accessible ad libitum in the food receptacle.

Tasks (1)-(4) took place after successful operant conditioning. They were presented on average six days apart in the order of increasing complexity (i.e. T_N, T_C, T_I, T_R). During the days in between PET sessions, the rats repeated the last training step.

For the combination of behavior with metabolic PET imaging, rats were briefly anesthetized for intraperitoneal injections of [^{18}F]fluorodeoxyglucose (FDG; 1.7-2.1 mCi; 500 µl injection volume; stock solution in 228 mM Na-phosphate buffer, diluted as needed with 0.9 % NaCl). Five minutes after tracer injection, rats started to perform one of the five tasks in the operant chamber for 30 min (**Figure 9**). As a glucose analogue, FDG is incorporated by active brain cells and is subsequently phosphorylated by hexokinase, but cannot be further metabolized because of the missing hydroxyl group (Wienhard, 2002). The process of trapping is an indicator of the state of metabolic activity of tissue, which can be measured during a scan under anesthesia after the behavioral task. Fifty min after FDG administration (i.e. 15 min after the end of the behavioral task), animals were anaesthetized by inhalation of isoflurane (5 %, delivered in 70 % N_2O and 30 % O_2), and placed in the animal holder of a Focus 220 micro PET scanner (CTI/Siemens Knoxville, TN; resolution at center of field of view: 1.4 mm). Breathing rate was kept at 50-70 per min by adjusting isoflurane concentration (1.5-2.5 %). Body temperature was held at 37 °C with a feedback-

controlled flow of warm water through the animal holder. Glucose concentration was measured in a blood sample collected from the tail vein at 60 min after FDG injection, using a blood glucose level meter (One Touch Ultra). Emission data were recorded over 30 min in list mode, starting 60 min after FDG injection. Following Fourier rebinning, data were reconstructed using the iterative OSEM3D/MAP procedure (Qi et al., 1998), resulting in voxel sizes of 0.38 x 0.38 x 0.82 mm. RTs and ERs measured during FDG accumulation were compared over tasks (1)-(4) using one-way repeated measures ANOVA with post hoc comparison and Holm-Sidak correction. Each task provided one factor level, except task (1)T_R, which yielded two factor levels, one for compatible and the other for incompatible trials.

Figure 9: **Schedule of the Simon task combined with PET**. The animals received an intraperitoneal injection of 2 mCi FDG during a brief anesthesia. After five minutes, rats performed a Simon task in an operant chamber for 30 min. During this period FDG accumulated in cells with high metabolic activity. MicroPET scans took place under isoflurane inhalation anesthesia in a Focus 220 microPET scanner (CTI/Siemens Knoxville, TN) with a resolution at center of field of view of 1.4 mm. Emission data

Behavioral block

All rats conducted three basic Simon tests, each with different relative probabilities of incompatible trials: (1) 20 % I; (2) 50 % I; identical to T_R in the PET block; (3) 80 % I. For example, in the 80 % I condition a rat trained to associate the 15 kHz tone with the left response side and the 10 kHz tone with the right response side heard the following in randomized order: 15 kHz - right speaker (I) - 40 % of trials; 10 kHz - left speaker (I) - 40 % of trials; 15 kHz - left speaker (C) - 10 % of trials; 10 kHz - right speaker (C) - 10 % of trials.

Rats received one test session per day, in randomized order balanced between animals. Effects of conflict probability and condition on RT and ER were estimated with two-way repeated measures ANOVA (see results for factorial design) and Holm-Sidak corrected post hoc comparison. Statistical computations were conducted with Sigma Plot (version 11.0, Systat Software, Inc.). Significance level (α-level) was set at $p<0.05$.

MRI scans

Because the animals were in an inverse day-night-rhythm, they were carried to the scanner in optically opaque boxes. MRI scans were performed in a 4.7-T BioSpec animal scanner (Bruker BioSpin, Ettlingen, Germany) using a quadrature transmit/receive birdcage coil (Rapid Biomedical, Rimpar, Germany) with an inner diameter of 38 mm. A relaxation enhancement (RARE) sequence was used: RARE factor = 8, TR/TE = 5000/14.0 ms, averages = 2, matrix size = 256 x 256, FOV = 4.6 x 4.6 cm^2, 21 slices, slice thickness = 1.3 mm, interslice interval = 1.8 mm.

In preparation of the scanning, the rats were anesthetized with Isofluran and fixated in the MRI scanner. Inhalation anesthesia procedures were the same as those used for µPET scans.

Imaging data analysis and statistics

MRI and PET data were analyzed using the imaging tool VINCI (Vollmar et al., 2007). MR images were manually co-registered on a master brain derived from the atlas of Swanson (Swanson, 2003) and examined for structural abnormalities. PET images were then manually co-registered on the corresponding MR images. With the help of the master brain, individual MR images, and the brain atlas of Paxinos and Watson (2005), three-dimensional volumes of interest (VOIs) corresponding to defined brain areas (**Table 2**) were drawn section by section in the transverse/coronal plane. Section

thickness was identical with z-dimension of voxels (0.815 mm). For intensity normalization, every image was divided by the respective mean value of a whole brain VOI (ratio normalization; Arndt et al., 1996). Normalized metabolic activity was then assessed in the VOIs of individual brain areas. Because of chewing food reward pellets during operant behavior, high FDG uptake of temporal muscles was unavailable, and substantial spillover of radioactivity obscured some parts of the lateral cerebral cortex (**Figure 10**). With a threshold function we determined the outline of muscle activity and used this to draw a muscle artifact mask and to adjust VOIs, if necessary.

In order to assess task-relevant regional brain activation, we compared PET sessions with each other. For analyzing brain activation associated with the task condition in general, we compared T_I (Simon task with 100 % incompatible trials) with the resting state control R_S by calculating $100 \times T_I/R_S$ (i.e. percent normalized metabolic activity of T_I versus R_S). Metabolic activation associated solely with conflict processing was assessed by displaying percent metabolic activity of T_R, T_I, and T_C versus the no-conflict control T_N ($100 \times T_R/T_N$; $100 \times T_I/T_N$; $100 \times T_C/T_N$). T_N was chosen as reference condition, because the bilateral stimulus presentation provides ambiguous spatial information, and therefore no conflict occurs. In T_C, on the other hand, there is no conflict, either, but it cannot be ruled out that facilitatory processes (e.g. facilitation of the automatic route) may take place. For this reason, normalized metabolic activity during the incompatible control task T_I was furthermore compared to the compatible control task T_C as well. Using the one-sample t-test, we compared the resulting relative VOI activities with $\mu=100$ % (i.e. no change relative to T_N or T_C). In addition to the VOI analysis, we compared matched voxels of the four tests using one-way repeated measures ANOVA. For post-hoc comparison, the Holm-Sidak method was used with T_N serving as control. Voxels from T_I were additionaly compared to T_C with the help of the paired t-test. Finally, correlation analyses were run between task-related activity changes on the one hand, and RT and ER on the other hand, using the Pearson product-moment correlation test. As the rat brain comprises approx. 19,000 voxels, voxel-based statistical calculations include multiple comparisons associated with a considerable increase in the type I ER. P-values were corrected for multiple comparisons using the Benjamini-Hochberg control of false discovery rate. However, as in previous PET studies with low degrees of freedom (e.g., Nichols and Hayasaka, 2003; Rocke et al., 2005), all individual voxel comparisons missed significance if using the false discovery

rate procedure. Uncorrected significant p-values were between 0.01 and 0.05, therefore we set a threshold of p=0.02 (corresponding to $F(3,9)=5.51$), as proposed by Genovese et al. (2002).

Figure 10: (**A**) **Example of a structural MRI and (B) the corresponding PET image.** (**C**) Fusion of the two images shows muscle artifacts on the lateral aspects of the brain (arrowheads). This leads to covering of the lateral cortical regions in the atlas (**D**). Abbreviations: Cg1, Cg2: anterior cingulate cortex area 1 and 2; LS: lateral septum; lStr: dorsolateral striatum; M1: motor cortex; M2: premotor cortex; MS/DB: medial septum anddiagonal band of Broca; mStr: mediodorsal striatum. Scale bars: 1 cm.

4.1.3. Results

Test schedule
Seven animals were used for behavioral PET, and successfully conducted R_S and T_I. One rat died during the PET block, and two refused to work reliably in the PET situation, so that four rats completed all five PET tests. For the subsequent behavioral block, these four plus four additional animals were used

1) Behavioral block

Behavioral data
We start with reporting the behavioral data, because occurrence of a stable Simon effect in the behavioral experiments undisturbed from PET procedures is the prerequisite for all further analyses. The different probabilities of incompatible trials are important for the following PET block as well, since a pronounced Simon effect with a high rate of incompatible trials would suggest a high conflict level in the PET T_I condition (100 % incompatible trials). The analysis of the Simon tasks with three different frequencies of incompatible trials revealed a Simon effect for both RT (n=8); mean values calculated

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for all three Simon tasks: RT[C]=315 ms; RT[I]=351 ms) and ER (mean values for all three Simon tasks : ER[C]=7 %; ER[I]=14 %; **Figure 11**). This was confirmed by 2-way repeated measures ANOVA with the factors "condition" (factor levels: I, C) and "probability of incompatible trials" (factor levels: 20 % I, 50 % I, 80 % I), indicating a significant main effect of the factor "condition" on RT ($F_{(1,14)}$=16.8, p=0.005) and ER ($F_{(1,14)}$=10.5, p=0.014). Post-hoc comparison showed that RT[C] was significantly shorter than RT[I] in the 20 % I (p=0.003) and 80 % I task (p=0.029), while ER[C] was significantly lower than ER[I] in 20 % I (p=0.001), 50 % I (p=0.016) and 80% I task (p=0.029). Conflict probability had no significant main effect on RT and ER.

Figure 11: **Reaction times (A) and error rates (B) in compatible (black) versus incompatible (grey) trials at different conflict probabilities, measured in the behavioral block.** Values shown are means ± SEM (n=8).

2) Behavioral PET imaging block

Behavioral data

Here we evaluated if rats showed a Simon effect in the PET condition, where tracer injection, scanner noise, etc. may have compromised conflict processing. Most important are results of T_R, where compatible and incompatible trials can be compared directly. Four animals were tested repetitively in combination with PET in all conditions. Rats conducted 128 - 246 trials per session. Blood glucose levels at the start of the scan (123 - 192 mg/dl) did not correlate significantly with trial numbers (R=0.17, p=0.53, Pearson product moment correlation test), suggesting that the number of consumed food pellets during the task did not bias global cerebral FDG uptake. T_R yielded results comparable to those of the behavioral block (**Figure 12**). RTs were on

average 33 ms shorter in compatible compared to incompatible trials (t=2.17, p=0.059; one-tailed paired t-test). In the control tasks consisting of only one type of condition (T_C, T_I, and T_N), RTs were similar to those in incompatible trials of T_R and did not differ significantly across tests (F(4,12)=1.82, p=0.19, one-way repeated measures ANOVA). To further assess whether T_I is a valid control with high conflict level we compared average RTs during the first, middle, and last third (10 min each) of the task. During the first third, RTs were higher than in the other tasks, but decreased significantly during the following 20 min (**Table 1**). This indicates a high conflict level at least in the first 10 min of the task.

ERs were on average 5.8 % lower in compatible compared to incompatible trials in T_R, leading to a significant main effect across tests (F(4,12)=4.58, p=0.0178) and a significant difference between compatible and incompatible trials of T_R after post-hoc comparison.

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Table 1: Reaction times during PET sessions (mean ± s.e.m.).

task	first third of trials	middle third of trials	last third of trials
T_R	326.2 ± 16.9 ms	297.2 ± 8.2 ms	303.2 ± 15.9 ms
T_C	351.9 ± 24.8 ms	323.1 ± 19.6 ms	338.5 ± 27.8 ms
T_I*	361.9 ± 24.7 ms	311.4 ± 5.1 ms	299.1 ± 15.7 ms
T_N	342.7 ± 18.5 ms	318.4 ± 19.2 ms	335.1 ± 36.1 ms

* F(3,6)=5.46; p=0.045 (one-way repeated measures ANOVA). One third comprises 10 min.
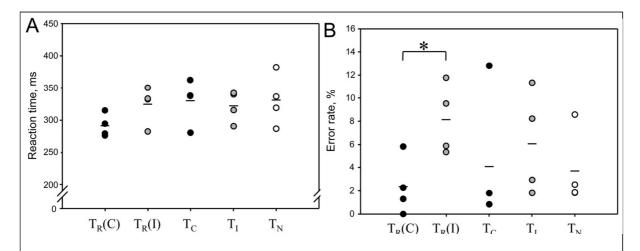
Figure 12: **Reaction times (A) and error rates (B) in the Simon tasks combined with PET imaging**. Each dot represents the result from one animal (n=4). Mean values are indicated by a short line. In the classical Simon task T_R, where compatible and incompatible trials were presented in randomized order, the results for compatible and incompatible trials were analyzed separately ($T_R(C)$ and $T_R(I)$). There was a significant Simon effect on error rate, but not on reaction times. $T_R(C)$ and $T_R(I)$ correspond to black and grey bars, respectively, of the condition "50 % incompatible trials" in Figure 11. Control tasks: T_C: compatible trials only; T_I: incompatible trials only; T_N: neutral trials only.

Subtractive approach

We first describe metabolic patterns associated with the Simon task in general. To examine task related brain activations in general, we compared metabolic activity in one of the basic Simon task variants (T_I) with resting state R_S (n=7; **Figure 13**). We found a significant task related decrease of metabolic activity in the left prefrontal cortex (VOI statistics: one-sample t-test; t=-3.33, p=0.0157), right prelimbic cortex (t=-2.74, p=0.0338), right posterior cingulate region Cg1 (t=-3.66, p=0.0106) and decreased metabolic activity bilaterally in the posterior Cg2 (left: t=-3.41, p=0.0144; right: t=-3.24, p=0.0176). Furthermore, metabolic activity was decreased bilaterally in the retrosplenial granular cortex (left: t=-2.65, p=0.0382; right: t=2.54, p=0.0442), medial septum (t=-4.22, p=0.0055), bilaterally in the lateral septum (left: t=-4.71, p=0.0033; right: t=-3.86, p=0.0084) and in the hypothalamus (t<-3.0, p<0.022). A task related increase of metabolic activity was additionally found in the left lateral striatum (t=3.22, p=0.0182).

Figure 13: **Subtractive approach**: Percent change of metabolic activity in Simon tasks with 100 % incompatible trials (T_I) relative to resting state (R_S). Grand average from n=7 animals with mean changes projected onto transverse and horizontal sections of a master brain. Coordinates are mm from Bregma. Areas obscured by muscle artifacts are masked and significant VOIs are indicated by asterisks.

Next, we report metabolic activity changes related to conflict processing. We looked for metabolic activity changes relative to T_N, which were visible in T_I and T_R, but not in T_C (**Figure 14**, columns 1-3; **Table 2**). In the right prelimbic cortex FDG uptake was significantly increased during T_R at the VOI level (t=15.26, p=0.0006) as well as during T_I and T_R at voxel level (F(3,9)>4.3, p<0.0378). FDG uptake also increased significantly in the right ventrolateral striatum, on the border to the entorhinal cortex, during T_R (F(3,9)>4.98, p<0.0264 for voxels). In T_I, there was a non-significant average increase of FDG uptake of more than 10 % in the same region. Metabolic activity decreased in voxels of the left dorsocentral striatum during T_I and T_R (F(3,9)>4.5, p<0.0349). Brain activity changes related to potential automatic route facilitation caused by compatible trials should be visible in T_C and T_R, but not in T_I. We found decreased metabolic activity in right olfactory tubercle voxels during T_C and T_R (F(3,9)>4.6, p<0.0334).

The cerebrometabolic correlates of automatic route suppression may become most obvious if we analyze T_I relative to T_C (**Figure 14**, column 4). Here, we found increased FDG uptake in the right Cg1 region of the anterior cingulate cortex, (t>-3.39, p<0.0428 for voxels), right orbitofrontal cortex (t>-3.29; p<0.0462 for voxels), right basal forebrain and nucleus accumbens (t>-3.50, p<0.0393 for voxels), right dorsolateral striatum (t=-3.57, p=0.0374 for one voxel; t=13.4711, p=0.0006 for VOI), right amygdala (t>-3.35, p<0.0440 for voxels), right subthalamic region (t>-4.11, p<0.0261 for voxels), right mediodorsal thalamus (t>-3.37, p<0.0433 for voxels), and left lateral hippocampus (t>-5.22, p<0.0137 for voxels). FDG uptake decreased in the left tenia tecta (t>3.19, p<0.0497 for voxels), right lateral septum (t>4.06, p<0.0270 for voxels), left dorsocentral striatum (t=5.45, p=0.0122 for voxels), left hippocampus (t=3.75, p=0.0331 for voxels), and left mediodorsal thalamus (t>3.47, p<0.0403 for voxels).

Figure 14: **Subtractive approach**: Percent change of metabolic activity in Simon tasks with randomized presentation of compatible and incompatible trials (50 % each; T_R), 100 % compatible trials (T_C), and 100 % incompatible trials (T_I). Values are relative to T_N (column 1-3) or T_C (column 4). Grand average from n=4 animals with mean changes projected onto transverse sections of a master brain. Column 5: Coordinates (mm from Bregma) and analyzed VOIs. Areas obscured by muscle artifacts are masked. Significant voxels (uncorrected p<0.05) are shown in green and (uncorrected p<0.02) in yellow, significant VOIs are indicated by asterisks.
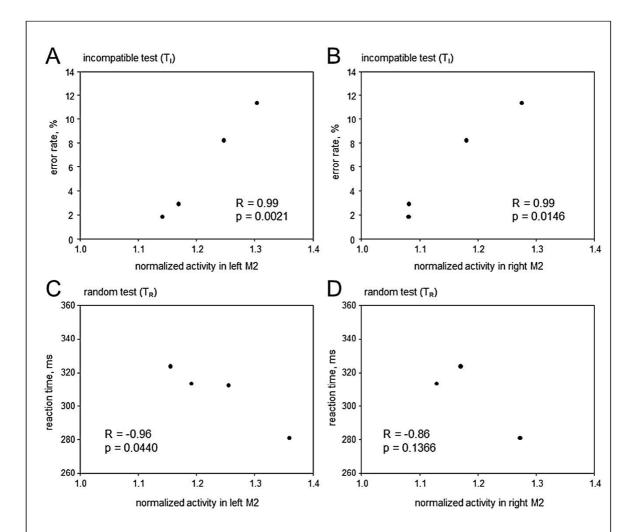
Table 2: VOI-analysis of relative metabolic activity during the Simon task (T_R) and control tasks (T_C and T_I).

Brain area	T_R vs. T_N	T_C vs. T_N	T_I vs. T_N	T_I vs. T_C
rostral anterior cingulate cortex (Cg1)[1]	l: 99.7 r: 98.6	l: 98.9 r: 99.5	l: 96.3 r: 102.3	l: 97.4 r: 102.9
rostral anterior cingulate cortex (Cg2)[1]	l: 104.8 r: 97.4	l: 98.4 r: 97.9	l: 98.3 r: 98.8	l: 99.9 r: 101.2
frontal association cortex (FrA)	l: 107.1 r: 108.9	l: 101.7 r: 102.4	l: 104.4 r: 100.9	l: 104.1 r: 103.5
orbitofrontal cortex (OFC)	l: 100.4 r: 100.4	l: 99.4 r: 100.1	l: 97.2 r: 103.3	l: 97.8 r: 103.1
prelimbic cortex (pL)	l: 100.7 r: <u>106.5</u>; **p=0.0006**	l: 97.0 r: 103.4	l: 96.9 r: 102.7	l: 99.9 r: 99.9
anterior motor cortex (aM1)[1]	l: 101.5 r: 102.1	l: 98.0 r: 94.6	l: 103.3 r: <u>94.8</u>; **p=0.0440**	l: 106.7 r: 100.5
posterior motor cortex (pM1)[2]	l: 98.9 r: 101.1	l: 98.1 r: 99.0	l: 102.3 r: 98.7	l: 104.3 r: 99.9
anterior premotor cortex (aM2)[1]	l: 103.3 r: 103.3	l: 98.6 r: 99.4	l: 101.1 r: 99.9	l: 102.8 r: 100.5
posterior premotor cortex (pM2)[2]	l: 96.4 r: 100.8	l: <u>92.9</u>; **p=0.0251** r: 101.2	l: 95.0 r: 101.0	l: 102.2 r: 100.0
posterior parietal cortex (PPC)	l: 96.5 r: 98.0	l: 97.0 r: 97.1	l: 98.5 r: 98.7	l: 101.6 r: 102.3
hippocampus (Hip)	l: 102.1 r: 101.5	l: 100.8 r: 100.3	l: 99.3 r: 103.0	l: 98.5 r: 102.7
amygdala (Amy)	l: 108.5 r: 98.0	l: 106.2 r: 97.8	l: 103.7 r: 104.0	l: 97.8 r: 106.9
dorsomedial striatum (mStr)	l: 100.2 r: 101.3	l: 101.4 r: 101.3	l: 97.4 r: <u>105.7</u>; **p=0.0041**	l: 96.1 r: 104.4
dorsolateral striatum (lStr)	l: 99.5 r: 100.2	l: 100.0 r: 97.9	l: 99.6 r: 102.9	l: 99.5 r: <u>105.1</u>; **p=0.0006**
lateral septum (LS)	l: 100.0 r: 101.0	l: 102.4 r: 96.3	l: 94.6 r: 94.3	l: 92.4 r: 97.9
medial septum plus diagonal band of Broca	l: 92.0 r: 94.0	l: 91.1 r: 93.4	l: 93.2 r: 93.2	l: 103.1 r: 100.7
caudoventral auditory cortex (Te3V)	l: 116.6 r: 118.5	l: 104.6 r: 106.7	l: <u>117.6</u>: **p=0.0180** r: 109.2	l: 111.6 r: 105.6

[1]: up to 0.6 mm rostral from Bregma ; [2]: from 0.6 mm rostral to 2.0 mm caudal from Bregma
Given values represent % regional metabolic activity in the PET sessions T_R (50% incompatible and 50% compatible trials in randomized order), T_C (compatible trials only), and T_I (incompatible trials only) with respect to T_N (neutral trials only; column 2-4) or T_C (column 5). Underlined are values significantly different from 100%, i.e. from values in T_N (column 2-4) or T_C (column 5). Shown are p-values uncorrected for multiple testing. l: left hemisphere; r: right hemisphere.

Here the search was for areas where metabolic activity correlated with RT and/or ER during T_I and T_R, but not during T_C and T_N. The only area satisfying this precondition was the anterior premotor cortex aM2 (**Figure 15**), where metabolic activity was correlated to ER during T_I (left aM2: R=0.99, p=0.0021; right aM2: R=0.99, p=0.0146) and inversely correlated to RT during T_R (left aM2: R=-0.96, p=0.0440; right aM2: R=-0.86, p=0.1366). This indicates that with a higher aM2 activity animals will respond faster during conflicting situations but at the cost of a higher error probability.

Figure 15: Correlative approach: Error rates and reaction times in Simon tasks containing incompatible trials (T_I and T_R), plotted over metabolic activity in the left (A, C) and right (B, D) anterior premotor cortex (aM2) of four animals. Significant correlations were found in A, B, and C.

4.1.4. Discussion

The purpose of the current study was twofold, namely 1) to identify and study brain regions involved in conflict processing in rodents, and 2) to demonstrate that behavioral FDG-PET is a suitable tool to analyze behavior on a metabolic level in a complex cognitive test like the Simon task.

Behavior

Our study confirmed the results of Courtière et al. (2007) that rats performing a Simon task displayed longer RTs and produced more errors in incompatible compared to compatible trials. This was statistically significant in the behavioral block, for both RTs and ERs, while in the PET block only ERs were significantly elevated in incompatible trials. Taken together, behavioral results indicate that a cognitive conflict was present in incompatible trials, which was sufficient to produce metabolic changes in tasks with mixed compatible and incompatible trials in the PET situation. However, it is not possible to disentangle metabolic responses to compatible and incompatible trials within one PET session, because FDG-PET is a cumulative method. The uptake measured one hour after FDG injection is heavily weighted to the total metabolic activity prevailing in the preceding interval when the animals were performing the task. We thus had to conduct control sessions in our PET study consisting of either 100 % compatible (T_C) or 100 % incompatible trials (T_I). In humans, the Simon effect is strongly reduced or even reversed in tasks that include 80 % or more incompatible trials ("practice effect"; Stürmer et al., 2002; Melara et al., 2008; Iani et al., 2009), raising doubt whether the present experimental design generated a sufficient level of conflict in T_I. However, a practice effect was not found in our rats, which displayed a significant Simon effect additionally in tasks with 80 % incompatible trials, leading to the assumption that in T_I a high conflict level was present as well. It was therefore surprising that RTs during T_C and T_N were not reduced compared to T_I. This may be explained by an order effect, since PET sessions were performed in the sequence T_N, T_C, T_I, T_R, on average six days apart with training in between. It may be possible that rats further improved their performance during the PET block, so that a learning-induced decrease of RTs compensated a conflict-induced increase of RTs in T_I.

Simon task versus resting state

According to numerous studies, energy consumption in a given brain area is mainly determined by input activity (for review see: Ritter and Villringer, 2002; Raichle and Mintun, 2006) whereby excitatory and inhibitory inputs cannot be distinguished. Elevated FDG uptake can therefore be interpreted as increased afferent activity, independent of spike rate of the neurons in this area. In order to analyze brain activity changes during the Simon task in general, we compared FDG uptake during one of the Simon task variants with resting state (R_S). As Simon task we chose the task with 100 % incompatible trials (T_I), because it was conducted by the most animals. We found a significant decrease of FDG uptake during the task in brain areas recently assigned to the default mode network (DMN) in rats (Lu et al., 2011), namely the prelimbic and cingulate cortex as well as the retrosplenial cortex. This corroborates human PET and fMRI studies demonstrating that the DMN contains areas with a high resting state activity, for example the posterior cingulate and anterior medial prefrontal cortices, which decrease their activity in attention-demanding cognitive tasks (Greicius et al., 2003; Raichle et al., 2001). In addition, FDG uptake decreased in the septum and the hypothalamus during T_I compared to R_S. This may reflect a stress-induced activation of these areas during R_S (Sung et al., 2009), because rats had not yet started training when the resting state scan took place and were therefore not as familiar with the operant chamber as during the Simon task scans.

Comparison of different Simon task variants

Brain areas involved in conflict processing are supposed to be activated when automatic and intentional route processing lead to different responses. These areas should therefore change their metabolic activity during PET sessions involving incompatible trials (i.e. during T_R and T_I, but not during T_C). As reference, a no-conflict control (T_N; bilateral stimulus presentation, therefore ambiguous spatial information) was used, and reported activity changes were relative to T_N, unless otherwise stated. We found a conflict related increase of FDG uptake in the prelimbic cortex. This is in line with other conflict studies on rats (de Wit et al., 2006; Haddon and Killcross, 2006), where the prelimbic cortex, together with the anterior part of the cingulate cortex, was essential for inhibiting the incorrect, competing response (Chudasama et al., 2003) and therefore seems to be involved in conflict resolution. FDG uptake additionally changed in the

right ventrolateral striatum (increase) and the left dorsocentral striatum (decrease) during T_R and T_I, indicating that these areas are related to conflict processing as well.

During the PET sessions involving compatible trials (T_R and T_C), possible automatic route facilitation may be reflected by increased FDG uptake. However, we found no spots of increased FDG uptake visible in T_R and T_C but not in T_I. This indicates that facilitation, if present at all, was not strong enough to increase metabolic demand significantly. We therefore hoped to increase metabolic contrast between maximal conflict associated with automatic route suppression and minimal conflict possibly associated with automatic route facilitation by comparing T_I to T_C rather than to T_N. A decrease of FDG uptake in T_I versus T_C could then be interpreted as decrease of excitatory activity of serially coupled automatic route areas. An increase of FDG uptake could reflect (1) an increase of inhibitory activity during automatic route suppression, or (2) an increase of excitatory activity reflecting conflict monitoring or resolution which was not strong enough to be visible in T_I or T_R versus T_N. We found an increased FDG uptake in T_I relative to T_C in the right Cg1 region of the anterior cingulate cortex, which was not visible in T_I relative to T_N. This most likely indicates a conflict monitoring function in analogy to findings in human studies implicating this region as the main conflict monitoring area (Botvinick et al., 1999; Peterson et al., 2002; Botvinick et al., 2004; Kerns, 2006). Furthermore, we found a significant decrease in FDG uptake in the right posterior motor cortex (pM1), suggesting that automatic route suppression may occur during the last stage of audiomotor integration. This is in line with an event-related potential study in humans, reporting "late" automatic route suppression in the frontolateral motor cortex (Stürmer and Leuthold, 2003). The increase of metabolic activity in the right dorsomedial striatum during T_I can as well be interpreted as evidence for conflict resolution via automatic route suppression, because this area is involved in behavioral inhibition (Eagle and Baunez, 2010). Furthermore, a small spot of decreased FDG uptake was observed during T_I (compared to both T_N and T_C) and T_R in the dorsocentral striatum. This region receives projections from M2 and posterior parietal cortex (PPC; Cheatwood et al., 2005), and the fact that metabolic activity was additionally found to be lower in the ipsilateral M2 during T_I, may therefore indicate blocking of the motor basal ganglia loop (Alexander and Crutcher, 1990; Joel and Weiner, 2000) during automatic route suppression.

So far we have not explained activity changes occurring in both T_I and T_C but not in the other tasks, such as the decrease of activity found in the left aM2 and right aM1. We preferred 100 % incompatible (T_I) or 100 % compatible trials (T_C) over a mixed design, because metabolic patterns should be determined by one type of trials only. However, one could argue that in the homogeneous tasks T_C and T_I it could be possible for the animal to ignore the relevant stimulus dimension (pitch) and choose the correct response side with the help of the irrelevant stimulus dimension (side). In T_C, the animal has to respond always towards the stimulus side, and in T_I always towards the opposite side. Because the rats have never encountered homogeneous tasks before, this new stimulus side - response side association, if established at all, must have been developed during the PET session itself. Especially in the purely incompatible T_I task, we would then expect RTs to decrease continuously during the session, if the new rule based on stimulus side is easier for the animal than that based on pitch information. Indeed, we found a steady decrease of RTs during the T_I session. If the nature of audiomotor integration changes during the task, metabolic activity is supposed to be decreased in pathways related to the pitch-based intentional processing route compared to the neutral task T_N, where animals have to rely solely on pitch information. In such a scenario, the decrease of activity found in the left aM2 and right aM1 may therefore be interpreted as linked to learning a new rule rather than caused by automatic route suppression. Although the correlative analysis (see below) supports the role of aM2 (but not aM1) in conflict processing, further studies should rather use a high conflict control with 10 % compatible trials. The conflict level would still be high enough to determine metabolic patterns, but the rats will have to use the tone pitch - response side association throughout the whole task. Interpreting metabolic patterns as associated with learning a new association would then be ruled out completely.

Correlation of imaging and behavior

Conflict processing may not always be accompanied by profound changes of neuronal activity, which alter the average glucose metabolism by more than 5 %. Instead, activity changes may differ between animals, due to individual variations in conflict-processing capacity, or may remain below our detection threshold. Thus, a correlative approach might provide further insights: Metabolic activity of a brain area involved in conflict processing may be correlated with behavioral parameters during T_I and/or T_R, but not during T_C and T_N. Consistent with this account, the activity of the left aM2 was

inversely correlated to RTs during T_R, indicating that fast animals had high premotor activities. Furthermore, these findings correspond well with data from humans suggesting that the premotor cortex is involved in conflict resolution (Egner et al., 2007).

4.1.5. Synopsis

So far we have discussed activation patterns emerging in the Simon task versus resting state and comparisons between different variants of the Simon task independently from each other. But how can we interpret the finding that an area of the DMN, the prelimbic cortex, shows reduced metabolic activity during the Simon task with 100 % conflict trials when compared to resting state, but increased activity when compared to the no-conflict Simon task control with 100 % neutral trials (i.e., metabolic activity of prelimbic cortex: $R_S > T_I > T_N$)? Reduction of resting activity in the DMN during cognitive tasks is currently interpreted as attenuation of the brain's self-referential (excitatory) activity as a means of more effectively focusing on a task (Sheline et al., 2009). Our finding can therefore be explained in three different ways: (1) Metabolic activity changes comprise mainly alterations of excitatory input. DMN activity during T_N is therefore more strongly attenuated than during T_I. (2) DMN activity is attenuated likewise during T_I and T_N, but the prelimbic cortex is engaged in conflict processing during T_I, leading to a higher net activity. (3) Metabolic activity changes reflect additionally inhibitory input activity. The prelimbic cortex may receive strong inhibitory input during T_I, but not during T_N, which would lead to an even more effective attenuation of resting activity during T_I. Further studies are needed to decide which alternative may account for the observed activation patterns.

Our results demonstrate that spatial response conflicts occur in rats just as in humans. Our imaging results show remarkable similarities to the pattern of activated regions reported during conflict processing in human fMRI studies. The rat motor cortex (M1) may be part of the automatic route or involved in its facilitation, while premotor (M2), prelimbic and ACC may play a role in conflict resolution and/or monitoring. Moreover, conflict-induced automatic route suppression presumably occurs in M2 and M1 as well as in the dorsocentral striatum (i.e., on the motor side of audiomotor integration).

4.2. Experiment 2: Electrophysiological correlates of a rat Simon task

4.2.1. Purpose

The data of Experiment 1 show interesting insights into cross species comparison of conflict processing, and opens novel opportunities to investigate the anatomical basis of conflict processing in a rodent model. We obtained solid evidence that the ACCin rats may be part of an automatic route processing and therefore may play a role in conflict resolution or monitoring as has been shown in humans. Because of these results and the prominent role of the human ACC in literature we wanted to further investigate the role of the ACC in conflict processing in the rat brain. Although PET imaging gives sufficient spatial resolution to detect brain regions which are metabolically involved during conflict processing, its temporal resolution is limited to approx. 30 min in behavioral PET. A satisfactory explanation of how the rat brain processes response conflicts would have to demonstrate when the conflict occurs and how is it managed by the brain. Therefore, in our next study we use the technique of recording event related (local) field potentials (ERP; LFP) which allow us to determine when exactly during a conflicting trial conflict processing takes place in the rat brain. Furthermore, ERPs allow selective averaging of different stimulus conditions (i.e. compatible and incompatible), whereas PET only allow block designs. Animal models of ERPshave been developed for several tasks in order to gain further understanding of the psychobiological processes which underlie these waveforms. In the present study we used awake, freely moving, male Lister Hooded rats with permanently implanted electrodes for our auditory Simon paradigm in order to receive further insight into the electrophysiology of conflict and error processing in the rat ACC.

In the first step of this study we recorded stimulus-locked conflict-related LFPs during the task and compared the waveforms of conflicting and non-conflicting trials. If the neuronal mechanisms of rat conflict processing were similar to human mechanisms we should find conflict dependent modulations. The timeframe 200 ms after stimulus onset should be considered especially, as in humans the N200 is known to be modulated in conflicting situations.

In the second step we compared withdrawal-locked waveforms of correctly conducted trials and trials with error responses. We expected to find error-related components or waveform modulations like those found in humans (for example the ERN) 50 ms and the error related positivity (Pe) 300 ms after response). To examine electrophysiological

correlates of rat error processing we needed a task condition which generates a sufficient number of errors for the analysis but not so many that the rats lose their motivation during the task because of decreased rewards. Therefore we implemented a randomized task like T_R in Experiment1 with 50% incompatible and 50% compatible trials for LFP recording.

In Experiment 1 we found evidence that unlike humans, response conflicts in rats do not depend on the rate of conflicting trials. To further validate these findings we tested a new group of animals for behavioral modulations depending on different conflict rates. In addition, we conducted a behavioral task including neutral trials, and compared these with conflict and non-conflict trials. While during Experiment 1, we compared a test with 100 % neutral trials (T_N) with other tests, we now wanted to analyze neutral trials occurring among compatible and incompatible trials within one test. We predicted that the performance in neutral trials would be between the performance in incompatible and compatible trials. If this were the case our auditory Simon task rat model could be used to further examine sequential modulations of the Simon effect and the existence of an ancillary monitoring system as proposed by Stürmer et al. 2002. These analyses however are beyond the scope of this thesis and will be reported but not further discussed.

Furthermore, if electrophysiological correlates of conflict and/or error processing diverge for different conflict rates, this could indicate conflict adaptation processes.

4.2.2. Material and Methods

Animals and surgery

Twenty male Lister Hooded rats (Charles River Laboratories, UK) were used for operant conditioning. At the start of the training rats weighed 250-270g, were housed in pairs under an inverted 12:12 h light-dark cycle and restricted to 15 g food per animal per day. Water was available ad libitum. The first nine animals which reached a performance level of 90% correct trials and a minimal of 50 trials in 15 min, were used for surgery and conducted the Simon experiment with LFP recordings. Six electrodes consisting of stainless steel wires insulated with polymide (0.005 inc, Plastics One, Roanoke, VA) were used for LFP recording. The electrode wire was held in place by a prefabricated Teflon block, with three wires for each hemisphere in each block. The electrodes were attached to the Teflon block by Cyanacrylat glue and attached to the

skull with dental acrylic cement. In addition, four screws placed at several locations on the skull (see **Figure 16**.) provided extra support.

Before surgery, rats received and injection of Rimadyl (0,008ml/100g) for analgesie purposes. During surgery rats were anaesthetized by inhalation of isoflurane and received an injection of 0.1 ml atropine i.m. in order to reduce salivary secretion. Body temperature was held constant at 37°C by a self-regulating heating pad throughout surgery. The head of the rat was fixed in a stereotactic frame (Kopf) and adjusted until a flat skull position was obtained.

The electrodes were inserted intracortically aiming at the ACC, particularly the Cg 1 area. Stereotaxic coordinates of the recording sites were derived from the atlas of Paxinos and Watson (2007) and were 2.7 mm, 2.0 mm and 1.0 mm anterior, 0.4 mm lateral and 2.4 mm ventral to bregma. The electrodes of 3.8 mm length were inserted 2.0 mm lateral and tilted 64° towards the midline to avoid the blood sinus covering the ACC. The reference and the ground were screw electrodes and were placed over the cerebellum (**Figure 16 C**). After surgery the animals were housed individually. After a one week recovery period rats underwent a retraining phase for another week before the start of LFP recordings. The Animal Ethics Committee of the Radboud University Nijmegen gave approval for the procedures used in this study.

Figure 16: Schematic diagram of the head stage setup for cable connection on the rat skull. The head stage consist of a round connector (grey circle) and two Teflon blocks (white blocks) holding the electrodes in place. The system allows measurement of 6 channels (three on each hemisphere) plus reference and ground. **A**) Coordinates of the Teflon blocks for electrode implantation, transverse/coronal plane. B) Transverse plane, position of the stainless steel electrode in the rat brain C) head stage setup and electrode distribution seen from above. Stainless steel electrodes were implanted with 3 on each hemisphere aiming at the area of Cg1. Crossed circles represent screws, numbers represent channel number.

Apparatus for behavioral testing and LFP recording

The experiment was performed in four identical open-top operant chambers in which LFP recording could be conducted in freely moving animals. Each box measured 25 x 51 x 70 cm and was placed inside a sound-attenuating chamber. Both side and the back wall were made of clear Plexiglas and equipped with a 2 cm inner lining at the top to prevent the animals from jumping out of the box. The floor was made of opaque plastic. As in Experiment 1 the front wall of the Skinner box was provided with a central nose poke unit and two trough-like pellet receptacles on either side, equipped with light

barriers for measuring reaction and MTs. Pellet receptacles were connected to a motor-driven pellet dispenser, delivering 45-mg precision pellets (Bioserv) as reward. Two high frequency loudspeakers were placed behind the pellet receptacles to present the sound stimuli. The acoustic stimuli consisted of two 300 ms pure tones with carrier frequencies of 10 kHz and 15 kHz, respectively, and a sound pressure level of 70 dB (+/-2 dB, rise/fall time 5 ms). Rats were connected to the recording system with a counter-balanced swivel system allowing the animals to move freely. The plug of the cable had to be connected to the round connector of the head stage and fixated with a screw which was caught in the center hole of the round connector. This whole system was house made in cooperation with the technical staff of the Donders Institute.

All experiments took place during the animals' dark phase under red light and the operant chambers were cleaned with 70% ethanol after each usage.

Experimental procedures

Operant conditioning

The operant conditioning was conducted in a similar fashion to the protocol used in Experiment 1 for the shaping of the Simon task (S.35; **Figure 7**).

Behavioral protocol

The actual experimental phase started two weeks after surgery, with one week retraining. During the retraining the animals conducted the last training step of the shaping procedure (sound discrimination training, phase 3; **Figure 7. C**). The nine animals each carried out four Simon tests with different ratios of conflicting trials. All were concomitant with electrophysiological recording:

(1) Simon task with 50 % incompatible and 50 % compatible trials in randomized order ($T_{50\%I}$).

(2) Simon task with 80% compatible and 20% incompatible trials ($T_{20\%I}$).

(3) Simon task with 20% compatible and 80% incompatible trials ($T_{80\%I}$).

(4) Simon task with 20% compatible, 20% incompatible and 60% neutral trials ($T_{60\%N}$).

The tests were performed on ten consecutive days, starting with $T_{60\%N}$ and increasing conflict probability on each following day. On day five, rats performed again a $T_{60\%N}$ and afterwards the $T_{50\%I}$ test for five consecutive days.

The RT (**Figure 6**) was taken as the time between the start of the auditory stimulus and the withdrawal from the nose poke unit, while MT was taken as the time from nose withdrawal until entrance of the pellet receptacle. The trial was terminated if the nose was withdrawn within the first 1.5 s, while trials with RT >1 s or <100 ms were discarded offline. ER was taken as the percentage of wrong choices, and was arc-sine square root transformed before statistical analysis. Effects of conflict probability and condition on RT and ER were estimated with two-way repeated measures ANOVA (see results for factorial design) and Holm-Sidak corrected post hoc comparison. Statistical computations were conducted with Sigma Plot (version 11.0, Systat Software, Inc.). Significance level (α-level) was set at $p<0.05$.

Order effect

The experiments were arranged with increasing probability of conflicting trials. One could argue that the order of tests through the different test days could have led to an order effect. It is known that human subjects practicing an incompatible spatial mapping before performing a Simon task can eliminate or even reverse the Simon effect (Tagliabue, 2000; Proctor & Lu, 1999). For this reason we started our experiment order with $T_{60\%N}$ including 20%I and 20%C trials and continued with tests with increasing conflict probability. After $T_{80\%I}$ we performed again a $T_{60\%N}$ test to reset any order effect. The high amount of neutral trials should lead to the "reset" and the low amount of conflicting and non-conflicting trials (20%) should sustain a training level for both kinds of conditions. Afterwards we started the $T_{50\%I}$ block based on an assumed practice baseline. We favored this order over a randomized approach, as the latter assumes that ordering and practice effects should be suppressed arithmetically, which needs a high number of tests, subjects and trials. Because we were limited in all these variables, we preferred the increasing complexity approach. Therefore we could not completely exclude the possibility of conflict adjustments over the test days.

In order to achieve an appropriate number of error trials for our EEG averages to analyse error processing, we had to repeat the 50% incompatible task on five consecutive test days and calculated averaged RT, MT and EP for each animal over all test days.

Even though we found no general interaction of factors test day and compatibility (see behavioural analysis page 66) for ER and MT we found a general interaction for RT. However we could not isolate the groups which differ from each other as the post hoc test did not demonstrate a difference between the tests on test day one to test day 3. As the Simon effect itself was present on all test days, we argued that this was a suitable technique to produce a sufficient amount of error trials for statistical analysis. However, we cannot completely exclude the possibility of an influence of conflict adjustments over the five days of testing.

LFP recording

During the last two days of the retraining, rats were connected to the recording cables in order to habituate them to the connecting procedure. The recording cables were able to rotate freely by means of a swivel, allowing the animals to move freely. Signals from the active electrodes were measured by differential amplifiers together with the signals from the cerebellum reference electrode. A potential difference was measured between the output signal of the differential amplifier and the signal from the ground electrode. The signal was filtered with high-pass and low-pass filters set at 0.1 Hz and 500 Hz, respectively, and sampled at 1024 Hz. The acquisition software WINDAQ/Pro (DATAQ Instruments, Akron, OH) was used for data acquisition.

Histology

After completion of the behavioral tests, rats were anaesthetized with an overdose of sodium pentobarbital (0.8 – 1.0 ml, i.p.) and perfused with saline followed by paraformaldehyde and potassium ferro cyanide (2%). Before perfusion a small electrolytic lesion was made at the tip of the electrodes. This left an iron deposit, which reacted with the potassium ferro cyanide leading to a blue staining at the recording site. After the brains were removed and post-fixed in paraformaldehyde, they were sectioned coronally (40 μm) with a cryostat. Slices containing the electrode track were stained with cresyl violet.

LFP analysis

Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) was used for pre-processing. For averaging, the EEG was segmented into epochs ranging from 200 ms

before until 800 ms after event onset. There were three different events: Stimulus, reaction (= withdrawal from nose poke unit) and response (= entering a pellet feeder). Baseline correction was conducted 100 ms before events.

Statistical LFP analyses

Single-datapoint-analyses:

Data preprocessing and statistical testing was done using custom routines in Matlab 7.10.0 (TheMathWorks, Natick, MA) Subsequently, the data was downsampled to 250 Hz (from the initial 1024 Hz). To compare between conditions (errors. vs. correct trials / incompatible vs. compatible trials), we computed two-sided within-subject t-tests. Due to the absence of strong a priori hypotheses about time-ranges of interest, individual t-tests were computed for each datapoint following the event of interest (Stimulus, Withdrawal, Response). The resulting array of p-values was corrected for false positive using the false-discovery-rate correction method (FDR, Benjamini, Krieger & Yakutieli, 2006).

Bin-analyses:

Data preprocessing and statistical testing was done using custom routines in MatLab 7.10.0 (The Math Works, Natick, MA). To compare between conditions (errors. vs. correct trials / incompatible vs. compatible trials), two-sided within-subject t-tests were computed on 50 ms (Brass et al. 2005) wide bins beginning at the onset of the critical events (Stimulus, Withdrawal, Response) to 800 ms following the event (16 bins). This was done because of the absence of a strong a priori hypothesis concerning the time-ranges of interest. The resulting array of p-values was corrected for false positive using the false-discovery-rate correction method (FDR, Benjamini, Krieger & Yakutieli, 2006).

4.2.3. RESULTS

Histology

Histologically verified electrode locations are displayed in Figure 17. Electrodes reaching the region of the Cg1 were subdivided into three different groups (anterior; center; posterior; **Figure 17**) on each hemisphere (left hemisphere; right hemisphere) depending on their location relative to bregma. Electrodes between 3.7 mm and 3.2 mm

anterior to bregma were labelled Cg1 anterior, between 3.0 mm and 2.5 mm Cg1 center, between 2.2 mm and 1.5 mm Cg1 posterior.

Figure 17 : Electrode positions. Numbers represent anterior coordinates in mm, relative to bregma. Atlas plates are adapted from Paxinos and Watson. Abbreviations: Cg1, Cg2: anterior cingulate cortex area 1 and 2; M2: premotor cortex; PrL: prelimbic cortex. Electrodes were subdivided into three different groups depending on transversal location in the area of Cg1 (frontal/grey, center/blue, posterior/green). Red square shows the electrode pool which detected the LFPs shown in the rat EEG results. A) horizontal section, B) transversal section, C) sagittal section, left hemisphere. D) example of a histological section with an atlas

Behavioral analysis

A two-way repeated measures ANOVA on RTs, with the factors compatibility and conflict probability, revealed a significant main effect of compatibility [$F(1,16) = 88.88$, $p<0.001$] (**Figure 18a**.). Additionally, a Holm-Sidak post hoc test demonstrated significantly higher RTs for incompatible trials compared to compatible trials for all different conflict probabilities (**Figure 18b**.). The difference in mean RTs between incompatible and compatible trials was 82 ms in $T_{20\%}$, 85 ms in $T_{50\%}$ and 77 ms in $T_{80\%}$. There was no significant interaction between the factors compatibility and conflict probability [$F(1,16) = 0.093$, $p = 0.911$].

Similar results were found for MT [$F(1,16) = 32.62$, $p<0.001$], although the differences between the mean values of incompatible and compatible trials was smaller: 32 ms for $T_{20\%}$, 15 ms for $T_{50\%}$, and 17 ms for $T_{80\%}$). Although there was no main effect of conflict probability [$F(1,16) = 2.858$, $p=0.087$], the post hoc test indicated significantly higher MTs for incompatible trials in $T_{20\%I}$ compared to $T_{50\%I}$ and $T_{80\%I}$.

A repeated measure ANOVA on ER, with the two factors compatibility and conflict probability showed a significant main effect of compatibility [$F(1,16) = 9.86$, $p=0.014$] as well. The subsequent Holm-Sidak post hoc test revealed significantly higher ERs for incompatible trials in $T_{20\%}$ (+13%) and $T_{50\%}$ (+7%).

Although there was only a trend for an interaction between compatibility and conflict probability [$F(1,16) = 3.42$, $p=0.058$], the post hoc test indicated significantly lower ERs in incompatible trials for $T_{80\%}$ (6% error) compared to $T_{20\%}$ (14% error).

Figure 18: **Reaction times (A) movement times (B) and error rates (C) during four auditory Simon tasks with different conflict probability (x axis), combined with EEG recording**. Error bars represent SEM.

The evaluation of $T_{60\%N}$ led to similar outcomes as the three other tasks. There was a significant main effect of compatibility on RT [$F_{(2,16)} = 18.93$, $p<0.001$], MT [$F_{(2,16)} = 25.46$, $p=<0.001$] and ERs [$F_{(2,16)} = 14.25$, $p<0.001$]. The post hoc comparison confirmed significantly higher RTs and ERs during incompatible trials compared to compatible and neutral trials. There was no observable difference between compatible and neutral trials.

In order to achieve an appropriate number of error trials to analyse error processing by EEG, we repeated $T_{50\%}$ on five consecutive test days. The recordings of two animals on test day four had to be discarded due to technical problems and one animal lost the headstage after test day four and could not participate on test day 5. Due to the missing

values on test day four and five comparison between test days could only be performed for the first three test days. A two-way repeated measures ANOVA with the factors test day and compatibility revealed a tendency of factor interaction in MT [F=(3.527) p = 0.054], no interaction for ER [F(1.689), p = 0.216], but a factor interaction for RT [F(1,16) = 4.125, p= 0.036]. However, the post hoc comparison demonstrated no significant differences for the different tests. As we could not statistically prove a difference between RTs in incompatible and compatible trials between test days, we calculated averaged RT, MT and EP for each animal as an average over all test days (**Figure 19**). Similarly to our analysis for the first test day (see above) a one-way repeated measures ANOVA with the single factor compatibility demonstrated significant main effects on RT [F(1,8) =111.22, p<0.001], MT [F(1,8) =53.096, p<0.001] and ER [F(1,8) =20.01, p<0,002] with significantly lower values in compatible trials.

Figure 19: reaction times (RT), movement times (MT) and error rates (EP) for five consecutive test days of T50% with 50% conflict probability, combined with EEG recording. Error bars represent SEM.

EEG analysis

The shown LFPs are derived from the left posterior electrode subdivision. This pool of electrodes was the most representative for the modulations mentioned later. It contained the most correctly placed electrodes and demonstrated the highest amplitudes. All other pools demonstrated similar results, with amplitude increases from anterior to posterior

sites. Possible lateralization effects and clear localization of the modulation were difficult to investigate due to technical limitations which will be discussed later.

In accordance with the literature of conflict processing, the N2 is related to stimulus processing. Therefore, we compared stimulus-locked LFPs from the ACC of incompatible and compatible correct trials. The LFPs consisted of several early components and a relatively large negative component starting at 150 ms lasting for several hundred milliseconds. T-tests demonstrate significant differences in amplitude between incompatible and compatible trials after stimulus onset between 150 and 450 ms and 520 and 750 ms (p<0.05 uncorrected; **Figure 20**). When analyzed for time-bins, compared to incompatible trials LFPs for compatible trials showed a significantly more negative-going amplitude of -138µV (measured from baseline, 0µV) for compatible trials in the time range of 200 ms and 500 ms and a more positive-going deflection with an amplitude of -118µV between 550 ms and 800 ms. The detected differences were highest on the posterior electrodes (posterior Cg1). We found these results not only with averaged data but also on a single subject level (see example **Figure 21**).

Figure 20: **Grand-averaged LFPs elicited after stimulus onset in compatible (red line) and incompatible (black line) trials in the rat experiment T50%**. Grand average was averaged over five test days and recorded from electrode pool left, posterior Cg1 (red square in **Figure 17**).The time-bins between 200 and 500 ms and 550 and 800 ms show significant differences in amplitude between incompatible and compatible trials. Black blocks show uncorrected p values, gray underlay shows significant time-bins. Pre-stimulus baseline corrected.

S: stimulus onset

Figure 21: **single subject examples for LFPs elicited after stimulus onset in compatible (red line) and incompatible (black line) trials in the rat experiment T50%**. Grand average was averaged over five test days and recorded from electrode pool left, posterior Cg1 (red square in Figure 17).The time frames between 200 and 450 ms and 520 and 750 ms show significant differences in amplitude between incompatible and compatible trials. Yellow underlay show FDR corrected p values. Pre-stimulus baseline corrected. Abbr. Com: compatible trials, Inc: incompatible trials
S: stimulus onset

The time range of the observed negative amplitude was located in the vicinity of the MT. Furthermore, to discriminate between conflict processes and motor or reward delay processes, we analyzed the measured signals in relation to MTs. For this reason we divided MTs in slow and fast categories for compatible and incompatible correct trials by a within-subject median split **(Figure 22).** We found the highest amplitude (-170µV, measured from baseline) in fast compatible trials and the smallest amplitude in slow incompatible trials (-120µV). Because we could not see discreet peaks it was difficult to determine the correct latency. However, by inspecting the amplitudes one could observe

that the amplitude for fast compatible responses reaches its maximum earlier in time, followed by the amplitude for slow compatible responses (-150µV), which was in turn followed by the amplitude for fast incompatible responses (-130µV) and finally by the amplitude for slow incompatible responses (-120µV).

Figure 22: Grand-averaged LFPs elicited after stimulus onset and split by movement times for slow and fast movement time responses of compatible and incompatible trials. Grand average was averaged over five test days and recorded from electrode pool left, central Cg1. Fast movement times in compatible trials demonstrate the most negative activation, whereas slow responses in incompatible trials demonstrate the less negative activation. Blocks indicate regions where the p-value of a t-test was below 0.05. Black blocks = comparison compatible trials; grey block = comparison incompatible trials (no significant difference); dark grey = comparison of the most deviating amplitudes.
S: stimulus onset

As the ERN is supposed to be in relation to the actual erroneous response, we compared withdrawal-locked LFPs of correct and error trials. The LFPs consisted of a relatively large negative, slow component starting at 200 ms. Especially in the time frame between 190 and 320 ms significant differences in amplitude between correct and error

trials were evident (**Figure 23**. ;p<0.05). After time-bin analyses, we found a significantly higher amplitude for error trials in the time range between 250 and 400 ms in comparison to correct trials.

Figure 23: Grand-averaged LFPs elicited after withdrawal from the nose poke unit in erroneous (red line) and correct (black line) response trials in rat experiment T50%. Grand average was averaged over five test days (**A**) and recorded from electrode pool left, posterior Cg1 (red square in Figure 17). The time-bins between 250 and 400 ms show significant differences in amplitude between correct and error trials. Pre-stimulus baseline corrected
Black blocks show uncorrected p values. Gray underlay shows significant time-bins.
W: withdrawal

4.2.4. Discussion

Behavior

As predicted and in line with our first study (Marx et al., 2012) we were able to replicate the results of Courtiere et al. (2007) by demonstrating that rats performing a Simon task displayed longer RTs and produced more errors in incompatible compared to compatible trials. This was statistically significant for both RTs and ERs. In general, the observed Simon effect in Experiment 2 was stronger than in Experiment 1, while overall response times (RT+MT) were longer in Experiment 1 than in Experiment 2. This is in keeping with several studies on humans which show that the Simon effect decreases for slower responses (e.g., see Hommel 1994; Van der Lubbe & Verleger, 2002). Surprisingly in the present study we detected additional significant differences in MT between compatible and incompatible trials, which means that the Simon effect persisted within the period of actual movement. There are two possible explanations: 1) variations in the setup (i.e. technical differences) or 2) animals in both experiments adopted different response strategies. Both possibilities will be discussed below. If the animals demonstrate adaptation on the level of different response strategies, it may be possible that they also adapt their behavior in response to the actual conflict (i.e on a macroscopic scale.) over several trials.

Technical discussion

The occurrence of the Simon effect in both RT and MT could be explained by the fact that the general setup was slightly different in Experiment 1 compared to Experiment 2. Both were conducted in different laboratories (Experiment 1 was conducted at the MPI for Neurological Research, Cologne; whereas Experiment 2 at the Donders Institute for Brain and Cognition, Radboud University, Nijmegen) with the same protocols, but different hardware. While the speakers in Experiment 1 were above the Pellet through, in Experiment 2 they were slightly lower and part of the pellet through. This changed the angle of sound presentation to the rats and might therefore have resulted in better discriminability. This would have led to greater Simon effects (Hommel, 1994) at least in horizontal stimulus response arrangements.

Moreover, the nose poke unit was built slightly differently. While in Experiment 1 the nose poke unit was bole shaped with a photobeam inside, the nose poke unit in

Experiment 2 was a plate with a hole and a photo beam attached on the outside. Therefore, the actual nose poke recording could have been slightly different.

Another explanation might be that we used rats from two different suppliers (Experiment 1: Janvier; Experiment 2: Charles River). In general, this should not have an impact. It is common practice to use different suppliers in different studies as it is assumed that all animals of the same strain are nearly identical. Nevertheless, some researchers have demonstrated differences between the same rat strains from different suppliers (Palm et al. 2011). Experiments conducted in the MPI for neurological research (unpublished work) for example showed that animals of different supply origin have diverse hearing thresholds. Even though we cannot prove that this is the reason for the different observations, it remains an explanation that should be kept in mind.

It is worth noting that these set up differences had no general effect on the Simon task itself. These differences should only lead to reduced RTs without having an impact on the Simon effect, which is exactly what we observed. The Simon effect was present and detectable in both experiments. However, the fact that the Simon effect during Experiment 2 was observable in MT as well needs further discussion.

Regular choice tasks commonly used to produce the Simon effect are dissimilar to the experimental procedure we used in our task. Typically only the response time of a reaction is measured (i.e. RT+MT). This was not the case in our task, as here it was crucial that the head of the rat remains in place to ensure that all auditory stimuli reach the ear at the same angle and with the same interaural time difference. In order to accomplish this, we used a nose poke system. The advantages of this system, in comparison to others (e.g. lever system used by Courtiere) is the constant position of the rat, with particular focus on the position of the head which remains stable over all trials. This results in a more precise transition from the end of RT to the start of MT due to movement characteristics. A nose poke only requires head movement, whereas for a lever press, whole body movement is needed. Additionally, Courtiere demonstrates in his studies that the lever force has an effect on RT. With our nose poke approach we circumvent these problems. These disadvantages could have been the reason why the MTs, though measured in the study of Courtiere and colleagues, were not reported or discussed. Instead they focused solely on RT. This is regrettable if one considers results from other human studies, which reveal that separation of response times in a Simon

task leads to further insights into the chain of response processing and strategies (Hieätanen and Räma, 1995). In order to measure different time points precisely, which gives us the ability to differentiate between close time periods (i. e. separation of RT and MT), we favor the nose poke approach. The separation of reaction or decision and the motor part of the response gives us the opportunity to observe each process separately. In this manner it is possible to i) part the different correlated potentials from one another by time locking the ERPs on different time points and ii) have a closer look at response adaptations such as various strategies. Both will be discussed in the following paragraphs.

Response strategies

A more likely explanation for the observation that in Experiment 1 the Simon effect was only demonstrated for RTs, whereas in Experiment 2 it was evident for both RT and MT, apart from the technical variations, is that the rats adopted different response strategies in the two experiments.

Human studies have shown that whether facilitation or interference components show up in RT or in MT depends on the response strategy adopted by the subject (Rubici et al. 2000). One strategy is starting the movement and reaching for the target location as soon as the stimulus appears (fast response, R_{fast}). If this is the case, the Simon effect should be more pronounced in MT. The other strategy is to wait with the movement until the decision is completely programmed, in which case the Simon effect should be stronger in RT (slow response, R_{slow}). If error commission is of little consequence, then a fast response (R_{fast}) is a good strategy, but if the likelihood of punishment (or reward omission) increases, a change to more cautious strategies to avoid unpleasant consequences is of advantage.

Which plan is chosen depends on individual variability and on the instruction. Human subjects can be instructed to follow one or the other approach. In our case, the animals in Experiment 1 seemed to use the R_{slow} while animals in Experiment 2 used the R_{fast} strategy. As rats cannot be instructed to withhold their response until the decision is made, and have a huge motivation to receive a reward, intentionally the R_{fast} strategy should be preferred (i.e. speed over accuracy). This is the case for Experiment 2. On the other hand, animals in the first experiment committed more errors in general and therefore seemed to choose the safer strategy R_{slow} (i.e. accuracy over speed). This

could be an explanation for the difference in findings but has no effect on the general validity of either experiment. In both strategies the Simon effect still occurs at the response selection stage which is consistent with the dual route theory.

Nevertheless, the type of strategy used has to be determined as it will be important for our further ERP study. Depending on the implemented approach, conflict or error related components should show up at different time points during the ERP.

As it seems that rats show different adaptations in strategy on the level of responses (i.e. microscopic scale) it could furthermore be possible that they additionally adapt their behavior for long term strategy changes in response to the level of conflict (Macro-adjustments of behavior; Ridderinkhof et al., 2002) which will be discussed later.

In Experiment 1 we found no effect of conflict probability. In contrast, results of the second experiment showed decreased MTs in incompatible trials and decreased ERs if conflicting trials were frequent, which indicates a tendency to adapt to conflicting trials.

Conflict frequency

As we found different response strategies in both experiments, we additionally tested for long term strategy changes in response (Macro-adjustments of behavior) to changes in the task setting (i.e. increased or decreased conflict probability). In Experiment 1 we needed this information to be sure that we had a sufficiently high level of conflict in our PET experiment to detect metabolic changes. There we found no effect of conflict probability, which is contradictory to findings in human literature. In humans, the Simon effect is strongly reduced or even reversed in tasks that include 80 % or more incompatible trials ("practice effect"; Stürmer et al., 2002; Melara et al., 2008; Iani et al., 2009). To further corroborate this observation, we again tested for behavioral adjustments to conflict probability in the present study. In Experiment 2 the observed Simon effect in RT and ER was highest in $T_{20\%I}$, but similarly to Experiment 1 we found no general effect of conflict frequency. It seems, in line with what had been proposed by Courtiere, rats are not able to reduce the activation of the automatic response towards the stimulus (Courtiere, et al. 2008), even if the incompatible condition is frequent.

In Experiment 2 we found no general effect of conflict frequency either. However, the ANOVA on ERs just missed significance for factor conflict frequency (p=0.058) and the post hoc test for MT comparison demonstrated longer MTs for incompatible trials

during the low conflict test $T_{20\%I}$. These findings at least demonstrate a tendency towards conflict adjustments dependent on conflict frequency in rodents.

If this effect had been found for RTs as well, this could have been attributed to the strategic use of irrelevant stimulus information, which means that with increasing conflict probability the subject may have tended to suppress location based automatic route activation more strongly than for probability levels below 50%. Furthermore, attentional divergence effects would more likely be manifested in RT, although this appeared not to be the case in our experiments. Instead, conflict related adjustments were only observable in MTs of incompatible trials and in error proportion. This suggests that there are conflict adjustments or error avoidance processes that take place at a later stage during response processing. Therefore it is possible that processes which produce performance facilitation have to occur in the stage preceding the motor initiation and prior to the processes that cause response interference. This is in accordance with a theory which was established by Hietanen and Räma (Hietanen & Rämä, 1995) for a visual Simon task setup.

Taken together, the behavioral differences between Experiment 1 and 2 could be explained by means of different response strategies.

An additional explanation could be the general performance level which was lower in Experiment 1 during training (85%) and before they entered the test phase of the experiment. Animals in the second experiment were able to reach a level of 90% correct trials during training and entered the test phase after a 90% performance level. Potentially the animals in the first experiment could have had bigger problems overcoming the general Simon effect which leaves no cognitive capacity for incorporating the general conflict frequency. In contrast, animals in Experiment 2 were highly trained and committed less errors. Consequently further improvement of behavior could only be accomplished by behavioral adjustments.

Electrophysiology

Our PET study gave evidence of comparable networks in conflict and error processing including the ACC. Furthermore, regarding the analogies of the rat ACC connectivity and function which were described in the introduction, we predicted comparable electrophysiological correlates in error and conflict processing. We time-locked the ERPs to different response time points to separate the different correlated potentials

from one another. We time-locked the conflict related potentials to the stimulus, as it is thought that a conflict potential should precede the actual reaction. We did not expect the conflict related deflection to have the same polarity (positive/negative) as observed in human studies. Due to the fact that we measured intracortical LFPs in our rat study the polarity of the deflection is dependent on the layer in which the electrode is located and its depth. In terms of human EEG, which is derived from the scull, conflict related negative potentials like the discussed N2 (see: Introduction) are usually demonstrated. Thus we predicted a deflection in the time range of the N2 in particular.

While the conflict related potential should precede the reaction (i.e. the withdrawal in our setup) the error related potential should closely follow it. For this reason we locked the error related potentials to the time point of withdrawal. With the aforementioned constraints we expected a deflection in the time range of the ERN and/ or the P_e. These time locks will be discussed below.

Electrophysiology of conflict

The major finding from our conflict and non-conflict LFP comparison is an enhancement of a large negative deflection (which will be referred to as D_n) and a late positive deflection (D_p) in response to compatible trials compared to incompatible trials. Given that both components 1) varied with the level of conflict (compatible: non-conflict; incompatible: conflict), 2) have their source within the ACC or at least in its proximity due to the coverage of the LFP recording and 3) showed amplitudes in the time range between 150 ms and 450 ms for the D_n and between 520 ms and 750 ms for the D_p, the D_n might be comparable to the human N2 and D_p to the late positive component (LPC). The N2 is thought to be generated by the ACC (Carter and van Veen ,2007) and might reflect the resolution of a conflict between competing responses under uncertain conditions (Bland and Schaefer, 2011). ERP studies in literature demonstrated increased negative amplitudes (N2) for incompatible trials compared to compatible trials in conflict tasks like the Stroop (Liotti et al., 2000) and Flanker tasks. However, the negative component (D_n) we found was even more negative for compatible than for incompatible trials while the LPC was more positive for compatible trials. We may thus conclude that in our study positive amplitude is correlated to higher conflict.

A further explanation could be that the D_n reflects more motor or even premotor and transient activations, possibly correlated to the certainty of a correctly given response or rather, to a reward prediction. Results from our MT split calculation support this

account. Higher conflict and longer MTs, which indicate a higher level of uncertainty and predict a lower reward probability, or even high error likelihood, display lower negative amplitude. In contrast, low conflict with shorter MTs, indicating a high level of certainty and reward probability and a small error likelihood, display the highest negative amplitude. This could be confounded by the factor of reward delay as we found the highest MTs in the incompatible slow condition. A plausible assumption for our observations could be that higher certainty leads to a stronger activation in the motor system due to the increased invigoration. Another possible alternative explanation for Dn and conflict related activity has been put forward by Brown and Braver (Brown and Braver, 2005) who proposed that the ACC is a predictor of error likelihood and therefore a more downstream recipient of the conflict signal than the upstream conflict monitor. In this case, the conflict itself is detected and resolved upstream to the recorded signal. This implies that the decreased D_n in incompatible trials could demonstrate the consequences of a preceding (motor) inhibition of the automatic route, originated in areas of the premotor cortex and afterwards processed in the anterior cingulate cortex. The increased D_n in compatible trials then demonstrates a preceding activation/facilitation of the automatic route.

The conflict monitoring account of the ACC certainly cannot be ruled out in favor of the error likelihood account. Unfortunately, response conflict and error likelihood as well as reward probability and level of uncertainty are typically confounded variables, making it difficult to distinguish the theories empirically. Moreover, it is possible that we obtained these results because the LFP may display the general activity insufficiently. This means that a higher negativity does not necessarily mean more activity. Additionally, less negative variations from baseline activation can be correlated to increased conflict related activity. This possibility cannot be ruled out at this time.

However it may be concluded that there is a conflict/certainty/error predicting signal assessable in the proximity of the posterior part of the Cg1 area which reflects compatibility and certainty effects.

This is highly remarkable, as other studies on primates (macaques) could not demonstrate conflict-related signals carried by LFPs in the ACC (Emeric et al., 2008). Only error and feedback related potentials in the performance monitoring field potentials of the macaque ACC were found (Gemba et al., 1986, Emeric et al., 2008).

Electrophysiology of errors

A general problem of studying errors in an animal model is the fine line between accumulating enough errors for analysis and having the animal motivated enough to carry on with the task. This makes it difficult to receive an adequate amount of averages for error trials to display a clear LFP. This presents a statistical challenge, and is one of the reasons why our results for the error correct comparison do not reach significance. From what we know from previous literature we would have expected to find a ERN potential after the reaction in an erroneous response and a following Pe. Both components are thought to follow the reaction rather than to precede it. This is why we looked for withdrawal-related components, as the withdrawal represents the first time point of response selection. Unfortunately, we could not find an ERN like deflection. What we did observe was a negative sloping curve containing some peaks after withdrawal.

As the negative slope appeared not to be modulated by response type, we tested whether it could have been an effect of baseline correction. As the stimulus locked potentials included powerful activity in the time frame before withdrawal, this could have had an effect on the baseline correction. Every kind of baseline correction has different advantages and/or disadvantages. Pre-stimulus baselines, for example, seem to be dependent on certain factors like age (Falkenstein, 2000) and pre-response baselines are dependent on RT latencies. However, both kinds of baseline corrected ERPs usually differ between correct and erroneous responses (Morgan,1992; Hohnsbein,1998). Therefore we tried two ways of baseline correction: One 'normal' correction 100 ms before withdrawal and one baseline correction 50 ms before stimulus. This has however not led to crucial differences. In both cases one positive peak was more positive for error than for correct trials (time frame 200 ms to 280 ms), or at least showed a tendency to be more positive, as the statistical analyses confirm significant differences for the time-bins from 250 ms to 400 ms. Whether this component is Pe- like or not is difficult to determine because the Pe itself is insufficiently described in human literature.

Although the aforementioned LFP study with primates (Emeric et al., 2008) found no evidence of conflict-related LFPs in the ACC of primates, they were able to demonstrate error- and feedback-related potentials in a saccade countermanding task. The error-related positivity in the grand average began at 316 ms and peaked 424 ms after the

onset of the error saccade. Therefore the LFP technique appears to be an adequate measure of error-related potentials.

Two obvious reasons why we did not detect ERN like potentials are that error processing could be different in humans and primates in comparison to rats, or that it is task dependent. The second possibility is favored by the present investigation because the Simon effect was present in both reaction and MT. In comparison to a saccade countermand task, in our task the error processing appears to have occurred somewhere in between the reaction and MT. Hence, the ERN could have been blurred over these time points. The Pe on the other hand is a long lasting deflection which may therefore have persisted over time. These assumptions could be addressed by conducting a comparative Simon task in humans or primates (this will be addressed in Experiment 3). In conclusion, although we could not find significant error-related potentials in the LFP measured in the rat ACC, it is interesting that we found a tendency for Pe deflection. This makes the rat model a promising alternative to primate studies to investigate error related potentials.

Comparison to Imaging

With regards to Experiment 1, we cannot definitively reject or support either the error likelihood or the conflict monitoring account, as both receive support from our imaging results.

The theory of error likelihood, which maintains that the ACC is a more downstream recipient of the conflict signal is supported by the comparison of T_I and T_C in the PET experiment. We found a significant decrease in FDG uptake in the posterior motor cortex (pM1), suggesting that automatic route suppression may occur during the last stage of audio motor integration. This is in line with the conclusion we draw from our D_n. The D_n was a rather late component with a maximum at about 300 ms and gave evidence of a preceding (motor) inhibition/suppression of the automatic route in incompatible trials. It is possible that the measured component was not generated by the ACC were we had the tip of our electrodes. As LFPs have a range of approximately 3mm it is also possible that the component was generated by the anatomically very close posterior M1. This is especially supported by the fact that the component had its maximum at posterior ACC electrodes.

In favor for the conflict monitoring theory we argued that a higher negativity does not necessarily mean more activity in itself. Furthermore, less negative variations from

baseline activation could also be correlated with increased conflict related activity. This goes in line with the observations of our comparison of the DMN activity which demonstrated reduced activity during the cognitive task in contrast to baseline DMN activity. Particularly striking was a reduced activity in the ACC during the conflict task T_I in comparison to the non-conflict control task T_N. We propose three possible explanations:

(1) Metabolic activity changes comprise mainly alterations of excitatory input.

(2) DMN activity is attenuated likewise during T_I and T_N, but the prelimbic cortex is engaged in conflict processing, leading to a higher net activity.

(3) Metabolic activity changes reflect inhibitory input activity.

Nevertheless, all these explanations are applicable to our findings of decreased negative amplitude in incompatible trials and increased positive amplitude in compatible trials compared to baseline in the LFPs of the ACC if one assumes that the "baseline" in the ACC represents a general level of activity as a part of the DMN. Using the LFP technique we were able to measure compatible and incompatible activations at the same time point, albeit limited to certain areas (i.e. the ACC) and their proximity. If we take both these points into account we can raise three possible extended assumptions:

1. Activity changes in ACC comprise mainly alterations of excitatory input. Baseline activity in the ACC is weaker attenuated during incompatible and even less attenuated during compatible trials, which leads to a faster motor execution of the response in compatible trials.

2. The net activity is generally attenuated, but the ACC is engaged in conflict processing. A smaller deviation from baseline therefore demonstrates increased conflict processing.

3. Strong inhibitory activation during conflict in the ACC leads to suppression in incompatible but not compatible trials.

Considering these assumptions together it cannot be concluded with certainty whether the ACC is part of bottom up or top down control but it is quite clear that the ACC is part of conflict processing.

Further studies are needed to decide which alternative may account for the observed differences.

Comparison to human and primate literature

Unfortunately the results were more difficult to compare with human literature than expected (Yeung & Nieuwenhuis, 2009, Kopp et al., 1996). One factor was that the use of auditory stimuli is less common and less investigated in human literature (Wascher 2001, Leuthold & Schröter, 2006). Most studies use visual stimuli, although historically first tasks used to investigate the Simon task were auditory (Simon & Small, 1969; Simon & Rudell, 1967). Another point which could have resulted in differences between the species is the separation of the decision response and the motor response. The rats gave their first response by withdrawing their snout from the nose poke unit (withdrawal; decision) and their second response by moving to and entering the Pellet trough (motor response). In human Simon task studies the participants merely press a button for the response. Although these factors could have been amended for our rat study this would have led to various disadvantages. Rats are crepuscular and therefore less visual animals (Lashley, 1938;Wiesenfeld & Branchek, 1976; Artal et al, 1998). As such using visual stimuli may have led to higher variability in behavior due to the limited capabilities of the rat visual system.

The difficulties encountered during our attempt to reduce the Simon task to a simple model shows how advanced current discussions are in relation to the fundamental mechanisms that underlie this effect. Tracing it back to a straightforward system that mirrors the fundamental mechanisms involved in these cognitive tasks would be desirable. Without such model and without knowing the anatomical basis of the Simon effect it is difficult and imprudent to make statements about the mechanisms that form its basis. An easier comparable model which could also be extended to further species, such as pigeons, could be invaluable in decoding and understanding this phenomenon.

4.2.5. Synopsis

The results of this experiment would not have been possible without the high temporal resolution provided by the ERP technique, indicating exact temporal course and the highly dynamic nature of conflict and error monitoring and the possibility of selective averaging of different stimulus types within the same experimental block (incompatible, compatible). We found that rats seem to adopt different response strategies depending on their general performance level. Animals mostly used a speed over accuracy trade off which led to a Simon effect in RT and MT. Furthermore, they demonstrated a tendency for behavioral adjustments to the actual level of conflict

which was only detectable in MTs of incompatible trials and ER, which suggests that suppression of response execution processes (i.e. error avoidance) take place at a later stage of conflict processing (during MT). The measured LFP supported this, as we found a negative deflection (D_n), a potential modulated by conflict and a Pe-like potential modulated by error commitment. This demonstrated that there appeared to have been modulations in the LFPs recorded in the region or at least in the vicinity of the rat anterior cingulate cortex. The D_n was more negative for compatible trials, or rather compatible fast trials and less negative for incompatible trials which could indicate a neurophysiologic correlate to automatic route suppression for uncertain, error-likely trials. Therefore these data provide new information on the time course of the Simon task and are in line with previous PET findings where the rat ACCwas found to be related to conflict processing. In contrast, comparable studies on primates (macaques) were unable to demonstrate conflict-related signals carried by LFPs in the ACC (Emeric et al., 2008). This speaks in favor of the rat as an animal model for conflict related research compared to primate models.

Taken together, based on the present findings we were unable to conclude with certainty whether the rat ACC is part of bottom up or top down control. Further studies are needed to determine which alternative may account for the observed differences. However, it appears to be clear that the rat ACC forms part of conflict and possibly even of error related processing.

4.3. Experiment 3: A Cross-Species Simon-task:
Comparing conflict and error processing in rats and humans

"The difference in mind between man and the higher animals, great as it is, certainly is one of degree and not of kind."

The Descent of Man (Charles Darwin, 1871)

4.3.1. Purpose

Unfortunately, there is often little correspondence between human and animal studies. To assess whether a rat study is a good model for human behavior, animal and human studies should be directly comparable. To facilitate these comparisons the same dependent variables for both humans and animals should be manipulated. On this account this study used a variation of an auditory Simon task as a tool to investigate monitoring related electrophysiological correlates from a cross-species (rats-humans) point of view.

An auditory Simon task was used with four different stimulus frequencies (two low, two high). Compared to other human studies the present experiment used a setup which was designed to be as similar as possible to our rodent study in Experiment 2. Therefore a different type of response time measurement was used. Subjects had to keep a button pressed until the stimulus occurred, then had to release (RT) and to reach another button (MT). In this way we separated the measurement of response time into reaction and MT. For the sake of similarity, we additionally forced human subjects to use a speed over accuracy strategy as was employed in Experiment 2 with the rats. The speed over accuracy instruction and introducing four different stimuli were designed to lead to an accumulation of error trials to study error processing. In the rat study we used local field recordings during the task, to measure conflict- and error-related potentials. In this human study we performed electroencephalogram (EEG) recordings to serve the same purpose.

Additionally, with the different response time measurement we wanted to have a closer look at conflict frequencies adjustments, the effect of response strategy on the Simon effect and electrophysiological correlates separated in different steps to monitor

processing. We expected from these analyses to complete our results from Experiment 2. Similar results for response strategy would support our assumption of different types of response strategy. Adaptation to conflict frequency and similar results in our ERPs would argue for a special status of our modified auditory Simon task in comparison to other conflict task. Differences could give evidence to differences between the species.

Besides the cross species account, what makes it even more interesting is that most prior experiments that presented data on the physiological correlates of conflict processing in humans almost exclusively reported from the visual modality. It is assumed that humans possess a highly efficient visuospatial network which promotes reaching for a response. However, the Simon effect was initially reported for the auditory modality (Simon & Rudell, 1967). Wascher and colleagues (Wascher et al. 2001) were amongst the first to develop the theory that different processes underlie stimulus-response correspondence in the visual compared to the auditory modality instead of a supramodal connection. However, their study was also aimed at investigating the processes using visual stimuli. It is true that humans have a highly developed visual system, but the auditory system is less diverse between species. In all species the auditory dimension is tridimensional and sound localization is nearly similar simple and following the same principles, whereas the visual dimension is inherently divers, already due to the fact of different eye angle (for rats see: Block, 1969). As such, it could possibly better address basic, and species general processes of conflict processing.

Furthermore, by using the auditory modality for the task this study could additionally make an important contribution to the understanding of the physiological processes underling conflict processing in this modality.

4.3.2. Material and Methods

Participants

Nineteen neurologically and psychiatrically healthy volunteers (recruited from the institute's database) with normal or corrected-to-normal vision participated in the electroencephalogram (EEG) experiment. All participants were male, aged between 23 and 29 years and according to the Edinburgh Handedness Inventory (Oldfield, 1971) right handed. Participants gave written informed consent and received a payment of 10 Euros per hour of participation.

Auditory Simon task

Each trial started with a grey square which appeared in the centre of the screen. Upon seeing the square participants were required to continuously press a button in the centre of a response box. At button press the colour of the cross changed from grey to red and after a period of time between 1-1.5s the playback of one of four acoustic stimuli was triggered for 300 ms. According to tone pitch, the participant, after lifting his/her finger from the center button, had to press a response button on the reward side. (For 261.6 Hz or 329.6 Hz – button on the left, 1046.5 Hz or 1318.5 Hz – button on the right (**Figure 24**). 500 ms after pressing the response button participants received a feedback smiley depending on whether they had made a correct (green smiley) or wrong (red frowny) choice. After every 20 trials, participants received a "speed up!" feedback depending on their number of errors, reminding them of the "speed over accuracy rule". If they made one or more errors there was no "speed up!" feedback. 1400 trials were distributed in five blocks. Each block consisted of a different test which had different relative frequencies of incompatible trials: $T_{20\%I}$: 20% incompatible trials, $T_{50\%I}$: 50% incompatible trials, $T_{80\%I}$: 80% incompatible trials, $T_{60\%N}$: 20% incompatible, 20% compatible and 60% bilateral stimulus presentation. $T_{20\%I}$, $T_{80\%I}$ and $T_{60\%N}$ consisted of one block comprised of 300 trials. $T_{50\%I}$ consisted of two consecutive blocks with 250 trials each. All tests, apart from $T_{60\%N}$ were administered in pseudo-randomized order, counterbalanced across participants. $T_{60\%N}$ was always the last test and could be declined.

Participants were instructed to press every button with the right index finger and to give speed priority over accuracy. Correcting a response was not possible. After each block participants were able to take a break and to start the next block by a button press.

Figure 24: A) Stimulus layout and trial timing schematic. **B) Setup for the human Simon task.** Red arrow indicates button press or button release. Button press for 1-1.5s triggered the playback of a single acoustic stimulus from one of the speakers. Reaction time (RT) was measured from stimulus onset to button release (withdrawal). Movement time (MT) was measured from button release (red arrow up) to response-button press (red arrow down).

Behavioral analysis

The RT (**Figure 24 B**) was taken as the time between the start of the auditory stimulus and release of the response button, while MT was measured from button release to response-button press. ER was taken as the percentage of wrong choices, and was arc-sine square root transformed before statistical analysis. Effects of conflict probability and condition on RT and ER were estimated with two-way repeated measures ANOVA (see results for factorial design) and Holm-Sidak corrected post hoc comparison. Statistical computations were conducted with Sigma Plot (version 11.0, Systat Software, Inc.). Significance level (α-level) was set at $p<0.05$.

ERP Data Collection

The derived ERPs of experiment 2 were local field potentials, which were taken from the inside of the rat cortex. In contrast, the ERP of the EEG was derived from the scull surface of the subject. Therefore, the electrodes were mounted in an elastic cap (Easycap, Herrsching, Germany), containing 64 Ag/AgCl sintered electrodes plus reference and ground. The ground electrode was positioned at F2, which is central on the top of the head. This technique presumes that the derived potentials are summated over many parallel, simultaneously activated neurons. This is the reason why the EEG cannot be derived from the rat scull as it is too thick to allow potentials to pass through the bone. Additionally, intracortical measuring of ERPs is preferable in general for several reasons such as better signal to noise ratio. Obviously such a procedure is not advisable in a human study.

The experiment was conducted in a dimly lit, electrically and acoustically shielded chamber. For later correction of eye artifacts, evoked by the muscles around the eyes an electrooculogramm (EOG) measurement was taken.

The vertical electrooculogram (vEOG) was recorded from electrodes located above and below the left eye. The horizontal EOG (hEOG) was collected from electrodes positioned at the outer canthus of each eye. The general electrode impedance was kept below 5 kΩ. Potentials were referenced online on electrode CPz and later re-referenced off-line to the average activity at both Mastoids. The EEG was A-D converted with a 16-bit resolution at a sampling rate of 1000 Hz using BrainAmp MR plus amplifiers (Brain Products, Gilching, Germany).

Data analyses

Brain Vision Analyzer (Brain Products GmbH, Munich, Germany) was used for pre-processing. For averaging, the EEG was segmented into epochs ranging from 200 ms before until 800 ms after event onset. There were three different events: Stimulus, reaction and response. Baseline correction was conducted 100 ms before events.

ERP Data Statistics

Single-datapoint-analyses:

Data preprocessing and statistical testing was done using custom routines in MATLAB 7.10.0 (The Math Works, Natick, MA). Additional routines from the MATLAB toolbox (Delorme & Makeig, 2004) were used for the preprocessing of the human scalp EEG data. After import into MATLAB, the data was filtered using a .8 Hz high-pass and 40 Hz low-pass filter (two-way least-squares finite impulse response), then re-referenced to common average. For initial preprocessing, epochs were cut out ranging from 200 ms before the stimulus to 300 ms after the stimulus for each trial. Afterwards, following the recommendations from Delorme, Sejnowski & Makeig (2007) a combination of automated and visual rejection of non-stereotypical artifacts (gross movement and muscle artifacts) was performed. To this end, trials with a very improbable value distribution (> |5 SD| above the average distribution) were rejected from the dataset. Subsequently, an temporal infomax independent component analysis (ICA) was computed to separate stereotypical artifacts (eyeblinks, saccades, electrode artifcats, neck muscle artifacts, EKG) from the EEG signal. These artifacts were identified using automated criteria (see Wessel et al., 2012 for details) and eliminated by inverse matrix multiplication. The cleaned up datasets were then used for further averaging and statistical testing. Subsequently, the data was downsampled to 250 Hz (from the initial 1024 Hz). To compare between conditions (errors. vs. correct trials / incompatible vs. compatible trials), we computed two-sided within-subject t-tests. Due to the absence of strong a priori hypotheses about time-ranges of interest, individual t-tests were computed for each datapoint following the event of interest (Stimulus, Withdrawal, Response). The resulting array of p-values was corrected for false positive using the false-discovery-rate correction method (FDR, Benjamini, Krieger & Yakutieli, 2006).
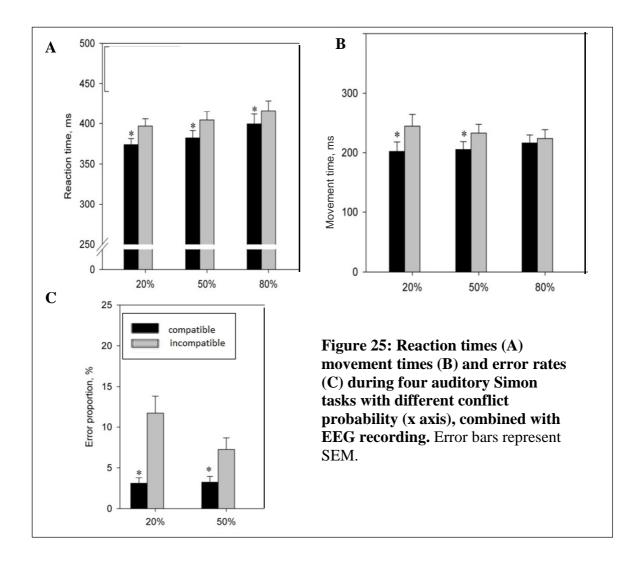
Bin-analyses:

Data preprocessing and statistical testing was done using custom routines in MatLab 7.10.0 (The Math Works, Natick, MA). Additional routines from the MATLAB toolbox (Delorme & Makeig, 2004) were used for the preprocessing of the human scalp EEG data. After import into MATLAB, the data was filtered using a .8 Hz high-pass and 40 Hz low-pass filter (two-way least-squares finite impulse response), then re-referenced to common average. For initial preprocessing, epochs were cut out ranging from 200 ms before the stimulus to 3000 ms after the stimulus for each trial. Afterwards, following the recommendations from Delorme, Sejnowski & Makeig (2007) a combination of automated and visual rejection of non-stereotypical artifacts (gross movement and muscle artifacts) was performed. To this end, trials with a very improbable value distribution (> |5 SD| above the average distribution) were rejected from the dataset. Subsequently, an temporal infomax independent component analysis (ICA) was computed to separate stereotypical artifacts (eyeblinks, saccades, electrode artifcats, neck muscle artifacts, EKG) from the EEG signal. These artifacts were identified using automated criteria (see Wessel et al., 2012 for details) and eliminated by inverse matrix multiplication. The cleaned up datasets were then used for further averaging and statistical testing. To compare between conditions (errors. vs. correct trials / incompatible vs. compatible trials), two-sided within-subject t-tests were computed on 50 ms wide bins (Brass et al. 2005) beginning at the onset of the critical events (Stimulus, Withdrawal, Response) to 800 ms following the event (16 bins). This was done because of the absence of a strong a priori hypothesis concerning the time-ranges of interest. The resulting array of p-values was corrected for false positive using the false-discovery-rate correction method (FDR, Benjamini, Krieger & Yakutieli, 2006).

4.3.3. Results

Behavioral Results

The results are presented in **Figure 25**. A two-way repeated measures ANOVA on RTs, with the factor compatibility and conflict probability, revealed a significant effect for compatibility [$F(1,38) =87,55$, $p<0.001$]. A Holm-Sidak post hoc test demonstrated significantly higher RTs for incompatible trials compared to compatible trials for all different conflict probabilities. The difference of means for $T_{20\%I}$ stands at 23 ms, for $T_{50\%I}$ 22 ms and for $T_{80\%I}$ 16 ms. There was no statistically significant interaction between compatibility and conflict probability [$F(1,38)=2.59$, $p = 0.088$] on RTs, but the post hoc comparison indicated a significant difference for compatible trials in $T_{80\%I}$ compared to $T_{20\%I}$.

For MTs we encountered a slightly different result. A significant difference between compatible and incompatible trials were present as well [$F(1,38) =47.46$, $p<0.001$], but we detected an overall dependence of condition on conflict probability [$F(1,38) =12.83$, $p<0.001$]. However, it was not possible to isolate which group differed from the others, as the post hoc comparison was not able to detect a difference. Nonetheless, comparing the MTs of compatible and incompatible trials indicated shorter MT in compatible trials if compatible trials are more frequent (20 % C: $T_{80\%I}= 202$ ms; 80 % C: $T_{20\%I}= 216$ ms) and shorter MTs in incompatible trials if conflict trials are more frequent (In $T_{20\%I} = 244$ ms; $T_{80\%I} = 223$ ms). A two-way repeated measures ANOVA on ERs, with compatibility and conflict probability as the two factors also exhibited a significant effect of compatibility [$F(1,38) = 27.41$, $p<0.001$] and an interaction between compatibility and conflict probability [$F(1,38) = 9.6$, $p<0.001$]. The subsequent Holm-Sidak post hoc test indicated significantly higher ERs in incompatible trials for $T_{20\%I}$ (12% error) compared to $T_{50\%I}$ (7% error) and $T_{80\%I}$ (7% error). To sum up, we had shorter MTs and higher ERs in incompatible trials for $T_{20\%I}$ compared to $T_{80\%I}$.
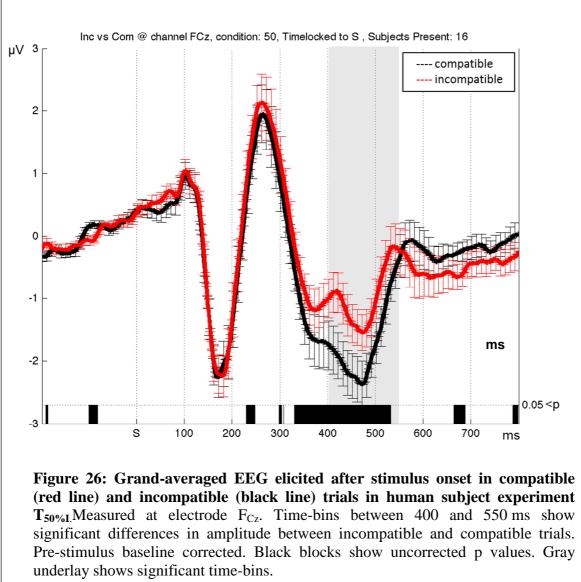
Figure 25: Reaction times (A) movement times (B) and error rates (C) during four auditory Simon tasks with different conflict probability (x axis), combined with EEG recording. Error bars represent SEM.

Statistical evaluation of the $T_{60\%N}$ (60% neutral trials) demonstrated a significant effect of compatibility on RT [$F_{(2,34)}$ =9,32, p<0.001], MT [$F_{(2,34)}$ = 19.27, p=<0.001] and ERs [$F_{(2,34)}$ =13,45, p<0.001].The post hoc comparison confirmed significantly higher RTs and ERs in incompatible trials compared to compatible and neutral trials.

ERP Results

Similar to Experiment 2 and in accordance with the literature of conflict processing we compared stimulus locked ERPs of compatible and incompatible correct trials. As conflict related potentials are known to be largest on central electrodes (Scheffers & Coles, 2000), we focused our observations on electrode FCz. The measured ERPs consisted of several early components and a relatively large negative component starting at 320 ms and lasting until 550 ms after stimulus onset. The components demonstrated significant differences in amplitude between incompatible and compatible
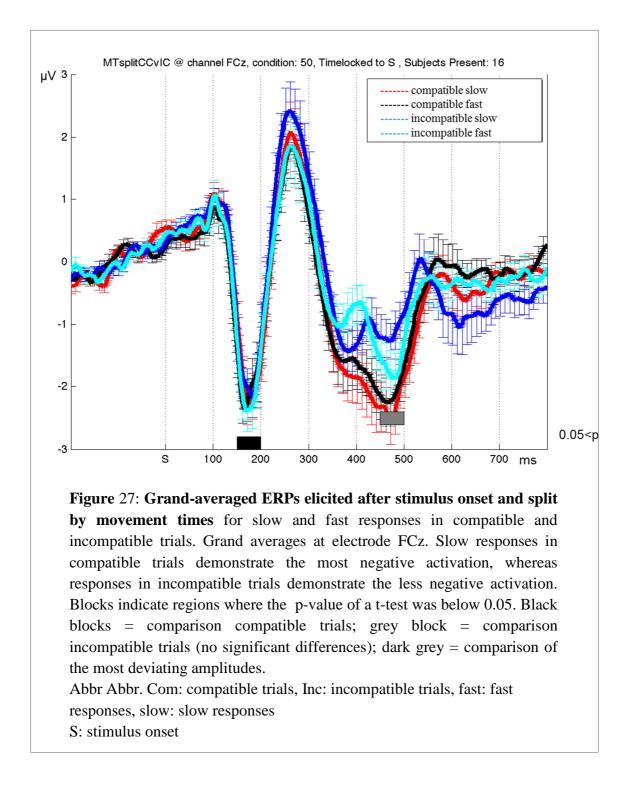
trials (p<0.05; **Figure 26**). If analyzed for time-bins, the large negative component was significantly more negative for compatible trials than for incompatible trials (400 ms to 550 ms). Furthermore a later, more positive difference between the signal for compatible and incompatible trials was observed at 680 ms after stimulus onset. But it was not significant in the time-bin analysis. The detected differences were on average highest for electrode FCz.

Figure 26: Grand-averaged EEG elicited after stimulus onset in compatible (red line) and incompatible (black line) trials in human subject experiment $T_{50\%I}$. Measured at electrode F_{Cz}. Time-bins between 400 and 550 ms show significant differences in amplitude between incompatible and compatible trials. Pre-stimulus baseline corrected. Black blocks show uncorrected p values. Gray underlay shows significant time-bins.
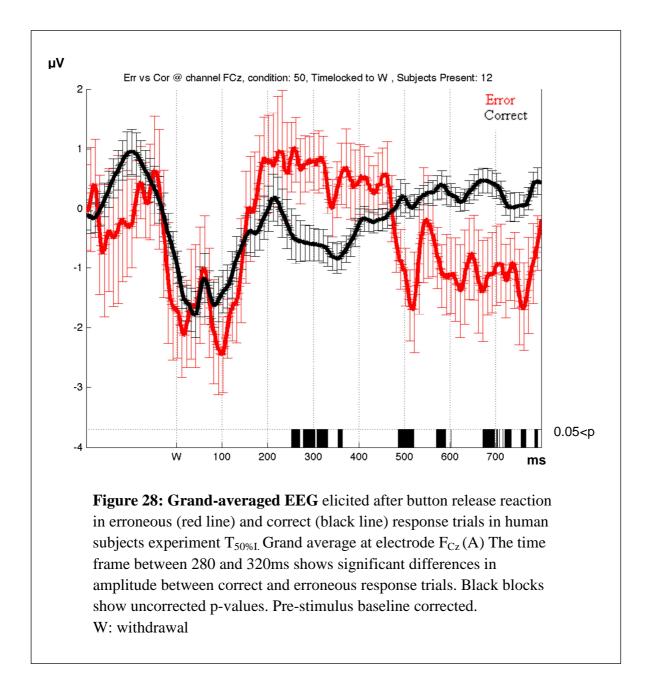S: Stimulus onset

In Experiment 2 we found evidence for possible modulations by uncertainty or motor responses by splitting up the measured signals for low and high MTs into slow and fast response categories. In order to examine the same for our human subjects, we performed a similar separation for compatible and incompatible correct trials (**Figure**

27). We found the highest amplitude (-2.5µV) in slow compatible trials and the smallest amplitude in slow incompatible trials (-1.25µV).

Figure 27: **Grand-averaged ERPs elicited after stimulus onset and split by movement times** for slow and fast responses in compatible and incompatible trials. Grand averages at electrode FCz. Slow responses in compatible trials demonstrate the most negative activation, whereas responses in incompatible trials demonstrate the less negative activation. Blocks indicate regions where the p-value of a t-test was below 0.05. Black blocks = comparison compatible trials; grey block = comparison incompatible trials (no significant differences); dark grey = comparison of the most deviating amplitudes.
Abbr Abbr. Com: compatible trials, Inc: incompatible trials, fast: fast responses, slow: slow responses
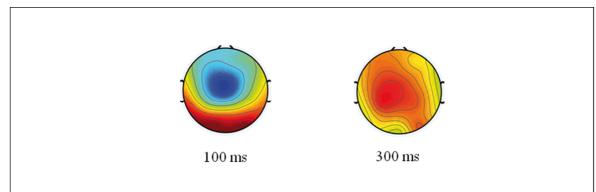S: stimulus onset

Similarly to Experiment 2 and in accordance with the prediction that error related potentials are locked to the actual erroneous response, we compared withdrawal-locked ERPs of correct and error trials. The ERP consisted of a relatively large positive, slow component for error trials starting at 100 ms until approximately 500 ms. But the time

period between 250 ms and 350 ms demonstrate particularly significant differences in amplitude between correct and error trials (**Figure 28**; p<0.05 black blocks show uncorrected p values). Unfortunately, these significant differences were not present after time-bin analysis.

Figure 28: Grand-averaged EEG elicited after button release reaction in erroneous (red line) and correct (black line) response trials in human subjects experiment $T_{50\%I}$. Grand average at electrode F_{Cz} (A) The time frame between 280 and 320ms shows significant differences in amplitude between correct and erroneous response trials. Black blocks show uncorrected p-values. Pre-stimulus baseline corrected.
W: withdrawal

In Experiment 2 due to the characteristics of the LFP measurement, we are able to assume that the measured potentials were elicited within or at least in the vicinity of the anterior cingulate cortex. In contrast, for human EEG measurements such assumptions can only be made after plotting scalp voltage maps which allow analysis of voltage values at different electrodes, making it possible to deduce the approximate area in

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which the potential was generated. While the ERN is known to have a negative frontro-central voltage distribution, the Pe is commonly associated with a negative parietal voltage distribution. The examination of the scalp voltage distribution within erroneous trials (**Figure 29**) demonstrated a negative fronto-central voltage change in the time frame of the ERN potential (100 ms) on the one hand and a positive parietal voltage change in the time frame of the Pe (300 ms) on the other.

100 ms   300 ms

Figure 29: **Scalp voltage maps** for the time point of the N_e like component (100 ms post button release) and the Pe like component (300 ms post button release) in the human subject experiment $T_{50\%}$. Both maps show topographic distribution of the waveforms elicited during erroneous response trials. Blue colors indicate negative voltages (max. 2.4µV, red colors indicate positive voltages (1µV).

4.3.4. Discussion

Behavior

Similarly to the experiments on rats, we found that humans performing our modified auditory Simon task display significantly longer RTs and higher ERs for incompatible trials (i.e. a Simon effect). This was statistically significant for both RTs and ERs and all levels of conflict frequency. In addition, we found a Simon effect for MTs too. However this effect was only significant for low conflict probabilities (20%, 50%). This, first of all, demonstrates the practicability of our cross-species version of the auditory Simon task at least from a behavioral perspective. Differences between the human and animal setups, response strategies, effects of conflict frequency and the electrophysiology results will be discussed in the following sections.

Technical discussion

As opposed to our rat study where we used two different auditory stimuli, in our human study we had to use four different stimuli, otherwise the task would not have been complex enough to accumulate a sufficient amount of errors. Nevertheless, in order to make the procedure as similar as possible auditory stimuli were utilized. We chose harmonic frequencies separated by one octave in order to prevent a spatial musical association of response codes namely the SMARC effect. It has been shown that tone pitch is often associated with a spatial component whereby high- and low-frequency pitches are assigned to high and low spatial locations respectively. (Trimble, 1934; Roffler & Buttler, 1968). This is also reported for the horizontal dimension, where low pitches are associated with the left and high pitches with the right direction (Mudd, 1963).

Even with the four stimuli approach the accumulated amount of errors was rather low (i.e. 7% to 12%), which led to a noisy error potential. We had similar difficulties with rats, as discussed above. However, with humans the problem was not related to motivation, more to the fact that the task was too easy even after using four rather than two stimuli. Increasing the number of trials to improve signal-to-noise ratio in averaged signals was also not possible, as the task already lasted over one hour One future solution may be to separate the different experiments into two sessions on two different days instead of doing all tasks in one session. In this case it would be possible to increase the amount of trials for each experiment but this arrangement may also result in logistical problems and higher participant costs.

Reference problem

Unfortunately, the EEG still presents the problem that maximal activity or maximal difference at a certain electrode does not indicate that the area where the signals were generated lies directly in the region below it. Different sources and different areas can generate the same distribution of potentials (Fender, 1987).

Furthermore, the general issue with EEG and LFP is the limitation of reference points. In general, attempt is being made to find a neutral point to form the basis of comparison for the potentials derived from active electrodes. The reference electrode is often far away from the active electrode, for example at the mastoids or on the tip of the nose. The measured potential at the electrode is generated somewhere between these two points, which can often be a large distance. This problem can be decreased if we use a

bipolar measurement. Here the voltage difference between two different proximity electrodes is measured. The advantage is that artifacts are reduced and the source of the potential can be further isolated. The disadvantage is, if the two electrodes are too close to each other they measure the same potential and cancel each other out when calculating the voltage difference. In our rat study the electrodes were too close to be adequate for bipolar results, therefore, we could not precisely determine whether the potentials were generated in the ACC itself. In the human study, because of the ability to calculate a voltage map, we were able to get closer to the possible source of generation. However, it still cannot be completely discounted that the point of generation lied somewhere else. To address this problem spatial source localization models need to be used or one has to resort to other techniques like imaging.

Response strategy

The finding that the Simon task was present in reaction as well as MTs was precisely what we predicted, as we instructed our human participants to choose a speed over accuracy response strategy. As mentioned above a strategy where the movement is started as soon as the stimulus appears (R_{fast}), leads to a Simon effect in MT (Rubici et al. 2000). The similar result in our rat study (Experiment 2) therefore supports our theory that similarly to humans, rats chose a speed over accuracy trade off and accept a higher probability of error commitment.

Conflict frequency

In our study the Simon effect in RT and in MT decreased with increasing level of conflict. Other studies report similar observations for RTs in several other conflict tasks (e.g. Gratton, Coles, & Donchin, 1992; Hommel, 1994; Logan & Zbrodoff, 1979). All this studies have a greater probability of incompatible trials leading to a smaller interference effect in common. Moreover, these studies mostly report general response times, as they do not separate reaction and MTs. Unlike these, we differentiated between reaction and MTs and found a significant effect for MTs only. This may demonstrate that humans have a general conflict adjustment process which occurs later during conflict processing (i.e. in MT instead of RT).

However, this conflict frequency adaptation is comparable to what we found for rats.

Conflict related adjustments were only observable in MTs of incompatible trials and in error proportion, indicating conflict adjustments or error avoidance processes that take place at a later stage of response processing.

This presents evidence that in humans as well as in rats processes which produce performance facilitation have to occur at a stage preceding motor initiation and prior to the processes that cause response interference.

Electrophysiology

In line with the rat study in Experiment 2 we locked the ERPs at different time points of the response process to separate the various correlated potentials from one another. We time locked the conflict related potentials to the stimulus, as it is thought that a conflict potential should precede the actual reaction and the error-related potential should closely follow the reaction, for which we locked the error-related potentials to the time point of the withdrawal. Both time locks will be discussed in the following paragraphs with reference to our rat study.

Electrophysiology of conflict

What one immediately notices is the similar distribution of negative and positive components found in the human and rat studies. Although these findings deviate from what might be expected based on recent literature (Yeung & Nieuwenhuis, 2009, Kopp et al.,1996) the similarity in pattern observed in our rat and human studies is remarkable. More specifically, we found a D_n and D_p in response to both compatible and incompatible trials. The only difference between the two studies were the latencies of the components. In rats the D_n started at 150 ms whereas the human D_n started at 400 ms.

As seen in Experiment 2 the D_p is also earlier in rats (520 ms to 750 ms) than in humans (680 ms). The human D_n was more negative for compatible than for incompatible trials and the D_p was more positive for compatible trials. It seems that in keeping with our rat study a more positive activation was correlated with conflict. To test the account that the human D_n reflects more motor or even premotor, and transient activations, which may be correlated with the certainty of a correctly given response or reward prediction, we conducted a MT split analysis just like in Experiment 2. Results showed that low MTs in compatible trials resulted in the most negative activation. For incompatible trials this was difficult to distinguish. In rats less effort and fast reward seemed to correlate

with high negative amplitude. In the human ERP we observed a similar distribution with more negative amplitude for incompatible fast than slow trials, but also an interference with a speed effect. There was an up-modulated positive peak with different latencies evident at the beginning of the negative deflection. Therefore the results of the MT split cannot be clearly separated. If we only look for the later part of the D_n which is not corrupted by the positive up-modulated peak, the distribution for incompatible trials is similar in humans compared to rats. This makes it tempting to argue that we, again, see similar effects as had been discussed in our rat experiment. Further experiments have to be carried out in order to separate a possible speed effect and/or certainty or reward prediction. Although the MT split approach did not lead to satisfying results, the similarities between our human conflict- related potential and the rat conflict-related potential and its modulation are remarkable, especially as they were both acquired using different techniques and different species.

Electrophysiology of errors

Unfortunately the error related ERP is extremely noisy due to the fact, that we had problems accumulating enough error in the task. Based on existing literature we would have expected to find an ERN potential after reaction in an erroneous response. Unfortunately we could not detect any such activity. In Experiment 2 we were also unable to find any evidence of an ERN like potential. We discussed several potential reasons for this. Two of which are that error processing is different in humans and rats or that it is task or setup dependent. If we had found an ERN Potential in Experiment 3 this could have argued for the first possibility. However that was not the case. Instead, in Experiment 3 we were not able to detect an ERN-like potential either. There may be several potential explanations for this. From a temporal point of view it may be that the time point of the button release (withdrawal) is too early to realize whether the response is correct or wrong. The conflict related potential starts rather late, which indicates that error processing has not yet started or ended. The examination of response related error potentials for the time point after the MT, when one of the two response buttons was pressed, led to non-satisfactory results. At this time point there was no ERN detectable, presumably because at this time feedback mechanisms are already initialized which may have masked the ERN.

Furthermore, it is possible that there is no discrete time point at which the system detects and differentiates between correct and error responses. Error information could

be processed somewhere between the time of button release (withdrawal) and response button press (response). One observation which argues for this is that we find a Simon effect in both reaction and MT and that this depends on the response strategy. If the error processing also occurs somewhere in-between the reaction and MT the ERN could be blurred over these time points. In contrast, the Pe, which is a long lasting deflection, is able to persist.

As a matter of fact we observed a Pe-like potential in Experiment 3 just like we did in Experiment 2. The ERP consisted of a large and slow positive deflection for errors starting at100 ms after button release to approx. 500 ms after button release. The time frame between 250 ms and 350 ms was significantly more positive in error trials than in correct trials. Further evidence that this deflection could be a Pe is the topographical distribution of the potential. The Pe has been observed to follow the ERN and to have its maximum amplitude over centroparietal electrodes. Our topographical map indicates exactly this distribution. Although we were not able to detect an ERN, the topographical analysis indicated a frontal, central processing in the time range of the ERN before the Pe deflection.

The fact that we could not find a discrete time point of error detection and/or ERN but a clear Pe could be considered an evidence of error monitoring. The Pe is argued to be related to error monitoring in more aware processes (Wessel, Danielmeier & Ullsperger, 2011). This fits with the assumption we made earlier that the time point of the button release (withdrawal) is too early to realize whether the response is correct or wrong, as the system is not aware of the error at this point. This however is highly speculative. Unfortunately, as the Pe is less studied than the ERN or the N2 it is difficult to pinpoint its actual functionality, which was also not the purpose of this study. What is more interesting is that we found a similar positive deflection in rats, in the same time range at 250 ms to 400 ms in our ACC LFPs. The exceptional similarity of both error-related potentials further supports our conclusion that the rat is a promising model for studying such potentials. Even with slightly different techniques and obviously extremely different species we find remarkably similar results. Further studies with this model will bring promising insights in error processing, which will be further discussed in the general discussion.

4.3.5. Synopsis

The results of this experiment demonstrate that it is crucial to use more comparative approaches in order to establish animal models. With only the results from Experiment 2 many question would remain unsolved. We were able to show that rats indeed demonstrate conflict and error related potentials presumably carried by the ACC, but with many differences, to what we find in human and primate literature (Heil et al., 2000; Liotti, et al., 2000; Kopp et al.,1996; Emeric, 2008). Conducting a highly comparable human experiment demonstrated that the rat findings were not alone in contradicting existing literature. In fact, human studies with exactly the same variables and modulations arrived at quite similar results. We obtained evidence that:

i) Conflict-related adjustments were only observable in MTs of incompatible trials and on error proportion, indicating conflict adjustments or error avoidance processes that take place at a later stage during response processing. This was supported by the electrophysiology results which demonstrated conflict related potentials later, after stimulus occurrence.

ii) the process of error detection is not assigned to a discrete time point. It appears more likely that the mismatch (conflict or error) could be processed somewhen during the time between the button release (withdrawal) and the response button press (response).

It seems that the differences were not due to differences between species but much more to specialties of the task itself. One distinction lies in the use of auditory stimuli, which is relatively rare, the other in the separation of response time into reaction and MT.

This study complements our results from Experiment 2 and demonstrates that it is essential to use a comparative approach to allow the assessment of cognitive processes in rats which appear to be activated in humans as well when performing cognitive conflict tasks. The validity of animal models can thus be enhanced considerably. By showing parallels between the two species our rat model offers great potential to further investigate neuropsychological and electrophysiological correlates of conflict and error processing.

5. General Discussion and future prospects

We acquired strong evidence that conflict and error processing takes place in the human prefrontal cortex, particularly the anterior cingulate cortex. Although we have found differences in processing between humans and rats, on a basic level they demonstrate highly similar behavioral, functional and electrophysiological modulations. This demonstrates that the rat model is a useful tool to get insights into a simplified version of the performance monitoring network. It has been shown that it is even preferable to more evolutionarily close models such as those that use monkeys, as these models have provided no evidence of conflict processing in the primate ACC. The present work does not exploit all promising options of the constructed setup. More results were not mentioned because of the sheer extent of possibilities. Further insights could be gained for example by analyzing neutral trials (Wühr & Ansorge, 2005). The comparison of trials where the stimulus was presented from both speakers (without any spatial dimension) with incompatible and compatible trials could give new insights into the nature of compatible trials especially. We could investigate whether there are any facilitation effects of the automatic route compared to neutral trials and discuss the subsequent consequences.

Until now we only looked at macroscopic adaptations like the general frequency of conflict trials. A closer look at microscopic differences could include the analysis of sequential modulations. It was shown that the Simon effect increases or decreases (Praamstra and Plat, 2001) or is even eliminated (Stürmer et al., 2002) depending on the preceding trial.

This has led to different theories about the dual route (Mordkoff, 1998; Stürmer et al., 2002; Wühr, 2005). Some theories propose that there is an additional ancillary monitoring system (Stürmer et al., 2002; Wühr & Ansorge, 2005), others suggest that it is an effect of feature integration (Hommel et al., 2004). Our model and the already collected data can be further analyzed to examine rat conflict processing and its effect on sequential modulation.

Furthermore, one of the key advantages of animal models in general, is that we can carry out more invasive investigations on the brain than what is possible in human studies. For example we have the possibility to further investigate anatomical brain studies, by lesioning brain areas found in our PET study which seem to be involved in the dual route processing like the ACC, the M1 or the M2. From a more neurochemical perspective we can have a closer look at such pathways by changing local

neurotransmitters or systemic neurotransmitters and hormones. For example, it is known that dopamine and the distribution of D1 and D2 receptors have a high influence on error processing. It is even possible to generate genotype rat mutants with different or no dopamine receptors.

To test whether some of the observed differences to literature, like more positive deflection for incompatible trials are due to a special exceptional position of the Simon task itself or our separation of the response time into reaction and MT, it would be worthwhile to test an adaptation of a Flanker task. This could be tested in a human EEG study. A Flanker task is a different version of a conflict assignment where participants have a target stimulus (for example an arrow) and distracting stimuli (arrows in different direction). When the distracting arrows point in the same direction as the target arrows this compatible condition leads to faster RTs and less errors, than incompatible trials where the distracting arrows point into different directions. There are several options of this task, with various types of target and flanker/distractor stimuli. This task is the most likely candidate for an adaptation to an animal model. Further conflict experiments like the Stroop task are less likely due to the need for semantic abilities.

The general auditory model of the Simon task will form part of the test battery of animal behavioral tests in the MPI of neurological research, for the testing of higher cognitive abilities in rat stroke models.

The occlusion of the anterior cerebral artery leads to ischemic lesions in the prefrontal cortex. With the help of this Simon task and several other behavioral tasks from the test battery it is possible to study the impact of this kind of stroke on behavior and its possible recovery.

As a final point, it can be concluded from the present observations that conflict processing and error processing can be assed in both humans and rats, using comparable tasks.

6. Conclusion

In conclusion, we have found remarkable similarities between animal and human behavior and electrophysiology. Both species demonstrate a valid occurrence of a Simon effect and seem to pursue similar response strategies. Both show a Simon effect in RT as well as in MT. In addition, both species demonstrate sustained differences in the modulation of the ERP depending on correct or incorrect responses starting at the time of response and prior to reward/no reward. This makes it tempting to speculate that the underlying cognitive error processing mechanisms are identical across species. Our paradigm offers a new approach in integrative, cross-species research and provides a useful rodent model for performance monitoring research.

7. References

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9. Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Herrn Prof. Dr. Büschges, Frau PD Dr. Heike Endepols und Herrn Prof. Dr. Markus Ullsperger betreut worden.

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