Serotonergic Modulation of Action Monitoring and Cognitive Control

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ABBREVIATIONS

5-HT	–	5-hydroxytroptophan, serotonin
5-HTT	–	serotonin reuptake transporter
5-HTTLPR	–	5-HTT-linked polymorphic region
aMCC	–	anterior midcingulate cortex
CNS	–	central nervous system
DA	–	dopamine
DRN	–	dorsal raphe nuclei
LC	–	locus coeruleus
mPFC	–	medial prefrontal cortex
MRN	–	median raphe nuclei
NAc	–	nucleus accumbens
NE	–	noradrenaline
OCD	–	obsessive compulsive disorder
PCS	–	post conflict slowing
PES	–	post error slowing
PFC	–	prefrontal cortex
PM	–	performance monitoring
pMFC	–	posterior medial frontal cortex
PSP	–	postsynaptic potential
SN	–	substantia nigra
SSRI	–	selective serotonin reuptake inhibitor
Trp	–	tryptophan
VTA	–	ventral tegmental area

CONTENTS

General Introduction and Outline of this Thesis

5-HT is one of the brains most diverse and intensely studied neurotransmitters and -modulators. An important role for 5-HT has been attributed to diverse functions such as inhibition, aversive conditioning, aggression, behavioral satiety, cognitive flexibility and learning, and it has been linked to many clinical disorders like obsessive compulsive disorder (OCD), depression, anxiety disorders, eating disorders, attention deficit hyperactivity disorder (ADHD), and substance dependence (Lucki, 1998; Müller and Homberg, 2014). Additionally, all the above mentioned disorders have also been linked to disturbances in performance monitoring (PM) – the ability of the brain to detect deviations from predicted sequences of events (Ullsperger et al., 2014a). While for other neurotransmitters, such as dopamine (DA), clearcut models have emerged from electrophysiological studies describing a connection between healthy functioning and diseased states (Schultz et al., 1997; Frank et al., 2004), a comparable model does not exist for 5-HT (Daw et al., 2002; Boureau and Dayan, 2011) and particularly findings regarding the role of 5-HT in PM are inconsistent. In part, this may be attributable to the complexity of the 5-HT system itself with more than 14 currently known receptor subtypes which sometimes have opposing functions (Hoyer et al., 2002; Boulougouris et al., 2008). This is complicated by a lack of knowledge about how agents interfering with serotonergic neurotransmission, especially selective serotonin reuptake inhibitors (SSRI), precisely act when given acutely (Cools et al., 2008). Additionally, because in humans the controlled study of effects of neuromodulators on higher cognitive functions has to rely mainly on pharmacological or dietary manipulations, another source of variation are genetic differences that interfere with these types of interventions. Many different aspects of the 5-HT system have been studied in this way, like tryptophan hydroxylase and the $5\text{-HT}_{2A/C}$ receptors, but by far most widely studied are polymorphisms regarding the serotonin reuptake transporter (5-HTT) (Veenstra-VanderWeele et al., 2000) located in an upstream promotor region called 5-HTT linked polymorphic region (5-HTTLPR). Since its discovery almost 20 years ago (Heils et al., 1996; Lesch et al., 1996), it has been found that the *in vitro* lower expressing, short (S) allele is associated with increased anxiety, a higher risk for depression in situations of multiple stressful life events, increased side effects and inferior response to SSRI treatment in depression (Caspi et al., 2003; Murphy et al., 2003; Schinka et al., 2004; Serretti et al., 2006a; Karg et al., 2011). However, the *in vitro* higher expressing, long (L) allele has been discussed as a risk factor for ADHD and may be associated with suboptimal impulse control (Curran et al., 2005; Faraone et al., 2005). Since its initial description, further functional single-nucleotide polymorphisms (SNPs) that subdivide S and L alleles more precisely, have been discovered and linked to certain disorders (Hu et al., 2006; Wendland et al., 2007). Yet, neither the neural underpinnings of these differential liabilities to disorders, the degree to which healthy subjects with either genotype display traits which confer such a risk and are measurable with tasks of PM functions, nor the question whether these genetic risk factors are transferred during ontogenetic development or differences in ongoing neurotransmission throughout life, are well understood.

The current study tries to tackle these questions by employing a pharmacogenetic approach and electrophysiological measures of PM functions. Two extreme groups regarding hypothesized 5-HTT mRNA expression were formed by comparing only homozygous subjects with the higher expressing allele at 5-HTTLPR and rs25531 to the homozygous low expressing genotype. For this investigation, both groups of healthy human subjects were then given a low dose of

a selective serotonin reuptake inhibitor (SSRI) intravenously in a double blind cross-over design prior to performing PM tasks. Comparisons performed in the placebo condition are thus informative about general effects of genetic variation of 5-HTT expression, but cannot be interpreted as direct evidence towards serotonergic involvement as it is unclear if effects are actually due to differences in 5-HT signaling or developmental / morphologic in nature. However, with the hypothesis that lower expressing subjects show higher extracellular 5-HT levels, as evidenced by rodent studies (Kalueff et al., 2010), findings that are directionally compatible when an effect of the SSRI and a genetic difference are seen, provide strong evidence for an involvement of the acute state of serotonergic neurotransmission.

In this thesis, following a recent review of PM functions, their electrophysiological correlates and time-courses (**Chapter 1**), a brief summary of the current knowledge about the serotonergic system and its role in PM is presented in **Chapter 2**. This includes presentation of a novel hypothesis of effects of acute SSRI administration that has been puzzling to clinicians and neuroscientists alike. Thereafter, characteristics of the study population are described and analyses of possible confounding side effects of the drug are discussed in **Chapter 3**. Then, the behavioral and electrophysiological results of three different PM-tasks are presented which were employed to explore different essential PM functions that have been ascribed to serotonergic modulation. The role of 5-HT in mediating inhibition following negatively valenced events, such as errors, is studied in a flanker paradigm, where also the error related potentials of the error-related negativity (ERN) and the error positivity (Pe) are analyzed (**Chapter 4**). Serotonergic involvement in attentional orienting and behavioral inhibition are studied in a novelty Go/NoGo task (**Chapter 5**). Here, the cost of behavioral inhibition were measured as the amount of slowing of the default response following inhibitory events and event related potentials (ERPs) associated with attentional orienting (P3a) and inhibitory control (N2, P3b) were analyzed. Thereafter, a new task designed to differentiate learning from real events compared to fictive events that could have happened given different choices are described to establish an understanding of the electrophysiological correlates of these events in **Chapter 6**. Using this framework, effects of the genetic and pharmacological variables are analyzed with their relationship to learning from real and fictive outcomes in **Chapter 7**. The final **Chapter 8** summarizes the findings of this thesis and provides an outlook for further studies on the role of 5-HT in PM.

CHAPTER

1

Neural Mechanisms and Temporal Dynamics of Performance Monitoring

Modified after:

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Introduction

In our changeable uncertain world it is inevitable that we make mistakes. However, adapting our behavior, cognition, and motivation helps us to compensate errors, to avoid repeating similar mistakes, and to make our future actions more efficient. For about two decades cognitive neuroscientists have focused on the question when, how, and based on what information the human brain determines the necessity to adapt and to recruit control. Evidence from neuroimaging, EEG, and invasive recordings in humans and non-human primates converges on the currently widely accepted view that this PM function is implemented by a cortico-subcortical network connected to the pMFC, comprising the anterior midcingulate cortex (aMCC), the pre-supplementary area (pre-SMA) and adjacent dorsomedial prefrontal cortex (Rushworth, 2008; Nee et al., 2010; Shenhav et al., 2013; Ullsperger et al., 2014a). Signal changes in the pMFC correlate with the necessity, type, and magnitude of adaptation. This information is signaled to other brain regions for implementation, such as the lateral frontal cortex (Kerns et al., 2004; Cavanagh et al., 2009; Rothe et al., 2011), motor and sensory areas (King et al., 2010; Danielmeier et al., 2011), and anterior insular cortex and the autonomic nervous system (Ullsperger et al., 2010; Wessel et al., 2011). Theories of PM differ predominantly with respect to the underlying information processing mechanism generating the adaptation signal. Here, we discuss two major accounts, the reinforcement learning (RL) theory (Holroyd and Coles, 2002) and its recent modifications (Holroyd et al., 2008) as well as the predicted response-outcome (PRO) theory (Alexander and Brown, 2011). Both models assume that adaptation is recruited based on a weighted prediction error (PE) signal, indicating a deviation of the action outcome from the expected outcome, but they differ as to whether this PE signal is signed and differentiates better- and worse-than-expected outcomes or whether the PE signal is unsigned and indicates surprise. Furthermore, the RL theory makes explicit assumptions on the neurobiological underpinnings of the PM signal in the pMFC. PM research has grown rapidly and extended into applied and clinical fields. Therefore, it is necessary to critically discuss the assumptions made by the theories in the light of the equally quickly growing neurobiological evidence at the cellular level. We will review recent human and animal work addressing (a) what signals are reflected in event-related EEG dynamics and how they fit with theories, (b) which brain structures and neurotransmitters are involved in the processes giving rise to PM and its EEG correlates, and (c) whether current theories are sound and testable. Before turning to these aspects, we will briefly describe the EEG dynamics related to PM.

Temporal Dynamics

A uniform sequence of EEG activity associated with performance monitoring

Since the discovery of the ERN and Pe in 1990, a number of further temporally and topographically defined event-related potentials (ERPs) have been linked to PM at different stages of goal-directed behavior. PM works continuously: During action selection it signals changes in expected action value, particularly increased costs requiring the recruitment of additional control. Action slips can be detected immediately when task rules are known. Otherwise, PM must be based on external sensory feedback on the action outcome.

Interestingly, while names and functional descriptions differ, a rather uniform sequence of ERP deflections is found, regardless of whether the eliciting event occurs prior to, during, or after the action (**Figure 1-1**): an early frontocentral negativity is immediately followed by a frontocentral positivity, succeeded by a later, more sustained parietal positivity. During action selection and execution, this sequence is reflected in the classical frontocentral N2, frontocentral P3a, and parietal P3b deflections elicited by the imperative stimulus. In reaction time tasks with fixed and well-trained rules, a frontocentral ERN, a frontocentral early Pe, and a parietal late Pe are found. When only feedback disambiguates the outcome, a feedback-locked sequence of the frontocentral feedback-related negativity (FRN), the frontocentral P3a, and parietal P3b is observed (see **Glossary** for definitions of the individual deflections). A common generator in the pMFC appears to contribute, at least in part, to the early frontocentral negativity-positivity complexes (Debener et al., 2005; Polich, 2007; Gentsch et al., 2009; Gruendler et al., 2011; Wessel et al., 2012; Hauser et al., 2014). This activity may reflect theta-band power increases phase-locked to the eliciting event (Nigbur et al., 2011) as reviewed elsewhere (Cavanagh et al., 2011; Cohen et al., 2011). The generators of the parietal P3b appear to be more widely distributed across the cortex encompassing the temporoparietal junction (Nieuwenhuis et al., 2005; Polich, 2007).

The classical approach focusing on peaks and troughs in grand mean ERP data has revealed important features of PM-related EEG activity. However, averaging leads to substantial loss of information and precludes tests of predictions at both trial-by-trial and within-trial levels made by mathematically formalized computational models from current PM theories. Another problem is the overlap of activity from different sources in the scalp-recorded EEG and the associated difficulties in independent quantification of each deflection. This has been addressed by means of source-separation techniques, for example independent component analysis (Debener et al., 2005) allowing to study the trial-by-trial time-course of activity in individual source networks. Source separation can be combined conveniently with regression-based approaches (Cohen and Cavanagh, 2011; Fischer and Ullsperger, 2013) or machine-learning algorithms (Steinhauser and Yeung, 2010) to link parametric predictors to cortical responses without a loss in temporal resolution. An appealing benefit of this technique is that it can be displayed in similar ways as classical ERP components as time-course and topographic activity (**Figure 1-1**), while it provides much richer information on the contributions of certain processes to the EEG time course independent of single peaks and arbitrarily chosen conventions for quantification. In model-based analyses (Mars et al., 2012), computational models are fitted to individual behavior to determine latent but behaviorally relevant parameters like expected value, PE, surprise, or

learning rate (**Glossary**), which then are used as predictors in the trial-by-trial EEG analyses (Cohen and Ranganath, 2007; Mars et al., 2008; Philiastides et al., 2010; Fischer and Ullsperger, 2013). If the model-based (or a statistically similar) parameter is indeed represented in cortical activity contributing to the EEG, statistical analysis will separate it from other EEG activity. This enables the temporal dynamics of this activity to be assessed in which theories can be tested more directly.

Synthesizing the results of classical and model-based EEG analyses, we propose that the family of early frontocentral negativities and positivities reflects fast alarm signals indicating incoming evidence for the potential necessity of action adaptation (Steinhauser and Yeung, 2010; Ullsperger et al., 2010; Wessel et al., 2011). The evidence may then accumulate giving rise to the later posterior positivity, which seems to represent a subjective evidence signal (O'Connell et al., 2012) or the outcome of task-related decision processes (Aston-Jones and Cohen, 2005a; Nieuwenhuis et al., 2005). When this signal exceeds a threshold, it may direct attention and conscious awareness to the decision to adapt and facilitate storing updated value representations in memory (**Box 1-1**). Notably, PM parameters do not only modulate these classical ERP peaks but may cause sustained shifts lasting several 100ms. For example, high learning rates, enhancing the impact of incoming evidence, shift feedback-related centroparietal EEG into a positive direction for about 400ms thereby differentially influencing the FRN, P3a, and P3b (Fischer and Ullsperger, 2013). The learning rate reflects a weighting factor depending on the statistical properties of the environment, since noisy, uncertain, and volatile environments influence the reliability and information content of feedback. These statistical properties seem to be tracked by the pMFC (Behrens et al., 2007; Jocham et al., 2009).

Although assumed to be generated in the pMFC, N2, ERN, and FRN signals may give rise to somewhat different scalp topographies even in model-based and source separation analyses (**Figure 1-1**). This may result from differential co-activation and functional connectivity of other brain regions with the pMFC depending on the kind of processed evidence and the appropriate adaptation at the given stage of the action.

Performance monitoring accounts of event-related EEG dynamics

Both theoretical RL and PRO theories discussed here, refer to pMFC –more specifically aMCC– activity and explicitly relate to the family of frontocentral negativities. Subsequent positivities, particularly the parietal P3b are not addressed by these models and will therefore be discussed separately in **Box 1-1**.

The RL theory (Holroyd et al., 2008) has had a strong impact on current understanding of pMFC function, since it integrates theoretical models and neurobiological findings on reinforcement learning with the field of PM. It suggests that the pMFC acts as a control filter recruiting necessary adjustments based on a reward PE (RPE) signal sent from a compartor (critic, in RL terms) residing in the basal ganglia. According to the theory, the striatum evaluates events associated with the current action as to whether they indicate a better or worse outcome than predicted. Thus, a signed RPE is calculated and conveyed to the pMFC. More negative RPEs (worse-than-expected outcomes) are proposed to be associated with a larger FRN and stronger hemodynamic signals. This logic can be transferred to the ERN and N2, which are related to preceding events that may predict a better or worse action outcome (Holroyd and Yeung, 2012).

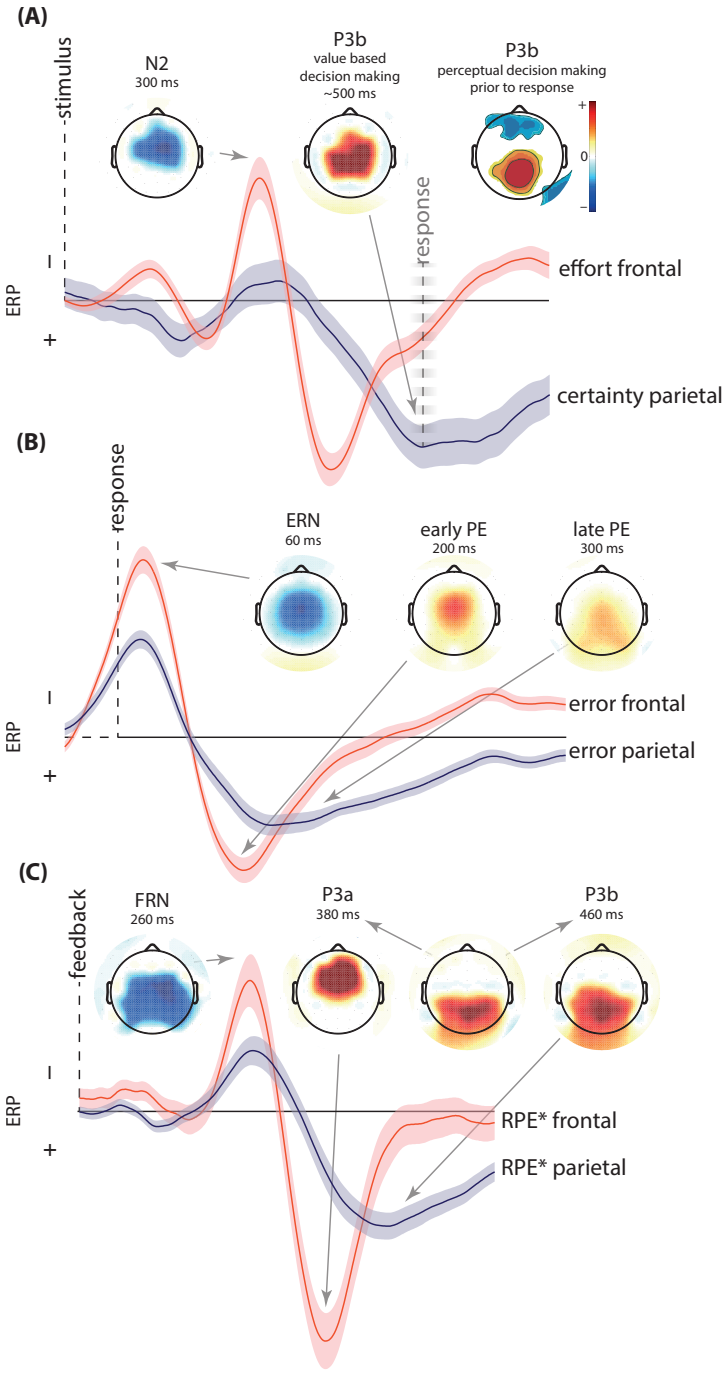

Figure 1-1. Schematic time-courses of regression weights and their topographies for selected correlates of performance-monitoring during (A) stimulus processing, (B) response generation, and (C) feedback evaluation as revealed by single-trial EEG multiple regression analysis. Time-courses represent prototypical temporal evolutions of these correlates at frontal (red curve) and parietal (blue curve) electrodes.

(A) Following stimulus presentation, the required effort or difficulty of a response (red curve) instigates a negative scalp potential – the N2 – followed by a short-latency positivity (P3a, not plotted here) in response conflict tasks. In this particular decision making task, subjective certainty of the given response covaries with stimulus locked EEG activity in parietal regions (blue curve). A similar activation pattern was observed in a perceptual decision making paradigm (O'Connell et al., 2012) preceding response onset, which is interpreted as the accumulation of evidence in favor of a decision.

(B) Incorrect responses in response conflict tasks again show a biphasic negative followed by positive covariation with frontocentral scalp activity – the ERN and early Pe. This is followed by a more parietal positivity (the late Pe) that may also index conscious accumulation of evidence for an erroneous response.

(C) Errors signaled via explicit feedback first elicit again a rapid negativity in the cortical potential – the FRN –followed by the frontal P3a (middle left) and the more posterior P3b (right) as correlates of the RPE. The base of both signals has been linked to learning rate estimates (middle right) that may index the amount of behavioral adaptation required. The conjunction of PE and learning rate correlates was also predictive of future adaptations (Fischer and Ullsperger, 2013).

* RPE has been multiplied with -1 for better comparability with (A) and (B) by showing correlations with unfavorable outcomes as negative. White areas in topography plots represent data points without effects, red colors positive, and blue colors negative covariations. Regression models included additional predictors to separate the described findings from reaction time, response hand, stimulus value or other adaptation effects. The schematic is based on a probabilistic learning paradigm and a flanker-task performed by the same subjects (Fischer and Ullsperger, 2013) and a perceptual decision making task (O'Connell et al., 2012). Parts of the Figure have been reproduced and modified, with permission, from (O'Connell et al., 2012; Fischer and Ullsperger, 2013).

The PRO model (Alexander and Brown, 2011) postulates that individual neuronal ensembles code the learned prediction of the probability and timing of the various possible outcomes of the action at hand. When an expected outcome occurs, the corresponding prediction signal is inhibited. In contrast, non-occurrence of predicted outcomes results in maximal pMFC activity. The PRO model is based on a generalized RL algorithm, which can predict multiple

possible action-outcome relationships simultaneously. It considers cognitive control as a result of evaluating probable and actual outcomes of one's actions. Thus, the PM signal should correlate with surprise.

In line with the RL theory, numerous studies show that the signed RPE modulates the frontocentral negativities (Walsh and Anderson, 2012). In model-based analyses the single-trial FRN covary positively with outcome valence and magnitude and negatively with expected outcome (Talmi et al., 2012; Fischer and Ullsperger, 2013), which is consistent with signed reward PE coding. Recent evidence suggests that the FRN tracks a model-free reward PE signal, even if model-based learning drives subjects' behavior (Walsh and Anderson, 2011). An inverse relationship between ERN and FRN has been shown at different stages of reinforcement learning: as learning progresses the error-related signal shifts forward in time from the feedback to the response (Holroyd and Coles, 2002; Krigolson et al., 2009), consistent with the principles of temporal difference error learning (Sutton and Barto, 1998).

However, N2, ERN, and even the FRN have also been shown to reflect unsigned PE or surprise, thus supporting the PRO theory. The N2 is increased on unexpected, valence-free action outcomes and rare potentially action-relevant stimuli (Folstein and Van Petten, 2007; Wessel et al., 2012). In tasks used to elicit the ERN, errors are always less frequent than correct responses and thus unexpected. FRN and feedback-related pMFC activity has been shown to be elicited by unexpected positive outcomes, the omission of expected punishment, or to correlate with surprise (Jessup et al., 2010; Amiez et al., 2012; Talmi et al., 2013; Hauser et al., 2014).

The proponents of the RL theory have suggested a modification that might account for the presence of both surprise and RPE signals in the same latency range of the ERP (Holroyd et al., 2008). Specifically, the negativity reflected in the FRN is understood as a purely surprise-related N2 deflection. Moreover, the frontocentral ERP in the latency range of the FRN is assumed to be modulated by positive (but not negative) RPEs, which gives rise to a so-called reward positivity or feedback correct-related positivity. A rigorous proof of this concept would be to show that negative reward PEs are not coded in the FRN. However, both negative as well as positive PEs have been demonstrated to

Box 1-1. The late centroparietal positivity reflects accumulation of evidence for adaptation

We propose that the late error positivity on response errors and the P3b response to action-relevant stimuli and to feedback reflect similar computations by the brain related to adaptive behavior, awareness of this behavior, and storage in memory. A recent model-based analysis applied to perceptual decision making suggests that the P3b reflects the formation of the decision itself, which was based on accumulated perceptual evidence leading to target detection (O'Connell et al., 2012). Similarly, the late Pe has been associated with accumulated evidence for having made a mistake, which when exceeding a threshold leads to its conscious perception and perhaps a deliberate shift in strategy (Steinhauser and Yeung, 2010; Ullsperger et al., 2010). In decision-making based on learned stimulus values, larger P3b was associated with higher subjective response certainty and higher tendency to repeat the current response in future trials (Fischer and Ullsperger, 2013). In contrast, a larger feedback P3b was driven by unfavorable outcomes and high learning rates and predicted a shift in behavior. Taken together, these examples share the notion that evidence in favor of a decision accumulates, which may drive the decision, attention to this decision, and, as a result, conscious awareness and storage of the decision parameters in memory.

At the neuronal level, phasic norepinephrine (NE) release, but also acetylcholine has been proposed to contribute to the P3 (Nieuwenhuis et al., 2005; Polich, 2007). According to the adaptive gain theory (Aston-Jones and Cohen, 2005), phasic NE responses of the locus coeruleus (**Figure 1-2**) enhance cortical responsivity to motivationally salient events and support optimization of task performance. This may result in orienting attention to incoming potentially action-relevant information reflected in the P3a, and subsequent accumulation of evidence in favor of or against adaptation, reflected in the P3b. Notably, evidence accumulation and its conscious appreciation can continue in the absence of or after an overt response.

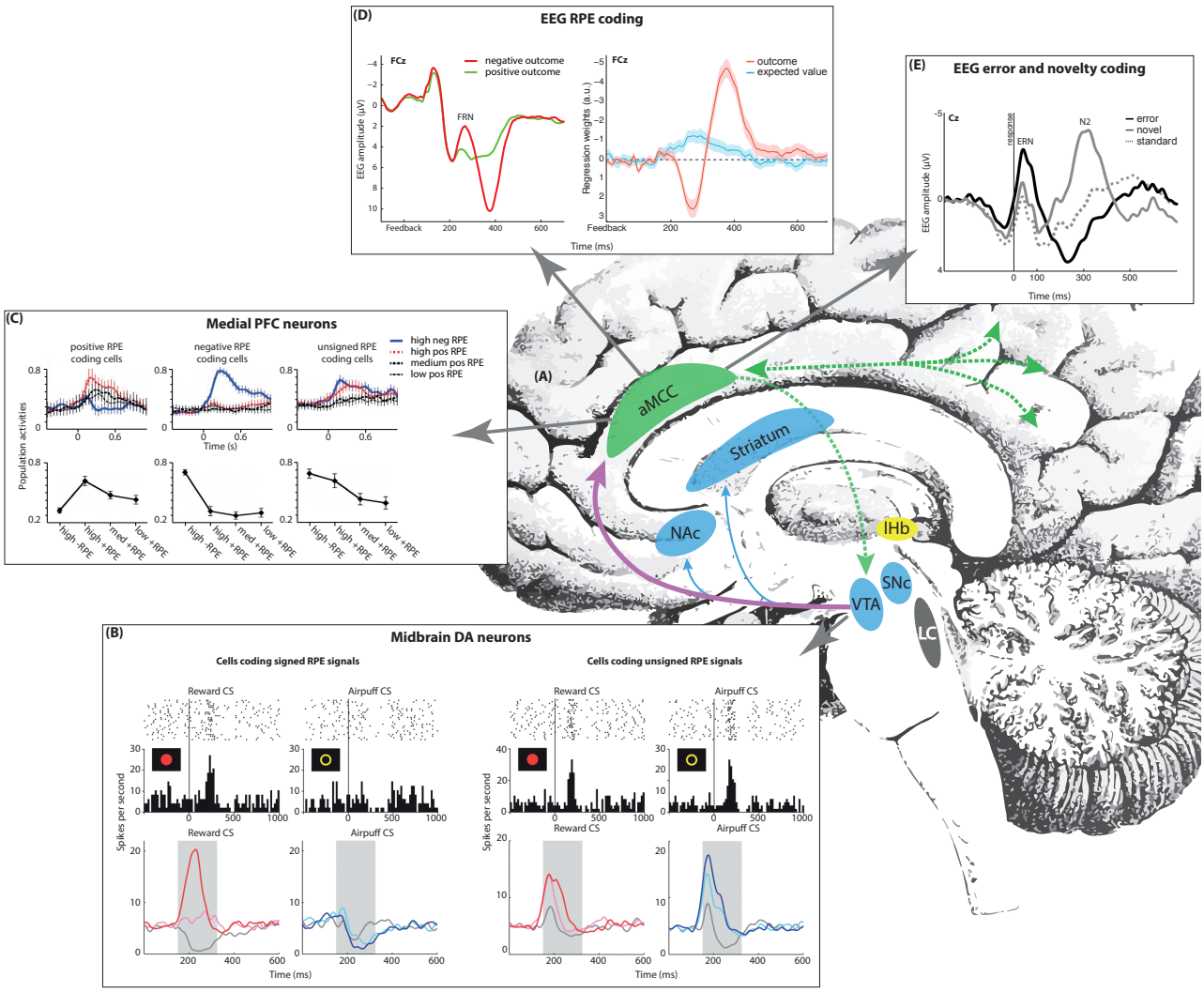

Figure 1-2. Neural implementation of performance monitoring and reinforcement learning processes – signed and unsigned motivational signals in midbrain structures and frontal cortex.

(A) Schematic depiction of selected relevant brain regions and model assumptions. RL theory suggests that aMCC activity is modulated based on VTA RPE signals via mesocortical DA projections (pink arrow). Activity time course of VTA neurons (B) and aMCC responses (C-E) in combinations with slow signal transduction from VTA to aMCC contradict a bottom-up dopaminergic signal causing aMCC responses. Alternatively, aMCC itself could generate PE signals based on sensory input via cortico-cortical projections and transfer them directly or indirectly via the striatum and habenula to the VTA and SNc (green arrows).

(B) Two types of midbrain DA neurons have been differentiated in monkeys. Classic DA neurons code a signed RPE signal for conditioned stimuli (CS) and their firing rate increases for rewards and decreases for aversive events (airpuff). The second type of DA neurons codes unsigned RPE signals and increases firing to both types of stimuli. Top row shows single cell activity for 100% reliable cues and bottom row shows averaged activity where line colors represent reliability of the cues (100% red/blue, 50% pink/ light blue, and 0% grey). Reprinted and modified, with permission, from (Matsumoto and Hikosaka, 2009).

(C) Medial PFC neurons in monkeys. Responses to positive and negative RPEs are displayed. Three types of cells were identified in the medial PFC: positive RPE coding cells (left column), negative RPE coding cells (middle column), and cells that code an unsigned RPE (right column). Averaged time courses (upper row) and averaged magnitude in the time window of 100-400 ms (lower row) after feedback onset are shown. Reprinted and modified, with permission, from (Matsumoto et al., 2007).

(D) EEG RPE coding. Grand average feedback-locked ERPs (left) for probabilistic favorable and unfavorable outcomes show more negative FRNs for losses. Single-trial regression (right) of outcome (win / loss) and expected value (-1 to 1) showed opposite signs of regression weights for both regressors in the FRN time window compatible with RPE coding. Reprinted, with permission, from (Fischer and Ullsperger, 2013).

(E) EEG error and novelty coding. Response errors (eliciting an ERN) and valence-free novel events (eliciting a later N2) were similarly coded in the EEG. In an accompanying fMRI experiment a conjunction of both event types revealed increased hemodynamic aMCC activity. Reprinted, with permission, from (Wessel et al., 2012).

modulate the FRN (Potts et al., 2006; Walsh and Anderson, 2012).

In addition to human EEG findings, neuroimaging and invasive recordings in monkeys and humans demonstrate coding of both, outcome valence and surprise, or, in model-based analyses, signed and unsigned PEs (Wang et al., 2005; Klein et al., 2007; Matsumoto et al., 2007; Jocham et al., 2009; Hayden et al., 2011; Wessel et al., 2012). For example, a study by Matsumoto and colleagues (Matsumoto et al., 2007) revealed the existence of different neurons in the monkey's medial prefrontal cortex that preferentially coded negative, positive, and unsigned PEs, respectively (**Figure 1-2B**). Thus, depending on task context, distribution of activity across these neuronal ensembles may vary, such that the EEG signal can reflect signed and/or unsigned PEs to a different degree.

It has been a matter of debate, how the signed and unsigned RPE signals are conveyed to the pMFC. According to the RL theory, the evaluative signal, determined in the striatum, is thought to influence dopaminergic midbrain neurons such that they can broadcast the RPE. Indeed, neurons in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) have been shown to display phasic changes in firing rate that code a signed RPE in monkeys (Schultz et al., 1997; Bayer and Glimcher, 2005) and humans (Zaghloul et al., 2009). This dopaminergic RPE teaching signal is conveyed to cortical and subcortical structures. In the striatum, it trains the critic to better predict outcomes. In the pMFC, it is used for the selection of appropriate adjustments. It was suggested that the phasic firing decrease of VTA neurons on worse-than-expected outcomes results in a reduction of dopamine (DA) release in the pMFC, which in turn disinhibits the apical dendrites of the pyramidal neurons (Holroyd et al., 2008). As these pyramidal neurons in the ventral bank of the cingulate sulcus are oriented in parallel and perpendicular to the scalp surface, their resulting dendritic excitatory postsynaptic potentials sum up to the frontocentral negativities in the EEG.

While the PRO model does not address the neurobiological source of the signals in the pMFC, a similar dopaminergic mechanism was assumed (Alexander and Brown, 2010), since a subset of VTA neurons has been shown to respond to salience and unexpectedness rather than to signed RPEs (Bromberg-Martin et al., 2010b). Alternatively, the unsigned PE signal might be calculated directly in the cortex in a DA-independent fashion (Ouden et al., 2012).

The neurobiological explanation put forward in the RL theory is appealing, but it makes a number of assumptions that are hard to test directly and some of which are questionable. Therefore, the proposed dopaminergic mechanism of ERN/FRN or reward positivity generation has been challenged repeatedly (Frank et al., 2007; Jocham and Ullsperger, 2009; Ullsperger et al., 2014a). In the following, we will discuss the raised issues and potential alternative mechanisms of information transfer between pMFC, striatum, and VTA/SNc. **Figure 1-2** summarizes the neurobiological evidence and model assumptions.

Do modulations of dopaminergic transmission influence PM?

Yes. Pharmacological studies in volunteers have shown that systemic administration of DA agonists enlarges and DA antagonists reduces the ERN (Jocham and Ullsperger, 2009; Barnes et al., 2013). However, it is unclear whether the ERN modulation reflects a direct DA effect in midcingulate cortex or a remote effect conveyed to the pMFC from the actual site of pharmacological action. A similar argument holds true for genetic association studies, some of which have found the ERN or FRN to be influenced by polymorphisms (**Glossary**) affecting the DA system (Ullsperger, 2010).

Intriguingly, in Parkinson's disease, when DA is depleted mostly from the striatum, the ERN amplitude is reduced and unresponsive to changes in medication (Willemssen et al., 2008). In sum, systemic pharmacological and genetic studies in humans suggest an involvement of DA in PM, but the exact mechanism of DA action in pMFC remains unclear.

Does the dopaminergic system code aversive events, and, if so, how?

Maybe. RL relies on positive and negative PEs. In most cases, unfavorable outcomes are particularly informative with respect to whether and which adaptation is needed. While it seems well established that mesolimbic and mesocortical DA neurons convey positive RPEs by increased burst activity, the coding of aversive events by the DA system has been a matter of intense debate (Bromberg-Martin et al., 2010b; Brooks and Berns, 2013; Fiorillo, 2013; Volman et al., 2013). Several views have been proposed, which are not mutually exclusive: (a) VTA neurons scale their phasic firing rate proportionally to the RPE; thus, negative RPEs lead to a reduction or cessation of firing (Schultz et al., 1997). With a tonic firing rate of 3-8 Hz (Bayer et al., 2007), however, the activity cannot be scaled down in a fine-grained fashion (Niv and Schoenbaum, 2008), and some studies failed to find changes in firing rate related to negative RPEs (Bayer and Glimcher, 2005). If the magnitude of the negative RPE were reflected in the duration of the firing pause (Bayer et al., 2007; Niv and Schoenbaum, 2008), the temporal precision of the signal would suffer dramatically. (b) Positive and negative RPEs may be coded by different VTA neurons. Indeed, a subset of VTA neurons seems to increase firing after aversive stimuli or stimuli predicting aversive outcomes (Mirenowicz and Schultz, 1996; Brischoux et al., 2009; Matsumoto and Hikosaka, 2009). However, given the difficulties in identifying dopaminergic neurons unequivocally in vivo (Ungless et al., 2004; Fields et al., 2007), it is questionable whether VTA neurons coding aversive events release DA (Ungless and Grace, 2012). (c) Some studies suggest that mesencephalic DA neurons do not code aversive events beyond being inhibited (Fiorillo, 2013). Instead, the serotonergic system has been proposed to code aversive outcomes, although no unequivocal negative RPE signal has been recorded from raphé neurons so far (Cools et al., 2010). Pharmacological interventions have failed to show an effect of serotonin on the ERN and other measures of PM (Endrass et al., 2008; Jocham and Ullsperger, 2009). Moreover, recent optogenetic work suggests that activity of GABAergic neurons within the VTA or those projecting to it contribute to aversive conditioning (Tan et al., 2012; Stamatakis et al., 2013), suggesting that at least the inhibition of DA neurons in VTA is important for learning from negative outcomes. Thus, there is evidence that the VTA codes aversive outcomes, but more research is needed.

Can a change in firing rate of DA neurons result in a fast change in PSPs?

No – at least not via a dopaminergic mechanism. DA is a neuromodulator whose synaptic action in the prefrontal cortex (PFC) is highly complex and depends on concomitant activity in other afferent neurons to the PFC target neurons as well as on the state of the target neuron itself (Seamans and Yang, 2004). In vivo and in vitro recordings suggest that DA release alone does not elicit rapid transient postsynaptic potentials (Fields et al., 2007). In combination with other inputs, however, e.g. when the VTA is stimulated at physiological frequencies, a rapid biphasic

EPSP/IPSP sequence is observed. Notably, this rapid response can be blocked by application of glutamatergic antagonists but not by DA receptor antagonists to the PFC (Lavin et al., 2005). Burst stimulation of the VTA resulted in a DA increase in PFC. However, as DA transporter expression in the PFC is low, the increase in extracellular DA evoked by stimulation of the VTA slowly decayed back to baseline within 2s (Lavin et al., 2005). Thus, the proposed mechanism of ERN/FRN generation through a sudden decrease in DA in aMCC (Holroyd et al., 2008) does not seem to fit with the current knowledge of a synaptic action of DA. In addition, the more recent interpretation that only DA release on positive RPEs would result in a reward positivity at the FRN latency (Holroyd et al., 2008) does not seem to be consistent with current knowledge on cortical DA action.

So how is the PE signal conveyed to the pMFC? One alternative explanation could be based on the fact that in rats fewer than 60% of VTA neurons are dopaminergic of which only 30-40% project to the PFC (Fields et al., 2007). Approximately 15% of VTA neurons appear to be glutamatergic (Volman et al., 2013). It has been suggested that dopaminergic VTA neurons may co-release glutamate (Chuhma et al., 2004; Lavin et al., 2005; Fields et al., 2007). Based on firing patterns of VTA neurons, one would expect that glutamate is released more on positive RPEs and less on negative RPEs. Reduced glutamate levels on negative RPEs seem at odds with the proposal that ERN and FRN are elicited by disinhibition of midcingulate pyramidal neurons. Interestingly, 25% of VTA neurons are GABAergic. Many of them are interneurons, but GABAergic projections from the VTA to PFC (Carr and Sesack, 2000) and striatum (van Zessen et al., 2012) have been demonstrated. One could speculate that via such inhibitory GABAergic projections positive and negative RPEs could rapidly inhibit and disinhibit, respectively, aMCC neurons thereby modulating the frontocentral negativities.

Which region processes outcome information and PE first?

In contrast to the original RL theory (Holroyd et al., 2008), it has been speculated that the PE is sent from the midcingulate cortex to the brain stem (Jocham and Ullsperger, 2009) rather than vice versa. A number of top-down pathways could mediate this information flow either directly, or via the striatum and habenula to the VTA and SNc. Also top-down connections to the locus coeruleus (LC) are known, where surprise appears to be associated with phasic responses of norepinephrinergic neurons (Aston-Jones and Cohen, 2005b; Nieuwenhuis et al., 2005). We are not aware of simultaneous recordings from pMFC and VTA (or LC), which could answer the question in which direction information is conveyed. Separate studies recording either from the cingulate or VTA neurons suggest similar latencies of phasic firing increase after reward or reward-predicting cues in instrumental learning tasks (e.g., maximal spiking around 200-300ms in VTA (Matsumoto and Hikosaka, 2007; Zaghloul et al., 2009) and 200-400ms in pMFC (Matsumoto et al., 2007)). However, recordings from the same animals performing the same tasks would be needed to draw firm conclusions. Notably, dopaminergic midbrain neurons are characterized by very low conduction velocities (as low as 0.5 m/s in rats and 1.2 m/s in monkeys (Schultz and Romo, 1987; Chuhma et al., 2004)) and are usually unmyelinated. Thus, a dopaminergic signal from VTA to pMFC would be significantly delayed, which seems inconsistent with the nearly identical latencies of RPE-related responses in VTA and pMFC and with the latency of the

FRN. On the other hand, changes in VTA neuron firing following stimulation of pMFC are observed after ~20 ms (Lodge, 2011).

Given that calculation of PEs appears to be a general principle applied throughout the human brain (Ouden et al., 2012), which is often independent of DA (e.g., in perceptual cortices), one may speculate that the signed and unsigned PE signals found in the mesencephalon and in the pMFC could be derived by parallel and to some extent independent computations.

Concluding Remarks

Current evidence and theoretical models converge on the notion that the pMFC, in connection with the basal ganglia and brain stem nuclei, extracts and weights information on the necessity of adaptation from motivationally salient events. This weighted adaptation signal is used to update value representations and to signal the need of motor, attentional, and affective/motivational adjustments to the appropriate brain regions (King et al., 2010; Danielmeier et al., 2011; Wessel et al., 2012). It appears that comparing predictions with evidence on upcoming and current action outcomes gives rise to both, signed and unsigned PEs. Weighted by parameters reflecting the statistics of the environment and reinforcement history, such as volatility, risk, and learning rate, these PEs seem to form the basis of the adaptation signal in the pMFC. Both, surprise and signed reward PEs, appear to play important roles for adaptation and learning. Surprise signals may allow to rapidly re-orient attention and prepare the organism for the necessity to adapt, as reflected, for example, in slowing after errors and other unexpected action outcomes (Wessel et al., 2012). Moreover, an unsigned PE signal would be sufficient to enhance selective attention based on known task-rules (King et al., 2010; Danielmeier et al., 2011). Surprise signals may furthermore be used to modulate the weighting factor applied to signed RPEs in reinforcement learning (Ouden et al., 2012; Roesch et al., 2012). The sign of the PE, indicating whether the outcome is better or worse than expected, is needed to determine the direction of RL, that is, to decide whether to repeat or avoid an action. Thus, it is conceivable that different PE signals in the PM system drive different types of adaptation. This could be tested using model-based EEG by disentangling the effects of surprise and reward PE and linking them to post-error adjustments. A potential problem may be that surprise and signed PEs are often highly correlated, particularly when frequencies of action outcomes are unequally distributed.

Despite the enormous progress in PM research, the neurobiological underpinnings are still remarkably poorly understood. Combining surface EEG, invasive recordings, and stimulation techniques will be needed to link the neuronal and systemic levels of research in this field. The proposed uniform sequence of processes and EEG correlates can serve as a basis for this future research in humans and animals addressing a number of important outstanding questions.

CHAPTER

2

An Introduction to the 5-HT System and a Suggestion for the SSRI Paradox

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Physiology and General Functions of the Serotonergic System

Anatomy, synthesis and signaling of 5-HT

Much of the current knowledge about the precise function of the serotonergic system is derived from animal studies and for the following sections, when human data is not available, descriptions will be based on studies of other species. 5-HT is a neurotransmitter which primates share with ancient predecessors as old as the avertebrate Aplysia (Goldsmith and Abrams, 1991). The name *serotonin* is derived from its ability to constrict peripheral vessels when released in high doses from blood platelets following injury (Rapport et al., 1948). Within the central nervous system (CNS), about half a million serotonergic cells reside almost exclusively in the raphe nuclei of the midbrain (**Glossary**), where they constitute around 40% of the population but some subregions, like the interfascicular part of the dorsal raphe nucleus (DRN), consist exclusively out of serotonergic cells (Hornung, 2003). The raphe nuclei provide serotonergic innervation to almost the entire forebrain, including the neocortex, but some regions, like amygdala, hippocampus, and striatum, are especially densely innervated. The frontal lobe is the most tightly innervated cortical region and also shows the highest density of 5-HT receptors which generally display a rostro-caudal gradient (Celada et al., 2013) suggesting an especially important role of 5-HT in higher cognitive and executive functions. The main source of input to raphe nuclei is the lateral habenula via excitatory amino acids (Hornung, 2013) and, at least the DRN, also receives modulatory input from the medial prefrontal cortex (mPFC) (Celada et al., 2001). Other afferent projections arrive from basal forebrain structures such as the amygdala but also include serotonergic projections between dorsal and median parts of raphe nuclei, dopaminergic projections from the substantia nigra (SN) and ventral tegmental area (VTA), and noradrenergic projections from the locus coeruleus (LC) (Molliver, 1987). Other more caudally located raphe nuclei in mid-pons and caudal medulla project within the brain-stem and to the spinal cord (Hornung, 2013). This pattern of connectivity renders the serotonergic system in an optimal position to modulate neural processing from sensory afferents, information evaluation itself to motor output – and indeed 5-HT has been implicated on all of these instances of brain functions (Lucki, 1998).

There appear to exist two rather distinct patterns of serotonergic innervation: in median raphe nuclei (MRN) serotonergic cells have thick, non-varicose, myelinated fibers that form well-defined synapses with their target cells (Törk, 1990). The dorsal raphe nuclei (DRN) neurons, that form the majority of the serotonergic input to the cortex, show highly branched, thin fibers with very small boutons and non-synaptic terminals that remain difficult to trace (Hensler, 2006). Both systems also differ in their respective target regions. The MRN project dominantly to septum and hippocampus while the striatum is mainly innervated by the DRN – most structures, however, receive input from MRN and DRN (Törk, 1990). In both areas cells are clustered in particular zones that show common targets and projection fibers send collaterals that innervate functionally related structures like amygdala and hippocampus or SN and putamen (Hensler, 2006). This implies rather specific functions for subsets of these serotonergic neurons as opposed to only a broad modulatory role.

5-HT is synthesized from the essential aminoacid trytophan (Trp) in a two-step process: first Trp is hydrolyzed to 5-hydroxytryptophan (5-HTP) by the rate-limiting enzyme tryptophan-hdyroxylase (TPH). This is followed by consecutive

decarboxylation to 5-HT via the unspecific aromatic amino acid decarboxylase (AADC) and the product is stored in presynaptic vesicles by the vesicular monoamine transporter type-2 (VMAT2). At physiologic pH levels, 5-HT is hydrophilic and thus cannot pass the lipophilic blood-brain-barrier. Therefore, it has to be synthesized in the brain independent of the peripheral 5-HT production. Two isoforms of TPH exist and TPH1 is mainly expressed in peripheral tissue while TPH2 seems to be exclusively expressed in 5-HT producing CNS cells (Walther et al., 2003). Thus, the distribution of 5-HT closely follows that of TPH2 in the brain and expression of TPH2 is an established marker of serotonergic cells.

The synthesis of 5-HT can be increased when demand is high: electrical stimulation of serotonergic cell's somata leads to a frequency dependent increase in TPH mRNA and consecutively increased 5-HT (Boadle-Biber, 1993) and also following partial desctruction of the 5-HT system increased TPH activity and concentrations are found in the remaining neurons (García-Osta et al., 2004). Additionally, as both 5-HT synthesizing enzymes are likely not saturated at physiologic levels, increased intake of Trp as well as 5-HTP leads to an increase of available 5-HT (Hensler, 2006). However, due to the unspecificity of AADC in the latter case, 5-HT can be found in non-serotonergic neurons following increased dietary intake of 5-HTP. Thus, 5-HT concentrations partly depend on the amount of peripherally available Trp and 5-HTP – which in turn depends on the activity of the peripheral AADC that can be blocked for example by peripheral decarboxylase inhibitors like levodopa (Turner et al., 2006).

The release of 5-HT via exocytosis is significantly modulated by firing rates of serotonergic cells within the raphe nuclei. An increase in firing rate leads to an increase of 5-HT release and vice versa for a decrease (Sharp et al., 1989). Therefore, drugs modulating raphe neuron firing rates can be expected to also modulate 5-HT release.

Currently, more than 14 different subtypes of serotonin receptors are known which, based on their pharmacological properties, are divided into three main families ($5\text{-HT}_{1\text{-}3}$). These serve excitatory as well as inhibitory functions via controlling ion channels and modulate phosphorylation via adenylyl cyclase and phospholipase C (Hornung, 2013). For many of these receptors physiological relevance has been demonstrated, sometimes in opposing ways (Celada et al., 2001), but others still have unknown functions (Hensler, 2006). Furthermore, 5-HT does not only act on postsynaptic neurons via classical synapses, but also is assumed to diffuse away from the point of release via volume conduction. Especially the thin, varicose projections of the DRN appear to function in such a manner (Hensler, 2006). Like DA neurons, the mostly unmyelinated 5-HT neurons transmit a slow signal and in rats, orthodromic 5-HT signaling from DRN to the PFC requires approximately 25 ms (Celada et al., 2013). Due to the sheer number of 5-HT receptors these will not be explicated here in more detail but, when specific functions can be related to receptor subtypes, will be named in the following sections.

Like for many monoaminergic neurotransmitters, the effects of 5-HT are terminated predominantly by binding to a specific transporter protein that spans the presynaptic membrane – the 5-HT transporter (5-HTT). mRNA expression of this transporter in the CNS has exclusively been found in serotonergic neurons but there is considerable homology between 5-HTT and other monoamine transporters and in situations where one is compromised, other transporters seem to incur their mutual substrates (Zahniser and Doolen, 2001). 5-HTT are mainly located perisynaptically and are not confined to the axon terminals which again suggests that 5-HT can diffuse away from its point of release to a certain degree (Tao-Cheng and Zhou, 1999). After its reuptake into the presynaptic neuron, 5-HT is either relocalized

into storage vesicles by VMAT2 or metabolized preferentially by the monoamine oxidase A (MAO-A) to 5-hydroxyindoleacetic acid (5-HIAA). MOA-A is localized to the mitochondrial membrane and interestingly serotonergic neurons preferentially express MAO-B that is much less affine to 5-HT (Levitt et al., 1982). This likely serves the purpose of eliminating other monoamines that may accumulate during the partly unspecific synthesis of 5-HT.

Tight feedback mechanisms control the release of 5-HT via autoreceptors. 5-HT_{1A} autoreceptors are predominantly localized around serotonergic soma where their activation leads to reduced firing rates and thus serotonergic release of raphe neurons. This is achieved by opening potassium channels and consecutive hyperpolarization of the cells. The physiological significance of 5-HT_{1A} autoreceptors in vivo has been the matter of some debate. Administration of the potent 5-HT_{1A} antagonist WAY 100635 did not increase basal 5-HT DRN neuron firing rates in freely moving rats (Johnson et al., 2002). This effect, however, may be explained by an additional suppressive effect of that antagonist on LC neuronal firing which counteracts the firing rate increase mediated by blocking 5-HT_{1A} receptors (see below) (Haddjeri et al., 2004) and the current understanding is that 5-HT_{1A} autoreceptors effectively control 5-HT neuron firing and transmitter release both *in vitro* and *in vivo*. Another type of autoreceptors are the 5-$HT_{1B/D}$ family. It was initially assumed that the 5-HT_{1D} receptor was the human homologue of the 5-HT_{1B} receptor that was found to be expressed abundantly in rodents brains, but more recent evidence suggest that both receptors are expressed in rodents and humans alike and they are in most studies grouped as 5-$HT_{1B/D}$ receptors due to their similarities (Roth, 2008). These receptors are mainly found at the axonal end of serotonergic neurons where they reduce 5-HT release without affecting firing rate and also increase the activity of the 5-HTT (Daws et al., 2000). Note that both autoreceptors also serve as heteroceptors at the postsynaptic membrane.

The effects which 5-HT exerts on cortical neurons are mainly inhibitory but complex and depend on the ratio of serotonergic receptor subtypes present in a specific region. As mentioned before, 5-HT_{1A} receptors are inhibitory, but 5-HT_{2A} receptors have excitatory effects and decrease the permeability of potassium channels (Hensler, 2006). 5-HT_{2A} receptors appear to be localized mainly to the apical dendrites of cortical pyramidal neurons whereas 5-HT_{1A} receptors are found more pronounced around the axon hillock where they form axo-axonic contacts (Celada et al., 2013). This would put 5-HT in an optimal position to control the excitatory input and also limit the likelihood of action potential generation of cortical pyramidal cells. Indeed, application of 5-HT to human cortical neurons in vitro shows opposing effects regarding excitability depending on the receptor subtype most prominent (Newberry et al., 1999) but seems to also be dose dependent. This dose dependency may result from the sensitivity of GABAergic interneurons to 5-HT which themselves are inhibited by 5-HT via 5-HT_{1A} receptors (Celada et al., 2013). In addition, roughly 80% of cortical pyramidal neurons co-express 5-HT_{1A} and 5-HT_{2A} receptor mRNA (Santana et al., 2004). Even within the same cortical region, some neurons are inhibited, some are excited, and some display bi-phasic responses to 5-HT whereby an early hyperpolarization is followed by a longer lasting depolarization (Celada et al., 2013). It was recently found that this effect may be less dependent on the investigated area, but crucially depend on the projection region of the pyramidal neurons themselves. While corticopontine-projecting neurons were universally inhibited by 5-HT, commissural neurons showed mainly excitatory responses (Avesar and Gulledge, 2012). This would suggest that 5-HT decreases corticopontine output but enhances intracortical information processing.

In sum, the serotonergic system shows characteristics of a system ideally located to generally modulate complex functioning, but its structure also strongly suggests projection- as well as region-dependent response-specificity.

Interactions with other neuromodulatory systems

It should be mentioned that all neuromodulatory systems function in complex interdependence of the state of each other and plenty interactions exist. The most well-studied interaction between the 5-HT system and other neuromodulatory agents is the suggestion of an opponent interaction with the dopaminergic system in that 5-HT exhibits tonic inhibitory control over DA release (Hervé et al., 1987). This simple picture, however, does not hold as 5-HT can both inhibit and promote DA release in various dopaminergic regions depending on the 5-HT receptor subtype most prevalent (Porras et al., 2002). Optogenetic stimulation of DRN neuron terminals directly within VTA and NAc suggests that the glutamatergic part of the dual signal released from raphe neurons (see below) initially causes a fast EPSP in DA cells within this region which is followed by a longer lasting 5-HT mediated inhibition (Liu et al., 2014). Therefore, currently no clear conclusion can be drawn as to whether serotonergic neurons mainly inhibit or excite the DA system and likely both systems play intertwined roles by influencing differential aspects of reward processing like wanting and liking (Berridge et al., 2009) and the ability to wait for delayed rewards (Ranade et al., 2014).

The tonic firing rates of serotonergic neurons seems to crucially depend on NE levels within dorsal and possibly median raphe nuclei (Baraban and Aghajanian, 1980; Judge and Gartside, 2006) with higher NE levels associated with higher 5-HT neuron firing rates. Vice versa, serotonergic input can down-regulate LC neuron firing and destruction of the serotonergic system with 5,7-DHT leads to increased LC neuron firing rates (Szabo and Blier, 2001). The temporal dimension of these effects is less clear, as only prolonged treatment with paroxetine (Szabo et al., 1999) and citalopram (Szabo et al., 2000) led to significant decreases of LC neuron firing rates, whereas NA reuptake inhibitors, like desipramine, exhibit rapid effects on firing rates of raphe neurons (Szabo et al., 2000).

Additionally, it has been observed that administration of an SSRI, both systemically and directly to the brain, enhances release of acetylcholine from various brain regions (Consolo et al., 1994; Yamaguchi et al., 1997). It has been proposed that this mechanism explains the behavioral flexibility enhancing effects associated with higher 5-HT levels in some studies (Brown et al., 2012).

In sum, while it is important to bear in mind that none of the brains neuromodulators can in fact be influenced with complete specificity, a satisfactory picture of how neuromodulatory systems interact precisely is currently missing. Therefore, interpretations of most findings in this thesis will be focussed to explanations regarding the serotonergic system itself to limit the room of possible alternative explanations which otherwise appears unfeasible.

Is there a general function of 5-HT?

Naming one essential function for the main role that 5-HT serves in maintaining the homeostasis of an organism is probably most challenging among all central monoamine transmitters (Cools et al., 2008). Therefore, this paragraph

can only serve as a brief introduction into general ideas regarding 5-HTs role, while PM functions and their relation to 5-HT will be discussed in more detail in the following paragraphs and especially **Chapters 4-8**.

A common observation with regard to a general role of 5-HT is that although it is implicated in the control of many behaviors like feeding, circadian rhythms, sexual behavior, stress regulation, and escaping painful stimuli, these systems continue to function even when the serotonergic system is obliterated (Lucki, 1998). This led to the suggestion that 5-HT subserves mainly broad modulatory functions. This was further corroborated by early electrophysiological recordings of tonic firing rates of raphe neurons measured in cats which seemed to be mainly dependent upon general states of arousal and behavioral activation. They usually ranged from 0.5-2.5 Hz, with slightly higher firing rates seen in rodents (Rueter et al., 1997). During REM sleep, when muscle tone is lowest, presumed 5-HT neurons were found to be completely inhibited (Lucki, 1998) while they otherwise fired spontaneously and regularly – which was described as *clock like* (Judge and Gartside, 2006). These observations spoke to a general modulatory role of 5-HT with a low adaptive range and the idea that 5-HT facilitates motor output during wakeful stages. Wakefulness and motor activity, however, is confounded with plenty other variables that may drive this overall correlation. More recent findings show a broader adaptive range including much higher firing rates (Kocsis et al., 2006) and also microdialysis studies showed dramatic and specific regional changes of 5-HT concentrations in certain situations. In cats, exemplum gratum, different kinds of stressors showed independent effects on 5-HT levels in the striatum and hippocampus (Kirby et al., 1997). Electrophysiological recordings in freely moving rats showed activity increases in DRN neurons during specific actions in a rewarded two choice discrimination task. Many neurons showed movement related activity but this was specific to certain task situation, e.g. a nose poke into the odor- but not the water-delivery-unit (Ranade and Mainen, 2009). Furthermore, some neurons showed reward related activity and coded the time-point of expected delivery and some were active during the sampling of a specific odor, demonstrating a high degree of specificity of response patterns of raphe neurons. Therefore, the physiological effects of DRN neurons that are likely serotonergic are not restricted to a general modulation.

In this line, principal component analysis of different aspects coded in raphe neurons recorded in monkeys revealed that most variance of raphe signals was related to the coding of task and reward value, changing activity when progressing towards the reward. Furthermore, the actual reward delivery itself was coded by the same neurons (Nakamura et al., 2008; Bromberg-Martin et al., 2010a). Roughly half of these neurons increased and the other half decreased activity in association to the reward, suggesting that the raphe is an important locus in the brain‘s reward network.

It should be noted that identifying 5-HT neurons in electrophysiological recordings is complicated and that more recent studies found no correlation between the coding of certain task properties, like reward, and physiological characteristics classically ascribed to serotonergic neurons, like slow firing rates (Nakamura et al., 2008; Ranade and Mainen, 2009; Bromberg-Martin et al., 2010a). For most studies investigating DA systems, neurons are identified as dopaminergic if they encode an RPE signal (Cools et al., 2010). As no such clear cut coding scheme is known for 5-HT, it is likely that other neurons have been recorded in the raphe nuclei in the above mentioned studies in proportion to their normal distribution, that is between 30-50% serotonergic. A more elegant way of solving this problem has recently been developed employing optogenetic targeting of 5-HT neurons. A recent study in mice specifically

targeted 5-HT raphe neurons by recording Pet-1, an E26 transformation specific protein found selectively in serotonergic neurons, expressing cells (Liu et al., 2014). This enabled targeting of TPH2 expressing, serotonergic cells with ~97% sensitivity and ~92% specificity. These cells activity increased from baseline to about 20 Hz when mice were waiting for a delayed reward providing strong evidence for specific modulations of different functions.

However, so far it has been difficult to determine a common denominator of serotonergic functions. It has been suggested that 5-HT may shield motor output from distracting inhibitory events by disfacilitating the processing of salient events, as would be indicated by short bursts of activity to sensory signals (Ranade and Mainen, 2009; Bromberg-Martin et al., 2010a). Vice versa, abrupt orientation to sensory stimuli seems to suppress firing of serotonergic neurons (Baumgarten and Grozdanovic, 1995). This general role of 5-HT seems to be more specific when related to potentially punishing behaviors. Recently, it has been suggested that the serotonergic system monitors expected sensory input and internal states for relevant changes and conveys this information in order to facilitate *vigilance behavior* (Homberg, 2012). Another prominent suggestion positions the serotonergic system as a mediator of behavioral inhibition in the face of aversive expectations, suggestive of an opponency to the dopaminergic system (Daw et al., 2002; Dayan and Huys, 2008; Tops et al., 2009; Boureau and Dayan, 2011). It appears quite consistent that serotonergic activity increases during the delay period for a reward and may then inhibit impulsive behavior (Bromberg-Martin et al., 2010a; Liu et al., 2014). However, a unifying theory and a formalized model which could explain serotonergic effects in the diverse functions 5-HT has been implicated in, remain to be found despite these various suggestions. For a diversification of serotonergic signaling taking into account the idea that 5-HT neurons release dual signals including glutamate, see below.

Dual Serotonergic Signals: Key to Understanding Paradoxical Effects?

The paradox of SSRI action

Neuromodulatory systems arising from the midbrain provide monoaminergic innervation to almost the entire forebrain and have been identified as essential factors contributing to human behavior in health and disease. One of the major systems that have generated substantial research interest is the serotonergic (5-HT) system (Lucki, 1998). Among the functions modulated by 5-HT are emotional regulation, processing of reward and punishment, delay discounting and behavioral inhibition (Hariri and Holmes, 2006; Cools et al., 2008; Dayan and Huys, 2009; Boureau and Dayan, 2011). In order to understand the consequences of changes in neuromodulatory systems, it is essential to have a framework at hand which provides clear mechanistic insights on how to interpret the effects of manipulations – pharmacological, genetic or otherwise. Such an understanding requires the translation of animal research to well controlled human studies and patient populations. However, research tools that can be applied to animals, humans, and patient populations alike are sparse.

One of these are selective serotonin reuptake inhibitors (SSRI, **Glossary**), which are among the most widely prescribed drugs in psychiatric disorders as diverse as depression, anxiety, and eating disorders. They increase extracellular serotonin levels by blocking presynaptic serotonin transporters (5-HTT, **Glossary**), which usually translocate extracellular 5-HT back into the releasing neuron (Stahl, 1998). It has long been recognized that the acute and long-term effects of SSRIs differ (Cools et al., 2008; 2010), but the exact mechanisms to explain this discrepancy remains unclear. In the field of cognitive neuroscience, this has led to problems in interpreting results from pharmacological studies. For example, it has been shown that destruction of prefrontal serotonergic innervation in animal studies using the selective neurotoxin 5,7-DHT (see **Box 2-1** and **Glossary**) leads to pronounced impairments in an animal´s ability to reverse a learned response tendency (Clarke et al., 2004). Thus, serotonin is deemed essential for this cognitive capacity. Surprisingly, acute SSRI administration in healthy human subjects revealed the same pattern as destruction of the 5-HT system. SSRI increased the probability of inappropriate response switches and the number of reversal errors significantly (Chamberlain et al., 2006). In other words, it had the same effect as a *reduction* in 5-HT transmission following 5,7-DHT lesions (Cools et al., 2008). Studies on behavioral inhibition and impulsivity have likewise produced conflicting results. Animal studies have found that 5,7-DHT application leads to increased impulsive behavior (Harrison et al., 1997), genetic manipulations leading to increased 5-HT levels decrease impulsive behavior (Homberg et al., 2007), and 5-HT levels appear to be reduced in depressed patients attempting suicide (Linnoila and Virkkunen, 1992). Yet, SSRI application in human studies increased active responding, again suggesting a reduction of 5-HT rather than an increase (Guitart-Masip et al., 2013). Some studies, however, found effects of acute SSRI administration that are compatible with increased 5-HT neurotransmission. The ability to choose larger, but delayed rewards, is associated with increased 5-HT levels in animal studies (Miyazaki et al., 2014), and has also been reported to increase following acute SSRI administration in rats (Bizot et al., 1988; 1999), suggesting an increase in 5-HT.

Furthermore, despite clear clinical efficacy of prolonged SSRI administration in depressive and anxiety-related disorders, patients rarely benefit in the first weeks of treatment. In fact, increased anxiety and also a higher risk for

Box 2-1. Relevant techniques and their relation to dual signals

5,7-dihydroxytryptamine (5,7-DHT): the neurotoxin 5,7-DHT can be used to selectively destroy serotonergic raphe neurons in animal studies. This complete obliteration unselectively impairs both components of the dual signal. Thus, animal studies on cognitive impairments following the use of this technique can be used to infer the functional significance of the intact serotonergic dual signal.

Acute tryptophan depletion (ATD): a method to temporarily lower serotonin levels in the brain that is widely used in cognitive neuroscience and can be applied in human and animal studies alike. The amino acid tryptophan (Trp) is essential for the synthesis of 5-HT and by ingestion of a solution that lacks Trp but contains other amino acids with similar properties, the transport of Trp through the blood brain barrier is reduced. This consequently leads to lowered brain 5-HT levels on a time scale of approximately one to six hours (Fadda et al., 2000). The expected increase in raphe neuron firing rates due to less extracellular 5-HT has not yet been confirmed (Trulson, 1985) and thus ATD may decrease the serotonergic component of the dual signal but appears to leave the glutamatergic component unaffected. However, the specificity of this technique is not undisputed (van Donkelaar et al., 2011).

Optogenetics: a technique in which a gene encoding a light sensitive ion channel is introduced into specific neuronal populations and consecutively stimulated with light of a certain spectrum. Light stimulation can be used either to excite or inhibit a neuron depending on the ion channel expressed. In the case of excitation, activation will correspond to the frequency of optical stimulation (Boyden et al., 2005; Zhang et al., 2007). Neuronal specificity can be obtained by introducing a promoter that is activated only in certain neuronal populations, e.g. tryptophan hydroxylase 2 which is almost exclusively expressed in 5-HT neurons. When either combined with pharmacological inhibition of serotonergic or glutamatergic receptors or experimental animals that lack one of these receptor types, the dual signal can be dissected into both components (Liu et al., 2014).

suicide have been reported (Burghardt and Bauer, 2013; Miller et al., 2014). These discrepancies are puzzling because the most pronounced effect of SSRI administration, an increase in extracellular 5-HT levels, is evident immediately after first administration of the drug.

Physiology of serotonergic activity and SSRI effects

The 5-HT_{1A} receptor acts as an inhibitory hetero- and autoreceptor (**Glossary**) and controls the generation of action potentials of serotonergic neurons. Its relevance for 5-HT signaling has been demonstrated both *in vitro* and *in vivo* (Haddjeri et al., 2004), and its activation increases the permeability of potassium channels which causes hyperpolarization (Hensler, 2006). Its distribution appears to be especially dense within the raphe nuclei themselves (Ito et al., 1999), where most serotonergic neurons reside (Hornung, 2013). It has long since been recognized that acute administration of antidepressant drugs reduces raphe neuron firing rates mediated by these autoreceptors which become activated by the increased extracellular 5-HT levels (Scuvée-Moreau and Dresse, 1979; Gartside et al., 1995). Importantly, this decrease in firing rates is dose dependent, where only high doses cause a complete cessation of raphe neuron firing (Hajós et al., 1995). However, although this negative feedback mechanism effectively controls serotonergic neuron firing rates and, partly, 5-HT release, acute administration of SSRIs quickly and strongly increases extracellular 5-HT levels in most projection regions, as evidenced by microdialysis studies (Marek et al., 2005). Although the amount of increase appears to be region-specific (Beyer and Cremers, 2008) and dose dependent, with some studies reporting no change in 5-HT levels at low SSRI doses (Invernizzi et al., 1992), an actual decrease in 5-HT levels has not been reported following SSRI administration (Beyer and Cremers, 2008). This raises the question of how studies involving acute SSRI administration find effects compatible with decreases rather than increases of serotonergic transmission (Chamberlain et al., 2006; Guitart-Masip et al., 2013).

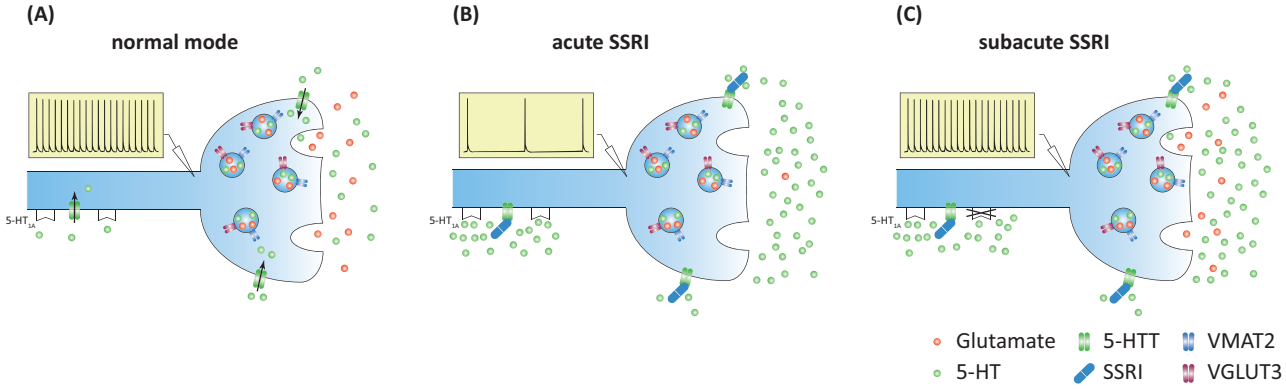

Figure 2-1 Schematic of normal serotonergic signaling (A), hypothesized effects of acute (B), and sub-acute (C) SSRI administration. Without perturbations, serotonergic neurons co-release glutamate and 5-HT as a dual signal in an exocytotic manner by merging presynaptic vesicles with the cell membrane. 5-HT and glutamate are stored in these vesicles by unique transporters (VMAT2 and VGLUT3, see Glossary) known to be co-expressed in serotonergic neurons (Mestikawy et al., 2011). The release is triggered upon arrival of action potentials (schematic recording depicted in the yellow box) (Liu et al., 2014). Acute administration of an SSRI (B) blocks the 5-HTT in the projection region (right), which leads to an increase of extracellular 5-HT levels. However, because 5-HTTs control the reuptake not only in projection regions but also within the raphe nuclei themselves, this also leads to increased 5-HT levels in vicinity to 5-HT_{1A} autoreceptors (left) (Invernizzi et al., 1992). These autoreceptors affect the neurons firing rate by causing hyperpolarization and thus, acute SSRI administration decreases firing rates (Scuvée-Moreau and Dresse, 1979; Gartside et al., 1995; Hajós et al., 1995). In turn, less glutamate and 5-HT is released from the neuron, but due to the blockade of the 5-HTTs by the SSRI, only the glutamatergic component of the dual signal is decreased – while 5-HT is increased. When autoreceptors desensitize (C), approximately after 2 weeks of SSRI intake, firing rates return to baseline (Mansari et al., 2005). This restores the glutamatergic component of the dual signal while 5-HT levels remain elevated. This dissociation of the dual signal would explain the delayed onset of SSRI remedial effects in depression as well as contradictory findings in studies employing acute SSRI administration which suggested decreases in 5-HT neurotransmission.
5-HT = 5-hydroxytryptophan, 5-HTT = 5-HT transporter, SSRI = selective serotonin reuptake inhibitor, VMAT2 = vesicular

Relevance for short and long term drug effects

To explain the delayed onset of depressive symptom improvement with SSRI treatment, a desensitization or internalization of 5-HT_{1A} autoreceptors in the first weeks of treatment has been proposed (Blier and de Montigny, 1994). The idea holds that the increase of extracellular 5-HT is initially counteracted by autoreceptor activation and thus limited, while the desensitization later on allows for further increases. In fact, in depression, combined treatment with an SSRI and the 5-HT_{1A} receptor antagonist pindolol improves the speed of onset within the first 2 weeks of treatment, but not the overall remission rates later on (Ballesteros and Callado, 2004). Furthermore, selectively deactivating 5-HT_{1A} auto- but not hetero-receptors also mediates immediate antidepressant effects in mice (Bortolozzi et al., 2011). The desensitization appears to increase raphe neuron firing rates in a time window compatible with treatment response onsets in rats (Mansari et al., 2005) whereby degree of desensitization has also been shown to strongly correlate with treatment response in mice (Popa et al., 2010). However, this is not true for extracellular 5-HT levels, which appear to remain rather constant and even decline during the course of drug administration when measured repeatedly in monkeys (Smith et al., 2000; Anderson et al., 2007); and also do not correlate with treatment response in mice (Popa et al., 2010). Other rodent studies reported mixed results regarding 5-HT levels after prolonged SSRI treatment (Kreiss and Lucki, 1995; Gardier et al., 2004), which may be explained by depletion of 5-HT in the brain caused by prolonged 5-HTT blockade with SSRI (Caccia et al., 1992) or chronic consumption of drugs with

similar mechanisms (Müller and Homberg, 2014). Furthermore, in humans a dose-response association was not found for SSRI in the treatment of depression (Bollini et al., 1999). Therefore, something other than 5-HT levels itself seems to modulate long-term effects of SSRIs, and this seems to depend on firing rates of these neurons mediated by 5-HT_{1A} autoreceptors. Additionally, the proposed decreased serotonergic signaling (Cools et al., 2008) following acute SSRI challenges is unlikely to be caused by reduced extracellular 5-HT levels.

Serotonergic raphe neurons co-release glutamate

It has been increasingly recognized that the majority (~80%) of raphe neurons that express tryptophan (Trp) hydroxylase 2, the rate-limiting enzyme in 5-HT synthesis and a specific marker for serotonergic neurons, also express the vesicular glutamate transporter 3 (VGLUT3) (Hioki et al., 2009; Mestikawy et al., 2011). VGLUT3 stores glutamate into presynaptic vesicles from which it is then released via exocytosis following action potential induced depolarization (Liguz-Lecznar and Skangiel-Kramska, 2007). Knockout mice that lack VGLUT3 also show increased anxiety-related behavior and there is evidence that this effect is mediated by VGLUT3 in 5-HT neurons (Amilhon et al., 2010). Furthermore, it has also been observed that serotonergic neurons appear to co-release glutamate (Johnson, 1994), but only the recent development of optogenetic techniques specifically targeting serotonergic neurons confirmed this finding (Varga et al., 2009). The actions of both neurotransmitters appear to be on different time-scales with very fast (~3 ms) ionotropic glutamatergic excitatory transmission and slower (more than 100 ms) inhibitory metabotropic serotonergic effects. This offers the intriguing possibility of dual signaling within one monoaminergic system (Mestikawy et al., 2011).

Most recently, optogenetic activation of serotonergic raphe neurons in mice has revealed behaviorally dissociable serotonergic and glutamatergic effects (Liu et al., 2014). Selectively blocking the glutamatergic component of optogenetically stimulated 5-HT neurons, chemically using an AMPA receptor antagonist and in genetic VGLUT3 knock out strains, resulted in a pronounced decrease of the speed of instrumental learning and hedonic aspects of behavior, like sugar water ingestion or simple self-stimulation. Selective interference with the serotonergic component of the dual signal in similar ways only impaired the sustaining of motivation over longer times, for example when schedules to obtain stimulation required repeated actions.

Although this optogenetic study (Liu et al., 2014) is the first to clearly demonstrate this dissociation between glutamatergic and serotonergic effects, its results lead to two specific predictions that are relevant for commonly used behavioral paradigms. First, it suggests that tasks requiring sustained motivation or waiting for rewards, such as delay discounting paradigms, may be associated with the serotonergic component of SSRI administration. In contrast, reward-based learning paradigms may be associated with the glutamatergic component of the dual signal. This offers an intriguing potential explanation for the acute effects of SSRI administration and provides a testable framework for translating results between animal and human studies.

Glutamatergic and serotonergic components of raphe neuron signaling explain the SSRI paradox

If it is true that serotonergic neurons co-release glutamate and that these transmitters affect different behaviors, one can deduce the effects of acute SSRI administration. As noted above, SSRI administration rapidly increases extracellular 5-HT levels. This is especially pronounced within the raphe nuclei themselves, but not restricted to them (**Figure 2-1A**). This increase, in turn, leads to a 5-HT_{1A}-autoreceptor-mediated decrease in the firing rates of these neurons, which then would decrease the release of both 5-HT and glutamate in projection regions (**Figure 2-1B**). However, due to the SSRI's blockade of the 5-HTT within these regions, SSRI administration still leads to an overall increase of 5-HT levels, as has been well documented (Beyer and Cremers, 2008). In contrast, the glutamatergic component of this signaling has to be restored over time. Thus, acute SSRI administration may effectively dissect glutamatergic and serotonergic signals until autoreceptors are desensitized and firing rates have returned to baseline (**Figure 2-1C**), which happens approximately 2 weeks after treatment onset (Mansari et al., 2005).

For depressed patients receiving an SSRI, this would mean that motivational components are initially boosted, while hedonic components of the reward system (Berridge et al., 2009) may even be hampered – a combination that could lead to the facilitation of suicidal thoughts or behavior in the early weeks of SSRI administration. Clinically, this initially observed increase in motivation is often counteracted by co-administering tranquilizers, such as benzodiazepines (Furukawa et al., 2002). Surprisingly, higher initial doses of SSRI in the treatment of depressed patients do not appear to lead to better outcomes (Bollini et al., 1999) but rather seem to be associated with increases in deliberate self-harm, at least in younger adults (Miller et al., 2014). This may be due to the fact that high doses lead to stronger 5-HT increases but likely also firing rate decreases (Hajós et al., 1995). Furthermore, a genetic association study of suicidal ideation of almost 2000 depressed patients treated with SSRIs revealed only two significantly associated genetic loci, both of which are located within genes encoding ionotropic glutamate receptors (GluR6 and AMPA3) (Laje et al., 2007). When firing rates return to baseline after desensitization, the glutamatergic component of the dual signal would be restored (while the 5-HT component is boosted) to pre-treatment levels, or even amplified if glutamate receptors are up-regulated in response to the decrease in glutamate release during the initial period of treatment. Another line of evidence supporting the dual signal theory is the observation that ketamine has immediate beneficial effects in depressed patients (Berman et al., 2000) which is supposedly mediated via an increased sensitivity of glutamatergic AMPA receptors (Caddy et al., 2014) (see Box: Open Questions).

The findings of slower learning and increased response switches following acute SSRI administration described earlier on (Chamberlain et al., 2006) are well compatible with the findings of hampered learning following selective blockade of the glutamatergic component of the dual signal during optogenetic raphe 5-HT neuron stimulation (Liu et al., 2014). Furthermore, acute Trp depletion (ATD, **Box 1-1** and **Glossary**) does not appear to alter raphe neuron firing (Trulson, 1985) and thus may only reduce the serotonergic component of the dual signal. This would be compatible with some negative findings for example in rats where lesions with 5,7-DHT produced reversal learning deficits (van der Plasse et al., 2007) but ATD did not (van der Plasse and Feenstra, 2008), or human studies that did not find behavioral effects on reversal learning (Evers et al., 2005).

In accordance with the idea of opposing effects of acute SSRI on glutamatergic and serotonergic components of 5-HT neuron signaling, it has been demonstrated that optogenetic stimulation of serotonergic raphe neurons increased the waiting ability for delayed rewards (Miyazaki et al., 2014). In rats, decreasing 5-HT synthesis with pCPA (p-Chlorophenylalanine) has the opposite effect (Denk et al., 2004) and it has been reported that acute SSRI administration increases waiting ability for delayed, larger rewards (Bizot et al., 1988; 1999). Furthermore, reducing the serotonergic component of the dual signal by ATD in humans also lead to a preference for immediate but lower value choices (Schweighofer et al., 2008). This would be compatible with immediate boosting of the serotonergic component of the dual signal by the SSRI.

Concluding remarks

Progress in understanding behavior and its neurochemical foundations crucially depends on translational research that links findings from animal studies across species and ultimately can guide treatment decisions in human populations.

The appreciation of dual serotonergic signals can help understanding so far puzzling results, namely that many studies of essential cognitive capabilities employing acute SSRI found results compatible with manipulations that decrease serotonergic neurotransmission in animal studies but measuring 5-HT levels following acute SSRI administration has not yet been reported to reduce serotonin levels. Among these cognitive capabilities are instrumental and reversal learning (Chamberlain et al., 2006) or behavioral inhibition (Guitart-Masip et al., 2013). Other abilities, like waiting for delayed rewards, may be more dependent on the general serotonergic tone. Furthermore it is suggested that the integrity of the dual signal is essentially important for SSRI to have remedial effects in the treatment of depression and that the lack of the glutamatergic component initially could mediate acute adverse effects such as increased suicidal ideation (Laje et al., 2007; Miller et al., 2014).

Clearly, other explanations for the paradoxical acute effects and the delayed onset of SSRI treatment responses already exist. These include shifts in tonic and burst firing rates (Best et al., 2011) and regulation of 5-HTT expression (Benmansour et al., 2002) which may differ between projection regions and the raphe itself (Müller and Homberg, 2014). Furthermore, it is possible that differences in acute doses affect 5-HT levels and autoreceptor activation differentially (Invernizzi et al., 1992) which may also relate to differences in the balance of glutamatergic and serotonergic components of the dual signal. However, the clear physiologic validation of the dual signaling idea, its compatibility with the delayed onset of the treatment response to SSRI, and its ability to explain inconclusive findings from human studies demonstrate its potential. This theoretical background will allow researchers to generate promising new hypotheses about SSRI interventions that are informed by a more precise characterization of both glutamatergic and serotonergic raphe neuron signals and physiologically plausible.

This offers a framework of directly testable predictions for a better understanding and interpretation of studies employing SSRI (and possibly other) challenges. Furthermore it opens potentials for new drug targets with the aim to bridge the onset delay of SSRI in depression, but possibly also increasing efficacy in the treatment by delineating the contributing factors of each aspect of the dual signal (see Open Questions box).

Box 2-2: Open Questions

The extent to which cognitive functions depend on the integrity of either component of the dual signal is widely unexplored (Liu et al., 2014). Optogenetic stimulation in combination with genetic and pharmacological knock-out of either component of the dual signal is an excellent approach to characterize such differences. Furthermore, given the increasing evidence towards co-release, human studies observing either acute SSRI effects in accordance with increased or decreased 5-HT neurotransmission can also serve to inform animal studies.

The distribution of raphe neurons that co-release glutamate, release only 5-HT, or even release only glutamate, appear to be projection specific, as shown in rodents (Mestikawy et al., 2011). This offers the intriguing possibility of different brain regions being differentially affected by dual serotonergic signaling, and thus SSRI administration. However, the distribution of these projections is only beginning to be delineated (Cools et al., 2008; Mestikawy et al., 2011).

The serotonergic and dopaminergic systems interact anatomically and functionally in a complex fashion (Cools et al., 2010; Boureau and Dayan, 2011). Interestingly, dopaminergic neurons also seem to co-release glutamate, suggesting a more general mechanism in monoaminergic systems allowing for synaptic action at different time scales (Lavin et al., 2005; Jocham and Ullsperger, 2009; Mestikawy et al., 2011; Ullsperger et al., 2014b).

How does the dual signal theory relate to known genetic polymorphisms that influence serotonergic transmission? For example, the 5-HTT linked polymorphic region (5-HTTLPR) has been shown to increase 5-HTT mRNA expression *in vitro* and has been linked to different behaviors in healthy and diseased populations (Caspi et al., 2003; Homberg and Lesch, 2011; Karg et al., 2011). However, its effects on neurotransmission *in vivo* are less clearly defined (Jedema et al., 2010; Murthy et al., 2010). Could a shift between both components of the dual signal help interpret these findings?

Serotonergic neurons appear to have rather low, clock-like tonic firing rates but also fire at much higher rates (Kocsis et al., 2006; Nakamura et al., 2008) and optogenetic stimulation at higher frequencies produces marked behavioral effects (Liu et al., 2014). To which extent are tonic and phasic firing of selectively targeted 5-HT neurons in dorsal and median raphe affected by 5-HT increases following SSRI application?

Increasing firing rates during initial SSRI treatment by blocking 5-HT_{1A} autoreceptors accelerates treatment onset in humans (Ballesteros and Callado, 2004) and animal models of depression (Bortolozzi et al., 2011). However, the unspecificity of the agent (pindolol) in humans prevents its routine clinical use, but an increasing role for glutamatergic drugs is being recognized. Ketamine, an NMDA receptor antagonist (Berman et al., 2000), and cycloserine, which acts as a functional NMDA receptor antagonist at high doses (Heresco-Levy et al., 2013), have demonstrated efficacy in depression, which is presumably mediated via up-regulation of AMPA receptors (Caddy et al., 2014). Could such a treatment supplementation also decrease initial side-effects of SSRI treatments, especially in subjects at higher risk for self harm? Could other drugs directly targeting AMPA receptors (ampakines) also help overcome the onset delay of SSRI with less unspecific effects?

Genetic association studies hint at glutamatergic receptors as mediators of suicidal ideation during SSRI treatment (Laje et al., 2007) – a major problem during SSRI treatments of wide importance for the field of psychiatry (Miller et al., 2014). Could patients at higher risk from suicide benefit more from a treatment targeted at both serotonergic and glutamatergic components of the dual signal?

Genetics and Pharmacogenetics of the Serotonergic System

Effects of 5-HTT polymorphisms

Almost two decades ago, Lesch and colleagues (Lesch et al., 1996) reported on a novel variable number tandem repeat polymorphism (VNTR, **Glossary**) in the promotor region of the 5-HTT gene (SLC6A4) on chromosome 17q11.1-q12 which was termed the 5-HTT linked polymorphic region (5-HTTLPR). The short (S) form showed 14 repetitions of a 20-23 basepair repeat unit as opposed to 16 in the long (L) form and about 50% reduced expression of 5-HTT mRNA within cell transfection models in vitro (but many subjects also show more than 16 repeats and these are mostly grouped to the long form) (Heils et al., 1996). Additionally, the S allele was associated with higher anxiety and neuroticism scores in a sample of healthy human subjects (Lesch et al., 1996). In Caucasian samples, the L allele was found to be the more common one (60-65%) and the S allele to be somewhat less common (35-40%) (Lesch et al., 1996; Hu et al., 2006; Wendland et al., 2007) but this differed between populations. African americans appear to have a higher prevalence of the L allele whereas it is less common in asian populations (Hu et al., 2006).

18 years later, this polymorphism has received great scientific interest and the original publication has been cited more than 4000 times (as indicated by google scholar). The interest in this polymorphism still stands even in the times were genome wide association studies (GWAS) and sequencing are readily available (Gelernter, 2014). Still, no fully accepted picture exists of the effects this polymorphism exerts on healthy or compromised 5-HT functioning. In part, this may be attributable to important newer findings demonstrating that what was believed to be the higher expressing L genotype, actually was modulated by an additional A > G SNP (rs25531). The presence of the G variant caused mRNA 5-HTT expression indifferent from the S genotype (**Figure 2-2A**). Furthermore, it had been believed that the S allele has a dominant mode of action, and most studies grouped S/L and SS subjects, but in fact a co-dominant expression seems to be the better model (**Figure 2-2B**; Hu et al., 2006). Another C > T SNP (rs25532) was later on shown to modulate 5-HTTLPR linked mRNA expression, and the lower expressing T variant showed up to 80% decreased expression (Wendland et al., 2007). Thus, the current state of knowledge is that homozygous subjects carrying the L_{AC}/L_{AC} genotype show the highest and homozygous subjects carrying the S/S genotype show the lowest 5-HTT mRNA expression, at least in vitro. For the study reported here, both these groups were selected and will here be referred to as *high expressing* LL and *low expressing* SS subjects, respectively (see **Chapter 3** for a detailed description of the study sample and genotyping procedures).

The association of the S allele with anxiety related personality traits and neuroticism seen in the initial study (Lesch et al., 1996) has since then been confirmed by many studies and meta-analyses (Serretti et al., 2006a 2006uz), although it seems that effects are particularly strong when measured with a neuroticism scale based on the five-factor model of personalty (Costa and McCrae, 1992; Schinka et al., 2004; Sen et al., 2004). The neural underpinning of this effect is thought to be mediated via amygdala hyper-reactivity that has been repeatedly demonstrated as increased BOLD responses to fearful stimuli in S allele carriers (Hariri and Holmes, 2006; Murphy et al., 2012). Furthermore, 5-HTT knock out mouse models ($Slc6a4^{-/-}$) have been established and the heterozygous $Slc6a4^{+/-}$ genotype is thought to resemble the human S genotype. The serotonin transporter deficient

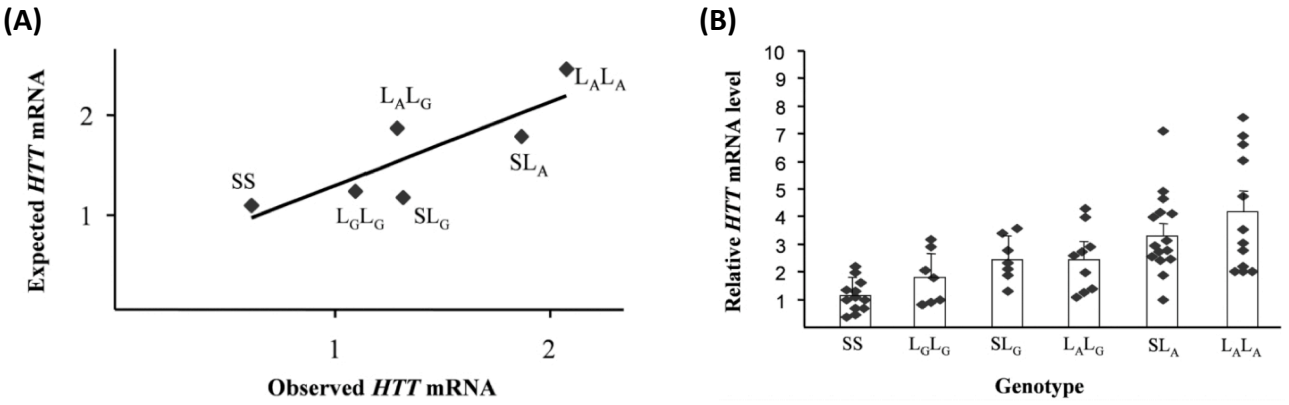

Figure 2-2. Modulation of 5-HTTLPR by rs25531.
(A) mRNA expression in lymphoblastoid cells lines is depicted normalized to SS homozygous subjects. The L_G allele is found to function similar to the S allele and by far the most profound differences are seen between homzygous SS and L_AL_A subjects.
(B) The six tri-allelic 5-HTTLPR genotypes are well modeled assuming a co-dominant expression mode ($r = 0.84$, $p = 0.38$).
From: (Hu et al., 2006).

genotypes have repeatedly been demonstrated to display increased anxiety related phenotypes and startle responses (Murphy et al., 2008). On the contrary, they are also associated with decreased aggression in that both Slc6a4$^{-/-}$ and $^{+/-}$ rats as well as mice show reduced aggressive behavior towards intruders (Homberg et al., 2007; Murphy et al., 2008). These observations led to the suggestion of 5-HTTLPR being a susceptibility factor for anxiety and affective disorders. Additionally, the heterozygous knock out mice display increased extracellular 5-HT levels (Kalueff et al., 2010) and about 33% reduced raphe neuron firing rates, likely because of increased 5-HT_{1A} receptor mediated inhibition (Gobbi et al., 2001). How, and if, this translates into human studies, will be discussed below.

A widely influential paper extended the idea of genes as risk factors for disease by demonstrating dependence of the development of depression on an interaction of stressful live events with the genetic background of 5-HTTLPR. Carriers of at least one S allele demonstrated increased risk for depression only when multiple (>4) recent life traumas had been experienced (Caspi et al., 2003). Although not undisputed (Risch et al., 2009), this finding has been replicated in many studies and the most recent and complete meta analysis confirms this association (Karg et al., 2011). In non-human primates, early rearing conditions modulated 5-HT metabolism and interacted with a gene region thought to be the macaque homologue of 5-HTTLPR (Bennett et al., 2002). Such gene x environment interactions have since been investigated as especially important factors contributing to disease development as the idea of one gene causing a certain complex disease has been dropped (Serretti et al., 2006a).

One may wonder why this polymorphism was maintained through evolution with a quite high allele frequency of around 15% homozygous carriers if presence of an S allele decreases stress tolerance? It seems that while presence of an S allele increases risk for depression and anxiety related disorders, it also increases vigilance in general to external cues. This is likely beneficial under certain conditions and can lead to better harm avoidance and offset the negative consequences of the S allele in an evolutionary perspective (Homberg and Lesch, 2011). Therefore, it seems inappropriate to continue with the previously established, deficit oriented description of the S allele. Both genetic variants may be associated with advantages and disadvantages in certain tasks and conditions and extend behavioral

variability on a population level. In line with this, some studies suggest deficits for L allele carriers in emotional response inhibition (Roiser et al., 2006b), reversal learning and delay discounting (Jedema et al., 2010), and the allele allele has been identified as a quantitative trait locus for characteristics of ADHD (Curran et al., 2005), and an association with the onset of the disorder has been discussed (Faraone et al., 2005; but see: Landaas et al., 2010).

How do the highly robust *in vitro* findings of alteration of 5-HTT mRNA expression depending on 5-HTTLPR genotype (Murphy et al., 2008) mechanistically relate to the phenotypical differences observed? Two (not fully mutually exclusive) propositions exist. Either lower expression of 5-HTT leads to higher extracellular tonic 5-HT levels and consequently a general down-regulation of 5-HT receptors (including 5-HT_{1A} autoreceptors), possibly rendering this system more susceptible to perturbations, or 5-HT acts as a growth factor during ontogenesis and mediates the effects via developmental and possibly morphological changes.

An early post mortem study of human brains assessing 5-HTT mRNA levels and the amount of 5-HTT binding to the selective ligand [^{125}I]CIT indicated higher 5-HTT mRNA expression in LL compared to SL and SS carriers in DRN, MRN, and VTA (Little et al., 1998). For 5-HTT binding itself, the effects were less pronounced and SS subjects appeared to be comparable to LL subjects. The development of the non-competitive and highly selective 5-HTT ligand [^{11}C]DASB enabled to study the effects of 5-HTTLPR on 5-HTT levels *in vivo* independent of possible alterations of tonic 5-HT levels. Some of these SPECT studies found higher binding in LL subjects in the caudate nucleus (Kalbitzer et al., 2010), midbrain (Reimold et al., 2007), and putamen (Praschak-Rieder et al., 2007), but other studies could not confirm this (Parsey et al., 2006; Murthy et al., 2010). Given the fact that even the studies reporting positive effects do not replicate the findings in their respective other target structures, it seems unlikely that 5-HTTLPR significantly alters 5-HTT availability *in vivo* as measured with SPECT. On the other hand, morphological effects on amygdala grey matter volume as well as medial prefrontal regions such as ACC were found depending on 5-HTTLPR genotype (Canli et al., 2005; Pezawas et al., 2005). Additionally, a polymorphism thought to be the human homologue of 5-HTTLPR in rhesus macaques that displays comparable *in vitro* expression characteristics to the human 5-HTTLPR, showed similar behavioral and morphological features, but left 5-HTT and 5-HT_{1A} concentrations mainly unchanged as determined by [^{11}C]DASB SPECT (Jedema et al., 2010). It thus seems likely that the lower 5-HTT mRNA expression in S allele carriers is counteracted by some developmental mechanism but gives rise to a different equilibrium of 5-HT signaling. However, many studies agree that especially in the case of disturbances of the 5-HT system, differences between both genotypes become apparent (Caspi et al., 2003; Karg et al., 2011). While a negative life event may lead to such such a perturbation, clearly a pharmacological challenge could also be considered as such (Roiser et al., 2006a).

In short, the SS genotype is associated with harm-avoidant, anxiety related, negative personality traits, increased startle responses and possibly decreased aggression. On the other hand, the LL genotype may be more prone to impulse control disorders such as ADHD. However, the exact mechanisms whether these effects are mediated via structural brain changes or via direct influences on serotonergic neurotransmission remain highly debated, but it appears that differences become especially apparent when the normal mode of functioning is perturbated, via life events or pharmacological challenges.

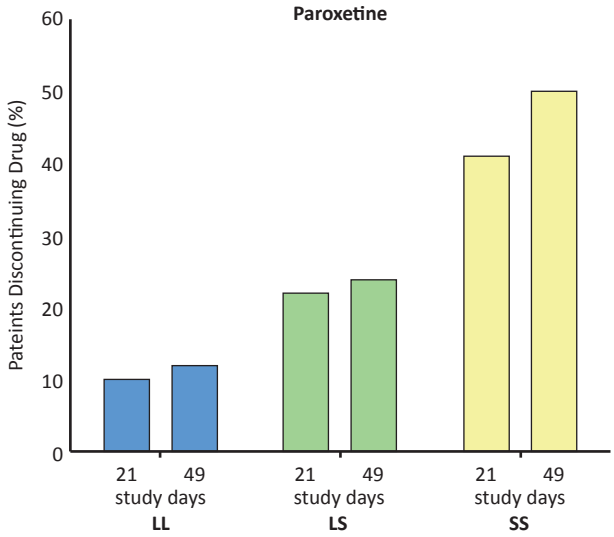

Figure 2-3. 5-HTTLPR modulates SSRI treatment discontinuation.
In 246 depressed patients (Murphy et al., 2004), SS subjects showed higher discontinuation rates due to adverse side effects.
Graphic from: (Murphy et al., 2008).

Interaction between 5-HTTLPR and SSRI response

Shortly after the discovery of the 5-HTTLPR, it was investigated as a modulator of the treatment response to SSRI in depression. An initial study conducted by Smeraldi and colleagues (Smeraldi et al., 1998) compared the SSRI treatment response depending on 5-HTTLPR genotype in the presence or absence of an additional 5-HT_{1A} receptor antagonist (pindolol) in a caucasian european sample. The results indicated that without pindolol, carriers of at least one L allele showed a better response to SSRI treatment and this effect was abolished by additional administration of pindolol, following which all subjects were comparable to the outcome of LL subjects. It was concluded that genetic differences in 5-HTT expression mainly determine the level of autoreceptor activation caused by SSRI. Since then, many studies were conducted in this field and the picture has become more complicated. While the largest study conducted so far (n = 1914) could not replicate these findings in a heterogenous sample (Kraft et al., 2007), subsequent re-analyses of the data in a non-hispanic caucasian sample replicated the effect described by Smeraldi et al. (1998). This finding was replicated in another noticeable sample of 795 european caucasians and could be differentiated from the treatment response to nortryptilin (a non-specific tricyclic antidepressant) (Huezo-Diaz et al., 2009). Especially studies in asian populations showed mixed results or even a better outcome for carriers of S alleles of 5-HTTLPR (Kim et al., 2000; Yoshida et al., 2002), which was also pointed out in a meta-analysis (Serretti et al., 2006b; Kato and Serretti, 2008) that confirmed solid effect sizes especially in caucasian subjects and absence of effects in asian studies. Notably, the S allele seems to be much more frequent in asian populations (Hu et al., 2006). Additionally, the occurrence of side effects and treatment discontinuation seems to be increased in subjects with lower expressing genotypes (Murphy et al., 2003) (**Figure 2-3**). In summary, sound data support a modulatory role of 5-HTTLPR on the mediate treatment response to SSRI in depressed patients of european caucasian origin but these findings may not generalize to other populations.

Regarding the underlying physiological interactions and the acute SSRI response, little data exist. Acute increases in prolactin levels in human subjects, a putative indicator of serotonergic neurotransmission, following administration of the tricyclic antidepressant clomipramine and the SSRI citalopram has been shown to be more pronounced in carriers

of the LL compared to the SS genotype (Whale et al., 2000; Peet, 2005). This has been attributed to more complete blockade of 5-HTTs in SS subjects which would lead primarily to earlier activation of 5-HT_{1A} autoreceptors and a consequent decrease of raphe neuron firing rates. In 5-HTT knockout mice, in which the heterozygous genotype has been proposed as a model of the human SS genotype (Bengel et al., 1998; Murphy et al., 2008), acute administration of SSRI impairs clearance of 5-HT stronger in the heterozygous compared to the wild type. Interestingly, both genotypes do not differ in their baseline clearance indicating some compensatory, likely developmental mechanism (Montañez et al., 2004).

The impact of these findings of interactions between genetic factors and pharmacological interventions on higher cognitive functions is currently not clearly predictable. It likely depends on the ratio of auto- and post-synaptic receptors in projection areas and may be different depending on the function studied. As pointed out before, even without genetic variation (as in in-bread mouse models), the effect of SSRI seems to differ depending on the exact brain region studied. Therefore, it may well be possible to observe different SSRI effects depending on genotype – or the same effect in both groups. However, the most evident prediction would be a greater increase of 5-HT in projection areas in LL subjects due to lesser autoreceptor activation at least in low- to midrange doses. Additionally, when naïve group differences are observed, these should likely be reduced following 5-HTT blockade. It may even be speculated that low doses of SSRI could lead to an increase in serotonergic transmission in LL subjects but a decrease in SS subjects.

It should be mentioned that other ways of manipulating serotonergic neurotransmission apart from SSRI are subject to the same limitations and problems. For example, an acute decrease of available 5-HT due to dietary Trp depletion clearly also effects decreased autoreceptor activation and can interact with 5-HTTLPR genotype (Roiser et al., 2006a) and has even been questioned with regard to its specificity in general (van Donkelaar et al., 2011).

Specific 5-HT Functions and their Relation to the PM Tasks of this Thesis

This paragraph will only serve as brief introduction to the specific functions ascribed to 5-HT that motivated the use of the different tasks analyzed in this thesis. The chapters reporting the results of the tasks then focus on the specific questions in more detail.

Behavioral Inhibition

For a long time, 5-HT has been implicated in behavioral inhibition based mainly on observations derived from rodent studies (Soubrié, 1986) and it is thought that higher 5-HT levels are associated with more behavioral inhibition. This is corroborated by patient studies where lower 5-HT levels are associated with disinhibition. For example, post mortem studies showed decreased 5-HT in depressed patients that committed suicide and lower 5-HT was found in aggression resulting from alcohol (Asberg et al., 1976; Linnoila and Virkkunen, 1992).

Under lab conditions, three different tasks are usually employed to measure behavioral inhibition: the Continuous Performance Task (CPT), Go/NoGo paradigms, and Stop-Signal Tasks – but across tasks, results regarding 5-HT differ considerably. In the CPT behavioral inhibitory dyscontrol is measured as responses to non-target stimuli presented in a rapid sequence that alternates standard and target stimuli. On the other hand, attentional impulsivity is thought to be reflected in the number of failures to attend to target stimuli (Homberg, 2012). The CPT and its rodent analogon, the five-choice serial reaction time task (5-CSRTT), mainly confirmed serotonergic influences on behavioral inhibition whereas the data for attentional impulsivity seems more ambiguous. For example, globally increased extracellular 5-HT levels in 5-HTT knock out rats led to improved inhibition of premature responses (Homberg et al., 2007) while lesioning the 5-HT system with 5,7-DHT (Harrison et al., 1997) or decreasing 5-HT neurotransmission with 5-$HT1_A$ (**Glossary**) agonists (Carli and Samanin, 2000) increased premature responding. In some studies, this seemed to be a trade off against attentional impulsivity (Harrison et al., 1997) that was found to decrease. Human data are less convincing here. ATD led to more impulsive behavior in the CPT, but this effect was confined to male participants and not modulated by 5-HTTLPR (Walderhaug et al., 2007). A consistent pattern in the animal literature seems to be that 5-HT2A antagonists and 5-HT_{2C} agonists increase premature responding (Boulougouris et al., 2008; Navarra et al., 2008; Winstanley, 2012), which would suggest that these opposing functions may cancel each other out and explain the mostly negative findings when general 5-HT levels are manipulated using SSRI or ATD.

In a Go/NoGo task, 5-7-DHT lesioned rats both responded faster to Go and less accurate to NoGo stimuli, indicating an overall effect on premature responding (Harrison et al., 1999). For human studies, behavioral results in this task are rare. Two independent fMRI studies with 9 and 12 participants, respectively, found an increase of inhibition related BOLD activity in the right inferior / orbitofrontal cortex following i.v. citalopram (Del-Ben et al., 2005) and a decreased BOLD signal in similar regions following ATD (Rubia et al., 2005), but both found no behavioral effects. A more recent study employed both ATD and high dose citalopram (50 mg) i.v. in combination with 5-HT_{2A} receptor PET in 24 human subjects in a Go/NoGo task with additional response alternation on target trials (Macoveanu et al., 2013). This revealed differential and interactive effects of both challenges in frontal brain regions, somewhat comparable to the

previously reported findings. All these studies appear underpowered to draw conclusions of negative results and also heavily suffer from the absence of behavioral findings which renders the interpretation of mere changes in brain activity problematic. All three studies argue that the ability to inhibit a response when task rules are rather simple may not be a sufficiently sensitive enough measure for human subjects. As to that regard, stop signal reaction times (SSRTs), which are usually titrated to each subject´s individual performance as to always lead to 50% failed inhibitions, are surely a more sensitive measure. However, even across different species, an association between 5-HT manipulations and SSRTs has repeatedly failed (Cools et al., 2005; Chamberlain et al., 2006; Eagle et al., 2008a; 2008b; Homberg, 2012; Winstanley, 2012). Employing the SSRT task, a human fMRI study using escitalopram did not report effects on behavioral measures, but increases in OFC and DLPFC responses associated with suppressing the initiated response (Drueke et al., 2013), similar to results obtained with the Go/NoGo paradigm described above. This suggests that while 5-HT seems to be important for selecting the appropriate action at least in animal models, it may be less involved in stopping an already prepared action – a function strongly associated with noradrenergic signaling (Chamberlain et al., 2006). In line with this, also an association study of 5-HTTLPR genotype and SSRT found no effects (Clark et al., 2005). These difficulties led to the suggestion that 5-HT mediates behavioral inhibition only in the context of affectively valenced tasks.

In this thesis 5HT effects on behavioral inhibition are reported which were found employing a more sensitive measure than commission or omission errors by calculating effects on RT increases following inhibitory events in **Chapter 5**. The task employed here was a Go/NoGo task with novel events, that themselves are known to induce inhibition (Wessel and Aron, 2013) which may maximize behavioral effects on inhibitory events.

General Inhibition or Aversive Coding?

While some researchers have argued that 5-HTs involvement in inhibition is sufficient to explain the data, others have stressed that its affective associations can better explain the inhibitory findings (Deakin and Graeff, 1991). It has long been acknowledged that depletion of 5-HT disinhibits previously punished behaviors (Soubrié, 1986). On the other hand, depression is associated with increased punishment processing and decreased behavioral vigor – yet it is thought to be related to lower 5-HT levels (Clark et al., 2009). Recent models of behavioral control suggest that 5-HT ties behavioral inhibition to aversive stimuli via a Pavlovian controller highlighting a role of 5-HT for avoiding punishment (Dayan and Huys, 2009; Boureau and Dayan, 2011) (**Figure 2-4**). The underlying idea is the suggestion of opponency between DA and 5-HT (see above) and while DA promotes both approach behavior and the coding of reward anticipation, it is suggested that 5-HT may fulfill the opposite function. Although it is difficult to disentangle inhibition from aversive processing as both are usually intertwined, there is evidence supporting a role of 5-HT especially in inhibition in the context of aversive processing. Some authors specifically suggested that 5-HT may code a punishment prediction error – that is the difference between expected and obtained punishment – which may be computationally more efficient coding than exploiting dips in DA neuron firing (Daw et al., 2002; Cools et al., 2008). However, direct coding of punishment prediction errors within the DRN or MRN has not been observed so far (Ullsperger et al., 2014b).

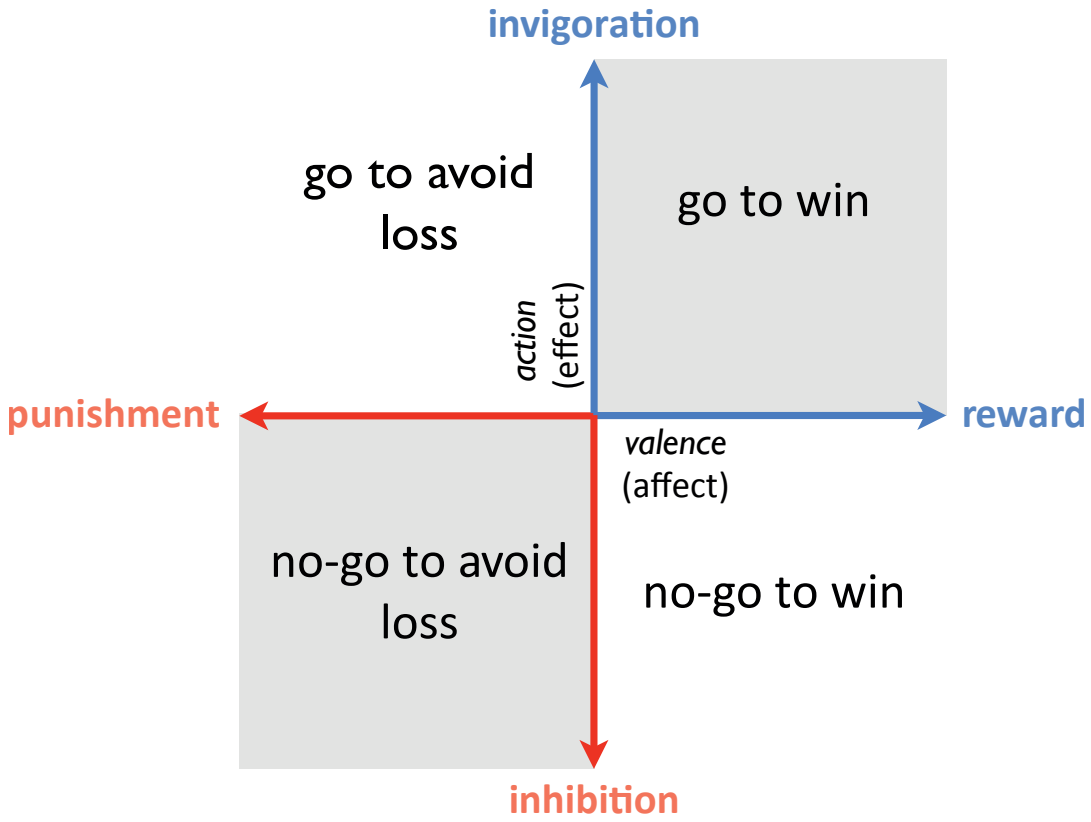

Figure 2-4. 5-HT in the Interplay of Affect and Effect.
It is assumed that DA controlled behavior (blue) leads to increased response vigor in the face of expected rewards and this mechanism is under the influence of a Pavlovian, hard-wired controller (grey quadrants). On the contrary, 5-HT (red) is thought to mediate inhibition in the face of expected punishments, which again is thought to be hard-wired. When both systems compete with each other (upper left and lower right quadrant), deficits in decision making are expected.
Modified from: (Boureau and Dayan, 2011)

Some studies have reported findings that support a role for 5-HT in inhibitory control especially in a punished context. Subjects usually speed up responding when their actions are evaluated with reward or punishment and ATD reduced this speeding while interacting with 5-HTTLPR genotype: LL subjects showed this speeding even when depleted of Trp, but SS subjects lost showed no more speeding when depleted of Trp (Roiser et al., 2006a). When crossing inhibition with aversion by either rewarding or punishing Go or NoGo responses in a Go/NoGo task, ATD led to a general reduction of RT slowing induced in punished blocks (Crockett et al., 2009). The effect of punishment to slow down responding in the immediately following trial, however, was not significantly affected. As general RT and RT in the rewarded blocks was not affected, the authors concluded that ATD affected inhibition only in the face of the predicted possibility of punishment and 5-HT may increase the avoidance of punished behavior. The general number of errors in this task was deemed insufficiently sensitive and not modulated by ATD. The authors replicated this finding in a modification of the task that required differential (left, right) responses following presentation of two stimuli out of which one was blockwise either only rewarded or rewarded and punished if wrong, but the other was never punished – which biased responses towards the unpunished stimulus and response (Crockett et al., 2012). Besides a replication of the previous finding, in that ATD reduced this bias seen on RT, ATD additionally abolished slowing induced by

punishment on the immediately following trial. Furthermore, the slowing effect of the presence of the punished stimulus extended also to the response that never was punished, suggesting a rather Pavlovian (Stimulus-Outcome) than instrumental (Stimulus-Response-Outcome) modulation. This finding, however, is contrasted by a study that employed a task that crossed valence and inhibition in which participants are required to make a Go response to win or avoid losing, or withhold responding to win or avoid losing depending on a previously shown stimulus (Guitart-Masip et al., 2013). It was found that acute SSRI administration increased a general bias towards performing a response, consistent with the idea that lower 5-HT activity disinhibits behavior in general. This finding, however, is further complicated by results of the same task where participants had to learn the correct response, which did not lead to significant effects of SSRI administration (Guitart-Masip et al., 2012). In sum, while some studies found evidence, especially when employing ATD, for a role of 5-HT in tying inhibition to the avoidance of punishments, others could not replicate this finding in a similar paradigm. Furthermore, it is unclear if this effect of 5-HT requires an external signal of punishment or failure, or extends to self detected performance errors. Additionally, the neural underpinnings of these effects have thus far remained widely unexplored.

A recent proposal for a unifying function of 5-HT is that it might mediate a shift between habitual stimulus-bound and goal-directed behavior by controlling vigilance (Homberg, 2012). According to this idea, the serotonergic system integrates both internal (e.g., hunger, action slips) and external information (e.g., reward, threat) and directly modulates vigilance mediated via top-down control of the prefrontal cortex over subcortical structures. Increased 5-HT levels lead to increased vigilance and thus more goal directed behavior and, for example, decreased delay discounting. Vice versa, decreased 5-HT levels are assumed to lead to decreased response inhibition, impaired reversal learning and increased delay discounting resembling habitual stimulus-bound behavior.

Serotonergic Modulation of Emotional Control

5-HT is one of the crucial neuromodulators affecting aversive processes as has been shown both in genetic association as well as in pharmacological studies in humans (Hariri and Holmes, 2006; Cools et al., 2008). A well replicated finding is higher sensitivity to negative events in subjects homozygous for the SS genotype which is accompanied by increased fMRI BOLD responses in the left and right amygdala (Hariri et al., 2002; Hariri and Holmes, 2006; Munafò et al., 2008) – although the effect may have been overestimated in previous studies (Murphy et al., 2012). Both functionally and structurally, these subjects show lower connectivity of the amygdala with the rostral ACC, which is interpreted as decreased inhibitory control over amygdala reactions (Pezawas et al., 2005). These findings extend to a modulation of the mood response to ATD by 5-HTTLPR. In healthy subjects, ATD induces depression related mood changes pronounced in subjects carrying at least one S allele at 5-HTTLPR (Neumeister et al., 2002; 2006) which may additionally be modulated by gender – women seem more sensitive to this effect (Walderhaug et al., 2007). Relatedly, following ATD, SS subjects lost incentive motivation measured as the response speeding under higher evaluated conditions. No such effects of ATD were seen in LL subjects – which thus again seemed more robust to 5-HT perturbations and their consequences (Roiser et al., 2006a). On the contrary, a Trp challenge with 0.8 g pure Trp

prevented the negative effects of stressors (cold exposure and forced backward counting) in SS subjects and had no effect in LL subjects, although the amount of stress induced was similar in both groups (Markus and Firk, 2009).

Research Questions of this Thesis

Given the evidence summarized above, it has been suggested that an overactive PM network that attributes increased attention to negative events and errors may serve as a link between the observed hyper-reactivity to negatively valenced stimuli for S allele carriers and the onset of depression (Hariri and Holmes, 2006). Here, we employed two different tasks to test different hypotheses derived from previous studies. Firstly, we employed a flanker paradigm to measure effects of RT slowing, or vigor decreases, induced by emotionally valenced internal events, namely errors, compared to less valenced events, namely difficulty of the preceding trial. This task also allows to measure EEG correlates (ERN, Pe) of the activity of the PM network while detecting and appraising the presence of an erroneous response (Ullsperger et al., 2014b) and results are reported in **Chapter 4**. Given the controversy about 5-HT´s role in approach or avoidance behavior, we also introduce a novel task that allows to analyze learning of the value of stimuli to translate these into either approach or avoidance without the possible confound of performing a Go response or not (Fischer and Ullsperger, 2013). This task further allowed to asses a possible role for 5-HT in the processing of and learning from fictive events that signaled the outcome of what would have happened if one would have chosen differently. This tests the hypothesis that given the difficulty of defining the precise role of 5-HT in human studies of learning mechanisms and reward processing, but its clear involvement in depression, 5-HT may modulate the appraisal of these fictive events. The task is introduced and exhaustively described in **Chapter 6** and results of 5-HT related factors are analyzed in **Chapter 7**.

Conclusions

Despite tremendous research efforts, a unified view of the role 5-HT plays in the homeostasis of an organism in general and in the field of research regarding PM has not been achieved. So far it is unclear if 5-HT influences inhibitory control in general, or solely in the face of aversive events. A role for 5-HT in learning has been proposed and underpinned by recent optogenetic studies which also suggest a dual signal of serotonergic signaling. While these findings appear in contrast with human studies employing SSRI action, this chapter proposes a solution for this apparent paradox based on feedback mechanisms that differentially affect the serotonergic and glutamatergic component of the raphe neuron dual signal.

Major factors that hinder progress in elucidation of the 5-HT system are the multitude of 5-HT receptor subtypes with partly antagonistic functions, high genetic variance in functional 5-HT related gene expression that may effect both ongoing neurotransmission as well as morphological aspects as a growth factor, and a lack of straightforward hypotheses regarding consequences of interventional manipulations within the 5-HT system, for example by solving the paradox of SSRI action. While there is no unique means to approach all of these problems at once, it appears that progress crucially depends on well controlled studies that account for genetic variance and employ highly specific perturbations to the 5-HT system in complex empirical designs which account for these factors. In the following, the results of a combined genetic and pharmacological double-blind crossover EEG study that controls for tri-allelic variants in 5-HTTLPR as well as SNP rs25532 in interaction with intravenous application of low-dose (10 mg) citalopram will be discussed.

CHAPTER

3

Study Procedures and Sample Description

Sample Description and Methods

Participants and Genotyping

Out of 878 subjects that had been genotyped for 5-HTTLPR polymorphisms, homozygous S and L caucasian subjects were invited to participate in the current study. 34 subjects (23 females) were included in the study after a second genotype analysis for rs25531 (Hu et al., 2006) and rs25532 (Wendland et al., 2007) had been carried out for LL subjects (see **Figure 3-1A** and below) and at both SNPs only the polymorphism with higher *in vitro* 5-HTT mRNA expression was included (A instead of G at rs25531 and C instead of T at rs25532). Exclusion criteria were any history of psychiatric or neurological disorders, drug abuse, more than moderate alcohol or nicotine consumption, Beck Depression Inventory (BDI) scores above 10, and age younger than 18 or older than 35 years. All procedures carried out were in accordance with the principles of the Helsinki Declaration and the study had been approved by the ethics committee of the Medical Faculty of the University of Cologne (Cologne, Germany).

Initial genotyping of all subjects was done using automated purification of genomic DNA conducted by means of the MagNA Pure LC System using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics). The 5−HTT region was amplified by PCR (Mastercycler). Primer sequences were 5'−TCCTCCGCTTTGGCGCCTCTTCC−3' and 5'−TGGGGGTTGCAGGGGAGATCCTG−3'. After an initial denaturation for 6 minutes at 94°C, 37 cycles of denaturing at 94°C for 30 s, annealing at 64°C for 1.5 minutes, and extension at 72°C for 1 minutes were followed by a final extension at 72°C for 5 minutes. PCR amplification was carried out in a final volume of 25 µl consisting of 50 ng genomic DNA, 0.2 mM of each desoxyribonucleotide, 0.5 µM of sense and antisense primers, 2.5 mM $MgCl^2$, 5.3 % DMSO, 1 U of Diamond Taq polymerase (Eurogentec) and the enzyme supplier's buffer. For genotyping, samples were loaded onto a 1.6 % agarose gel in a TBE solution, run for 1 hour 20 minutes at 170 V, and visualized by etidiumbromide under UV light. Samples were visualized and genotyped by at least two independent raters.

31% of the subjects were homozygous for the L and 17.7% for the S variant of 5-HTTLPR – matching the results of other studies (Hu et al., 2006). This distribution furthermore did not violate the Hardy Weinberg Equilibrium (p for violation = 0.354). LL subjects were then genotyped for rs25531 and rs25532 by direct sequencing of similarly obtained PCR-products as mentioned above. After enzymatic purification with Exonuclease I and Alkaline Phosphatase with both amplification primers, sequences were analyzed using SeqMan DNA−Star software (Lasergene). LL subjects that were not homozygous for the higher expressing forms at rs25531 and rs25532 were excluded and the LL group thus entails only the L_{AC}/L_{AC} genotype (Wendland et al., 2007) which thus, at least as derived from *in vitro* studies, should have higher 5-HTT mRNA expression levels.

Statistical & EEG Analyses, PM Tasks

Mixed linear models (MLM) were used to test main effects of factors *drug*, *genotype*, and their interactions (Gueorguieva and Krystal, 2004) if not stated otherwise. An additional factor coding the current *session* (first or second) was introduced to account for training effects due to task repetition, which may interact with the

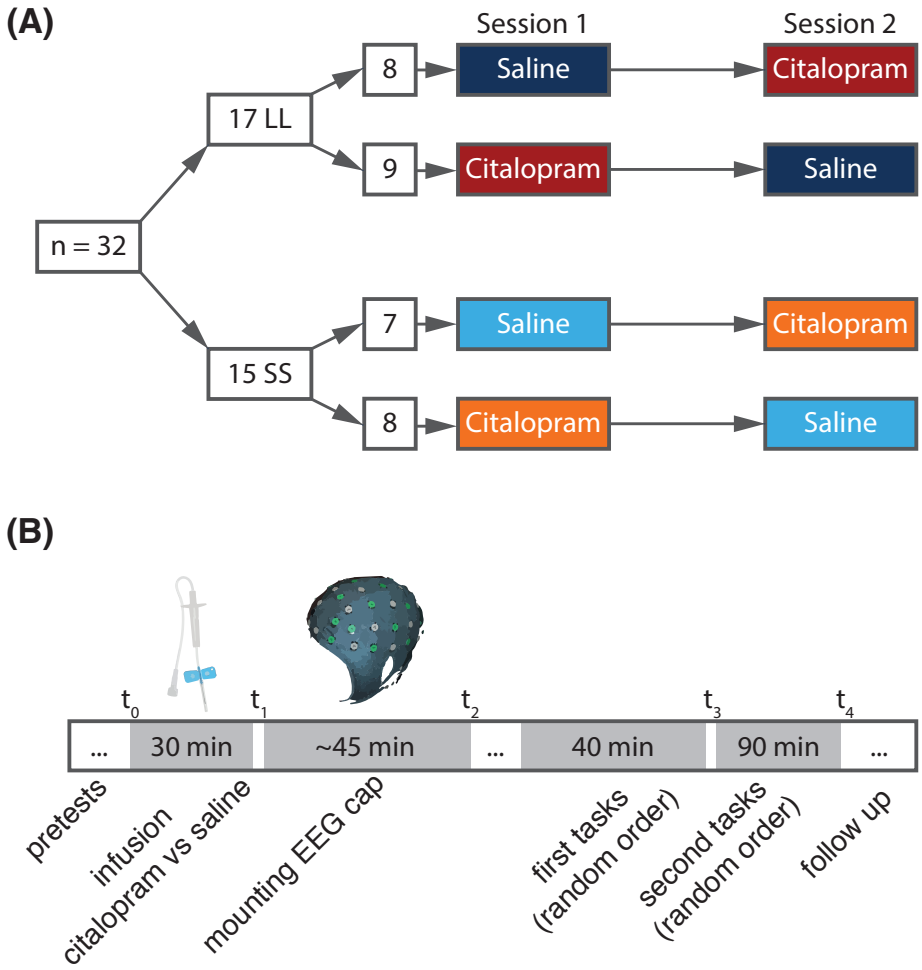

Figure 3-1. Study design and task structure.
(A) depicts schematically the study protocol. Order of drug administration was counterbalanced between and within genetic groups. (B) demonstrates the time scale of study procedures. Subjects first received an infusion of saline or diluted citalopram over 30 minutes in double blind fashion. Blood pressure and heart rate were measured at 5 time points and results hereof are shown in **Figure 3-2**.

serotonergic system (Murphy et al., 2002). A compound symmetric covariance structure was chosen as it led to best model fits (Littell et al., 2000). Analyses were calculated using SPSS 22 (IBM) and interaction effects were further analyzed by post hoc contrasts of estimated marginal means applying Bonferroni correction implemented in SPSS.

Data were recorded continuously at a 500Hz sampling rate with BrainAmp MR plus amplifiers (Brain Products) and analyzed offline using EEGLAB 12.0 (Delorme and Makeig, 2004). 60 Ag/AgCl electrodes mounted in the extended 10-20 system in an elastic cap (Easycap) were recorded while impedances were kept below 5 kΩ. Electrodes at the left and right outer canthus and above and below the left eye captured eye movements. The ground electrode was positioned at F2 and data were online referenced to CPz and offline re−referenced to common average. The signal was bandpass filtered from 0.5 to 42Hz (if not otherwise specified) and epochs surrounding events of interest were extracted. Further analysis is described in more detail in the corresponding results sections for the associated task.

Subjects performed 4 different tasks in random, counterbalanced order. A modified Eriksen Flanker Task (**Chapter 4**), a modified NoGo-Oddball paradigm (**Chapter 5**), a novel task to disentangle real and fictive events (**Chapters 6** and **7**),

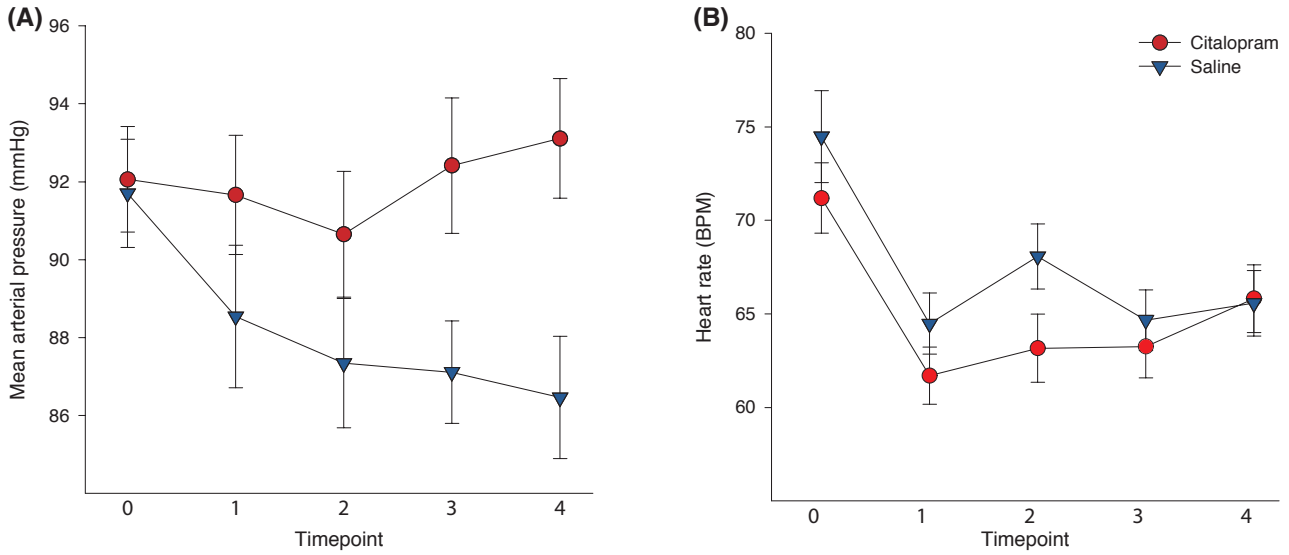

Figure 3-2. Drug effects on peripheral blood pressure and heart rate measurements.
(A) Citalopram showed a small but robust ($F_{1,192}$ = 16.95, p < 10^{-4}) increase of mean arterial pressure compared to baseline (t_0) of about 4 mmHg in a MLM analysis including factor *time point* (0 to 4). (B) Heart rate was not altered by the drug ($F_{1,197}$ = 1.57, p = 0.212). A trend towards lower heart rates in the second session was seen ($F_{1,196}$ = 3.49, p = 0.063) and no other factors were significant in both ML models.

and a simple reversal learning task. For the latter, data is not reported as the task turned out to not be sufficiently complex for repeated measures designs and subjects uniformly displayed ceiling, near optimal performance. Tasks will be described in the corresponding chapters in more detail.

Choice of Drug and Administration Protocol

Apart from escitalopram, that is not available as infusion solution, citalopram is the most selective SSRI and shows only minimal inhibition of DA and NE transporters. Its ratio inhibition constant of 3000 is significantly higher than that of paroxetine (280), sertraline (840), or fluoxetine (54) (Bezchlibnyk-Butler et al., 2000). Although citalopram does not show the highest affinity to the SERT among the class SSRIs, its intravenous application has been demonstrated to block more than 80% of 5-HTTs in brain regions high in 5-HT using PET even at low (< 10 mg) doses (Bezchlibnyk-Butler et al., 2000; Meyer et al., 2004). Intravenous application has several benefits over oral administration. It minimizes fluctuations in bio-availability of the drug due to first pass effects and enteral absorption, and also drastically reduces the required time until stable plasma levels can be expected to be reached, which for oral citalopram are observed after around 2-4 hours (Kragh-Sørensen et al., 1981). This effectively reduces the risk of side effects as well as it increases replicability and robustness of the study. Due to the dose-dependence of 5-$HT_{1B/D}$ autoreceptors (De Groote et al., 2002) and in order to minimize the risk of side effects, a rather low intravenous dose of 10 mg citalopram was chosen for the current study.

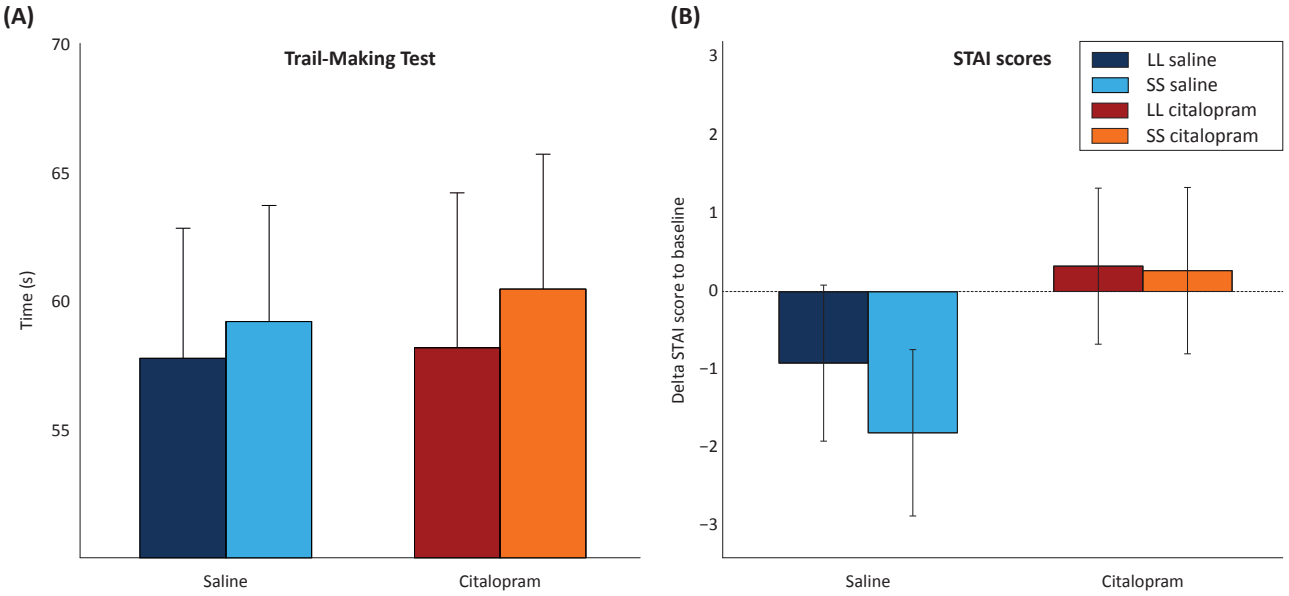

Figure 3-3. Assessment of unspecific drug effects.
(A) No difference was seen in the speed at which subjects completed a trail-making task at the end of each testing session (all ps > 0.2). (B) The state part of the STAI was filled out by subjects at the beginning and end of each test session. There was a trend towards a stronger reduction of STAI scores in the saline condition. A trend for a *session x drug* interaction was seen which was due to a strong difference between citalopram and saline condition when subjects participated in the experiment for the first time. No other effects were significant (all p > 0.1) and no differences in baseline scores were observed for placebo and verum conditions ($F < 1$).

When arriving at the lab, all participants completed a clinical interview with the physician in charge and completed baseline questionnaires (Table 1) and visual analogue scales (VAS) to asses mood changes (**Figure 3-4**) (Bond and Lader, 1974).

Pregnancy was ruled out by measuring HCG−β (< 0.1 U/l) in blood samples from female subjects 2 days before each test session to exclude possible fetal risks. Thereafter, in double-blind fashion either 10 mg of citalopram (Cipramil®) diluted in saline (250 ml) or saline alone were intravenously administered over a period of 30 minutes and the order of administration was counterbalanced (**Figure 3-1A**). Plasma levels of intravenous citalopram have been shown to remain constant over approximately 4 hours (Lotrich et al., 2004), within which time-frame all tests were carried out. The drug was well tolerated but one subject experienced side effects (nausea and vomiting) and was excluded from the study. Another subject was excluded during the first (placebo) test session because of an inability to follow task instructions.

EEG tasks were performed while the subjects were seated in a dimly lit, acoustically and electromagnetically shielded chamber. At the end of each session, subjects again filled out questionnaires and VASs and completed a trail-making-task in which numbers 1-90 had to be connected with a pencil in ascending order as fast as possible. This task served as a control to exclude detrimental effects of citalopram on visuomotor-coordination and different versions were used in the first and second test sessions (see below). Heart rate and blood pressure were measured immediately before the intravenous cannula was placed, when the infusion was completed, and three times thereafter. Analysis of these data showed a small but robust increase of peripheral mean arterial pressure by citalopram compared to saline

(average 4 mmHG) at all time points after the infusion while heart rates remained unchanged – matching 5-HTs physiologic characteristics (Veenstra-VanderWeele et al., 2000). Male subjects were re-invited to the second test session following a minimum of 7 days allowing at least 5 half life times of wash out of the drug (that is 33 hours for citalopram, Kragh-Sørensen et al., 1981). As estrogens seem to modulate the amount of 5-HTT binding sites (Krajnak et al., 2003), we re-invited female subjects at the same time within their menstrual cycles (i.e., after 27-31 days).

Table 1. Genotype details, demographics, and questionnaires

	LL (n = 17)	**SS** (n = 15)	**p for difference**
Genetics			
5−HTTLPR	homozygous long	homozygous short	
rs25531	A/A		
rs25532	C/C		
Demographics			
Gender (♀/♂)	12 / 5	10 / 5	
Age	23.8±0.5	23.9±0.9	0.920
Weight	67.8±2.1	65.7±0.7	0.518
Verbal IQ	105.8±10.1	110.8±9.0	0.154
Questionnaires			
BDI-II	2.85±0.7	4.03±0.8	0.300
EPQ-RS neuroticism	2.94±0.61	4.73±0.67	0.056
EPQ-RS psychoticism	2.59±0.33	3.2±0.47	0.288
EPQ-RS extraversion	9.24±0.71	8.07±0.87	0.302
EPQ-RS lie scale	2.76±0.50	0.4±0.13	**< 0.001**
BIS-11 total	62.3±2.2	65.4±2.1	0.306
BIS attentional	15.6±0.6	16.4±0.7	0.410
BIS motor	23.5±0.8	24.1±0.7	0.544
BIS nonplanning	23.2±1.4	25.5±1	0.187

Group LL included only the high expressing homozygous LAC genotype; group SS included C and T variants at rs25532. Groups did not differ with regard to demographics, IQ, and baseline depression scores. A significant difference was observed on the lie scale of the EPQ−RS indicating higher scores of social desirability in the LL group. Additionally, a trend towards higher neuroticism scores was seen in group SS, which is in accordance with previous studies (Schinka et al., 2004). However, impulsiveness as measured with the BIS−11 did not show group differences. Values represent mean±s.e. BDI = Beck Depression Inventory; EPQ−RS = Eysenck Personality Questionnaire Revised Short Scale; BIS−11 = Barratt Impulsiveness Scale.

Group Comparison and Unspecific Drug Effects

Both groups did not differ with regard to age, weight, or verbal IQ (Table 1). Scores of the BDI-II again indicated no group difference and all subjects scored sub-clinically. The neuroticism sub-scale of the Eysenck Personality Questionnaire (EPQ) indicated a trend towards higher anxiety in the SS group as is compatible with other studies (Lesch et al., 1996; Schinka et al., 2004). Impulsiveness assessed with the Barratt Impulsiveness Scale (BIS-11) did not differ between groups, which also agrees with the majority of other studies (Schinka et al., 2004). Additionally, we found a significantly higher score for LL subjects on the lie sub-scale of the EPQ indicating higher values of social desirability in that group. Citalopram did not prolong the time needed for completion of the trail making task (citalopram: 59±2s, saline: 58±2s, *drug* main effect $F_{1,32} < 1$, p = 0.59, **Figure 3-3A**) and neither were genetic group differences observed (*genotype* main effect $F_{1,32} < 1$, p = 0.44). Subjects were about 3 s faster in the second test session although different versions of the task were used, indicating a certain degree of learnability even in this basic test (*session* main effect $F_{1,32} = 10.08$, p = 0.004). No interactions between factors were observed (all ps > 0.1). Subjects could furthermore rate whether or not making mistakes in the flanker and Go/NoGo paradigm made them angry, how much they disliked to experience either real or fictive unfavorable outcomes and how much they liked favorable outcomes in the learning task (**Chapter 6**) via numbers from 1 to 10. For none of these items MLM analysis revealed a significant effect for factors medication, genotype, or their interaction (all ps > 0.1).

Baseline scores of the State-Trait Anxiety Index (STAI, Spielberger et al., 1983) assessed prior to the beginning of the study were not different between genetic groups (all ps > 0.2). STAI scores additionally assessed changes induced by the drug by subtracting baseline levels before each test session from the results attained at follow-up. MLM analysis of these scores showed a trend towards larger pre-post differences in the saline condition ($F_{1,32} = 3.39$, p = 0.075; **Figure 3-3B**). This trended to be further modulated by an interaction of *session x drug* ($F_{1,32} = 3.31$, p = 0.079) and this effect was larger in the first test session (ΔSTAI session 1 = 3.7 ± 1.0; session 2 = -0.4±0.4).

VAS were used to assess differences in self-reported mood changes. We first grouped the 16 items to three main factors (Bond and Lader, 1974): calmness, alertness, and contentedness. We then compared the differences in changes of subjective self reports between baseline level, determined by VAS completed at t_0, and VAS completed after the session at t_4, between citalopram and saline conditions including factors *genotype* and *session* into an MLM. We observed a trend for decreased calmness by citalopram administration ($F_{1,32} = 3.30$, p = 0.079), and no effects for the other two factors (ps > 0.20). Post hoc contrasts showed that effect on calmness was numerically larger in the SS (change: -0.81±0.48 cm, $F_{1,32} = 2.79$, p = 0.104) than LL group (change: -0.39±0.45 cm, $F_{1,32} = 0.76$, p = 0.390). No interactions between genotype and drug were observed for any item (ps > 0.50).

As this was a control analysis, we also report more detailed analyses of individual ratings and these results are reported without correction for multiple comparisons. **Figure 3-4** shows the effect of citalopram administration on this difference score as the effect (in cm) that drug administration had on the change of mood from baseline to the end of session for every item in the VAS. Exploratory tests of drug effects within both genetic groups via post hoc contrasts are also included into the graphic. We found a significant main effect of factor *drug* indicating a decrease in the subjective feeling of proficiency and trend level effects for increases in sadness and tension (**Figure 3-4**). While the

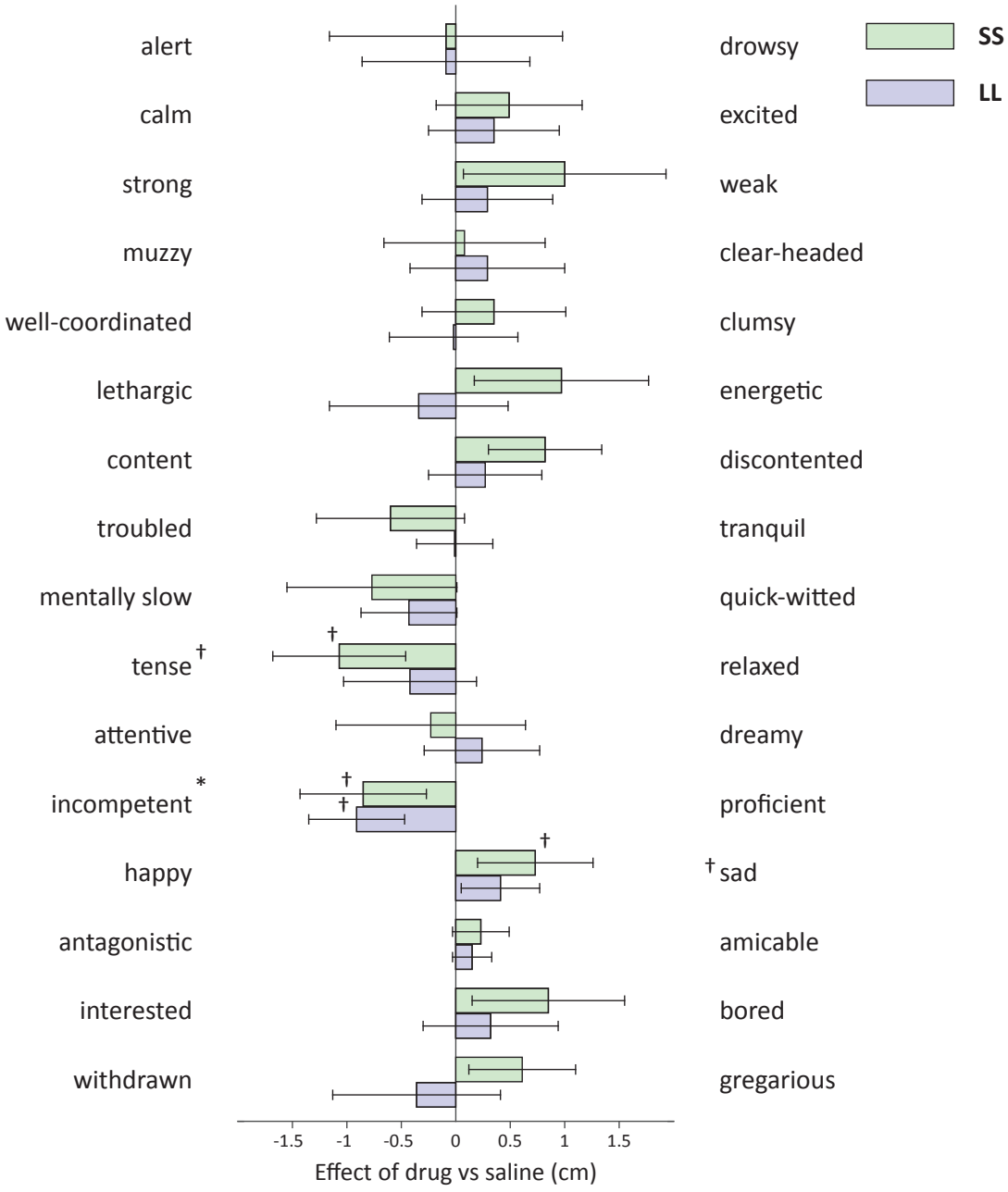

Figure 3-4. VAS for mood effects of the drug.
VAS were completed before and after each session by all subjects and are plotted as the difference (in cm) between mood changes compared to baseline depending on drug effects. Main effects of *drug* are marked at the respective item and post hoc comparisons of drug effects within genetic groups are marked at the respective bar. Overall, citalopram led to a decrease in the rating of feeling proficient ($F_{1,32}$ = 6.78, p = 0.014), and this effect was not different between genetic groups. Trends were seen for increased feelings of tension ($F_{1,32}$ = 3.56, p = 0.068) and an increase in sadness ($F_{1,32}$ = 4.05, p = 0.053). For both these effects, post hoc within group contrasts showed trends for SS subjects (ps < 0.079) and no effects in LL subjects (ps > 0.32), but effects were directionally compatible. Note that this analysis was not corrected for multiple comparisons as its main purpose was to exclude severe mood changes as confounding factors.
* mark p < 0.05, uncorrected; error bars = SE of raw data.

former effect was equally pronounced in SS and LL subjects, post hoc contrasts only showed trends for effects on sadness and tension in SS subjects. These findings are in accordance with the dual signal hypothesis of serotonergic

signaling, as both feelings of competence and happiness appear related to the glutamatergic, hedonic component of serotonin signaling which theoretically should be reduced by acute SSRI administration. Furthermore, despite not being significant and clearly exploratory, the numerically larger effect in SS subjects for sadness and tension would generally be in accordance with higher rates of side effects of SSRI treatment seen in this group.

Another exploratory comparison implied by the idea of differential drug effects would be to look for interactions between drug and genotype. We find no statistically significant interaction effect (all ps > 0.21) for any item. When we just compare directionality of drug effects (in order of amplitude of absolute difference), we found four items with directional differences (**Figure 3-4**). Citalopram numerically increased self-reports of feeling energetic, gregarious, attentive, and troubled for SS subjects, but had the opposite (numerical) effect, or none, on LL subjects. This analysis is reported for completeness rather than interpretability as we did not expect to find acute mood changes by administration of a single-dose of an SSRI in healthy young subjects and these kind of multi item analyses are extremely susceptible to type I errors.

Conclusion

In sum, these findings show that the drug had a measurable effect on peripheral cardiovascular parameters indicating effectiveness of the drug to alter physiological variables. Both groups were well matched and comparable regarding age, gender distribution, weight, and verbal IQ. The SS group showed a trend towards higher neuroticism scores, which fits well with previous studies (Lesch et al., 1996; Schinka et al., 2004). Additionally, the LL group was found to score higher in the lie sub-scale of the EPQ-RS which may indicate higher social desirability, but such a finding would clearly require replication in a larger sample to be interpretable and should not interfere with group comparisons of the measures focused on in this thesis. Furthermore, we found no evidence for sedatory side effects on visuomotor coordination in the trail-making-task that may confound analyses of drug effects. No significant changes were found on grouped VAS factors and neither were there any group differences in baseline state anxiety scores or interactions between *genotype* and *drug* on changes in STAI scores. In accordance with the dual signal hypothesis of serotonergic function, exploratory analyses of single items of the VAS showed a trend level effect of acute citalopram administration to lower subjective ratings of happiness, which are assumed to be dependent on the glutamatergic component of serotonergic signaling. Furthermore, subjective feelings of proficiency were decreased by the drug across genetic groups. Additionally, another trend level effect was observed for STAI scores, which reduced less strongly over the course of the experiment in the citalopram session, in accordance with the known effect of SSRI to initially induce anxiety (Burghardt and Bauer, 2013). However, it should be noted that this trend level effect was not an increase from baseline *per se*, but rather a less pronounced reduction of STAI scores compared to the unmedicated state. Thus, these data do not indicate major side-effects of the drug administered in this study that may limit interpretability of the study, and both genetic groups appear well comparable.

CHAPTER

4

5-HT Effects on Post-Error-Slowing

Submitted as:

Fischer, A. G., Endrass, T., Reuter, M., Kubisch, C., & Ullsperger, M. Serotonin Reuptake Inhibitors and Serotonin Transporter Genotype Modulate Performance Monitoring Functions but not their EEG Correlates.

Abstract

Serotonin (5-HT) has been hypothesized to be implicated in performance monitoring (PM) by promoting behavioral inhibition in the face of aversive events. However, it is unclear if this is restricted to external (punishment) or includes internal (response errors) events. The aim of the current study was to test whether higher 5-HT levels instigate inhibition specifically in the face of errors, measured as post-error slowing, and whether this is represented in electrophysiological correlates of error processing, namely error-related negativity (ERN) and positivity (Pe). Therefore, out of large sample of subjects (n = 878) two extreme groups were formed regarding hypothesized high and low 5-HT transporter (5-HTT) expression based on 5-HTTLPR and two additional single nucleotide polymorphisms (rs25531, rs25532). 16 higher (LL) and 15 lower (SS) expressing Caucasian subjects were administered the selective serotonin reuptake inhibitor (SSRI) citalopram intravenously in a double-blind cross over design. We found higher post-error slowing for SS subjects and SSRI administration increased slowing in both genetic groups, which both was not seen for post-conflict slowing. ERN and Pe were unaffected by genetic and pharmacological factors but ERN was de-coupled from behavioral adaptation by SSRI administration in the LL group. Thus, combined pharmacological and genetic evidence suggests that increased 5-HT levels lead to behavioral inhibition in the context of internal aversive events but electrophysiological correlates of PM appear unrelated to the 5-HT system. These findings are especially important as it has been suggested that genetic factors that influence serotonergic neurotransmission can increase the risk for depression via an overactive PM network.

Introduction

Serotonin (5-HT) has been implicated in the modulation of diverse neurocognitive functions in health and disease. Especially its role in behavioral inhibition and processing of aversive events has received considerable attention. It has been repeatedly reported that in the face of expected punishment, a reduction of 5-HT levels leads to a disinhibition of behavior (Soubrié, 1986; Crockett et al., 2009). However, it is unclear whether this effect is based on an increased impact of aversive events (Boureau and Dayan, 2011) or depends on a secondary mechanism that responds to such events with inhibition. The affective reaction to stress is modulated by a polymorphism (5-HTTLPR) at the 5-HT transporter (5-HTT) gene (SLC6A4) which interacts with acute manipulations of 5-HT levels (Roiser et al., 2006a; Markus and Firk, 2009) and in combination with traumatic life events acts as a risk factor for depression (Karg et al., 2011). Some studies suggest overactive error detection in a performance monitoring (PM) network modulated by 5-HT as a possible link between vulnerability factors and disease (Fallgatter et al., 2004; Holmes et al., 2010). It is assumed that subjects with lower 5-HTT expression attribute higher significance to negative events (Ma et al., 2014). However, genetic association studies have suffered from poor replicability while pharmacological challenges within the 5-HT system are inconclusive and complicated by the considerable degree of individual genetic variance (Veenstra-VanderWeele et al., 2000; Hu et al., 2006).

The error-related negativity (ERN) and error positivity (Pe) are EEG potentials thought to reflect early detection and evaluation of evidence for an erroneous response originating in the anterior midcingulate cortex (aMCC) (Steinhauser and Yeung, 2010; Ullsperger et al., 2014b). Following errors, subjects usually slow down their responses and increase their accuracy, a phenomenon known as post-error slowing (PES) (Danielmeier and Ullsperger, 2011). Single-trial ERN amplitudes have been shown to covary with the amount of slowing induced by errors, suggesting a direct link between error detection and adaptive implementation (Debener et al., 2005). Furthermore, following response conflict, subjects also show reaction slowing which has been termed post-conflict slowing (PCS) (Ullsperger et al., 2005; Verguts et al., 2010). While PES involves the evaluation of a subjectively aversive event, this is not the case for PCS.

Aim of the current study was to systematically examine effects of acute challenges and genetic variations of the 5-HT system on PES, PCS, and the electrophysiological correlates of error processing including the single-trial coupling between ERN and PES. We employed acute blockade of 5-HTTs via intravenous application of a selective serotonin reuptake inhibitor (SSRI) in a double-blind cross over design. The genetic background was controlled for by an extreme group approach: out of large sample of genotyped subjects (n = 878), two groups based on estimated highest and lowest 5-HTT expression were selected. One group consisted of homozygous high expressing L_{AC} allele carriers based on 5-HTTLPR, rs25531, and rs25532 (Hu et al., 2006; Wendland et al., 2007) and the other of homozygous S carriers. We hypothesized increased PES (Holmes et al., 2010) and not PCS, and possibly increased ERN / Pe amplitudes (Fallgatter et al., 2004; but see: Olvet et al., 2010) in S allele carriers, since PES involves evaluation of subjectively aversive events (Boureau and Dayan, 2011). As SSRI increase extracellular 5-HT akin to the hypothesized difference between S and L allele carriers (Murphy et al., 2008), we furthermore expected increased PES after drug administration.

Materials and Methods

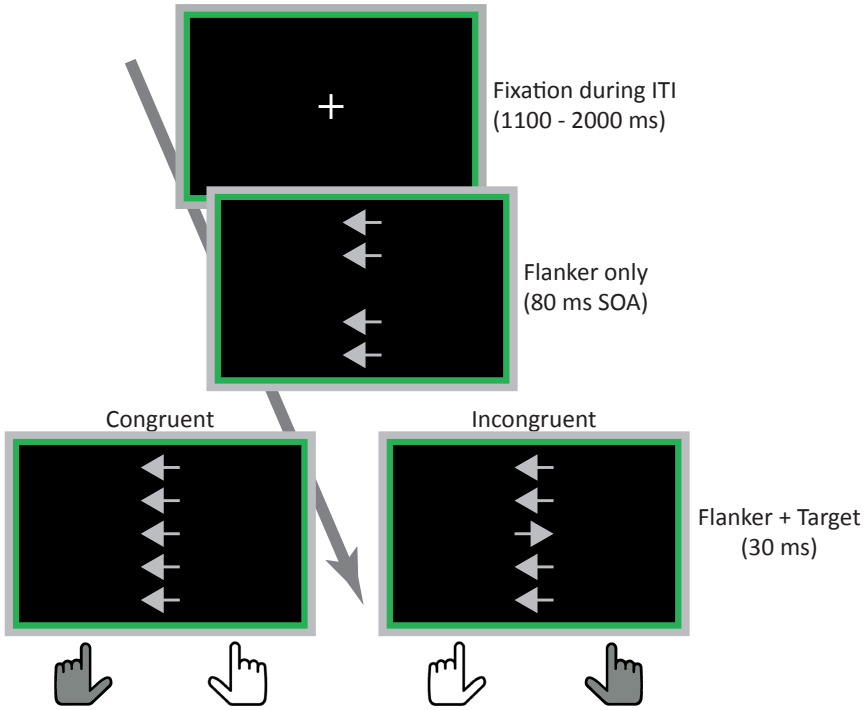

Figure 4-1. Flanker Task Details.
The Figure illustrates the timing of the flanker task with congruent and incongruent trials shown separately. The number of switches from one required response direction to the other, the total number of required responses with the left or right hand in congruent and incongruent condition, and the trial sequence between congruent and incongruent trials were exactly counterbalanced. Before the task began, all subjects completed 21 training trials.

Flanker task

A speeded version of the Eriksen Flanker task was used. Subjects had to respond in accordance to the direction of a centrally presented arrow (*target*) and ignore 4 flanking arrows (*flankers*) that appeared earlier on the screen with a stimulus onset asynchrony (SOA) of 80 ms (**Figure 4-1**). All stimuli stayed thereafter together on screen for 30 ms. On congruent trials, central and flanking arrows pointed to the same direction, whereas on incongruent trials both pointed into different directions – introducing the tendency to respond to the flanker‘s direction. Subjects were instructed to respond as fast and accurately as possible. A random inter-trial-interval (ITI) was used between 1100 and 2000 ms. Every 100 trials subjects received written feedback on screen about their performance and whether they should speed up their responses. Subjects were told to speed their responses up if the number of errors committed in the incongruent condition was below 20%. Additionally, the screen was bordered by a colored frame that encouraged subjects to speed up their responses up by changing the color from green to red with a delay of 12 trials when they did not make enough errors. Each subject completed 492 trials out of which half were incongruent. After each session, subjects had to indicate whether they got angry when committing an error on a scale from 1 (not at all) to 10 (very). Average scores were 6.7±0.3 suggesting that subjects experienced errors as aversive and reports were not different between genetic or drug conditions (all ps > 0.70).

EEG Analysis

The signal was bandpass filtered from 0.5 to 42Hz and epochs spanning from 1.5s before to 2s after response onset were extracted. Erroneous and correct trials were separately corrected for artifacts and epochs that contained deviations greater than 5 SDs of the mean probability distribution of each condition were automatically rejected. This was done as to not confuse the sometimes very high single trial ERN amplitudes with artifacts, and no more than 5% of the trials in each condition were removed. Epoched data were demeaned and submitted to Adaptive Mixture Independent Component Analysis (AMICA) (Palmer et al., 2012). Independent components reflecting uniform artifacts such as eye blinks were removed from the data and baseline correction from 300 to 100 ms before response onset was applied. Average and grandaverage waveforms were then calculated and ERP data was measured as described in the corresponding results.

To establish the relationship between early correlates of error processing and PES, multiple robust single trial regression analysis was used (Fischer and Ullsperger, 2013; Ullsperger et al., 2014b). The regression model used the following trials' RT for all error trials as the dependent variable while EEG activity and following congruency were used as predictor variables including their interaction. Single-trial data was smoothed with a running average of 10 ms before and after each datapoint and calculated for each datapoint between -250 and 500 ms surrounding the response at electrode FCz. Robust regression coefficients were scaled by their SDs and are thus comparable across subjects. Due to the central limit theorem, these coefficients can be assumed to be Gaussian under the null hypothesis and subjected to parametric statistical testing.

Results

Behavioral Effects

Neither the total number of errors (**Figure 4-2C**) nor the number of incompatible errors was modulated by genotype or drug (MLM all $p > 0.20$). Comparable to other studies, subjects in our task were slower on the more difficult incongruent trials (ΔRT +76±2 ms). Furthermore, subjects responded faster when they made an erroneous response (ΔRT -90±2 ms). When analyzed using MLMs, none of these factors interacted with *drug* or *genotype* (all $p > 0.29$).

An initial MLM analysis of overall median RTs in the task showed trends for higher reaction times under citalopram (360±3 ms) than saline condition (354±3 ms, $F_{1,32} = 3.3$, $p = 0.077$) and a trend towards faster RTs for subjects with genotype LL (351±4 ms) than SS (362±5 ms, $F_{1,32} = 3{,}45$, $p = 0.072$, **Figure 4-2A**). In order to investigate reaction time effects following errors, we included an additional factor *previous trial* (error / correct) into the model. This revealed higher RTs on post-error trials (+10±2 ms, $F_{1,96} = 16.9$, $p < 10^{-5}$) confirming PES. Additionally, main effects of drug ($F_{1,32} = 8.08$, $p = 0.005$) and genotype ($F_{1,32} = 5.53$, $p = 0.025$) were observed as well as trends towards interactions for *previous trial x drug* ($F_{1,96} = 3.56$, $p = 0.062$) and *previous trial x genotype* ($F_{1,96} = 3.80$, $p = 0.054$). Post hoc tests in post-error trials showed main effects for *drug* (citalopram +12±4 ms, $F_{1,96} = 11.2$, $p = 0.001$) and *genotype* (SS +20±7

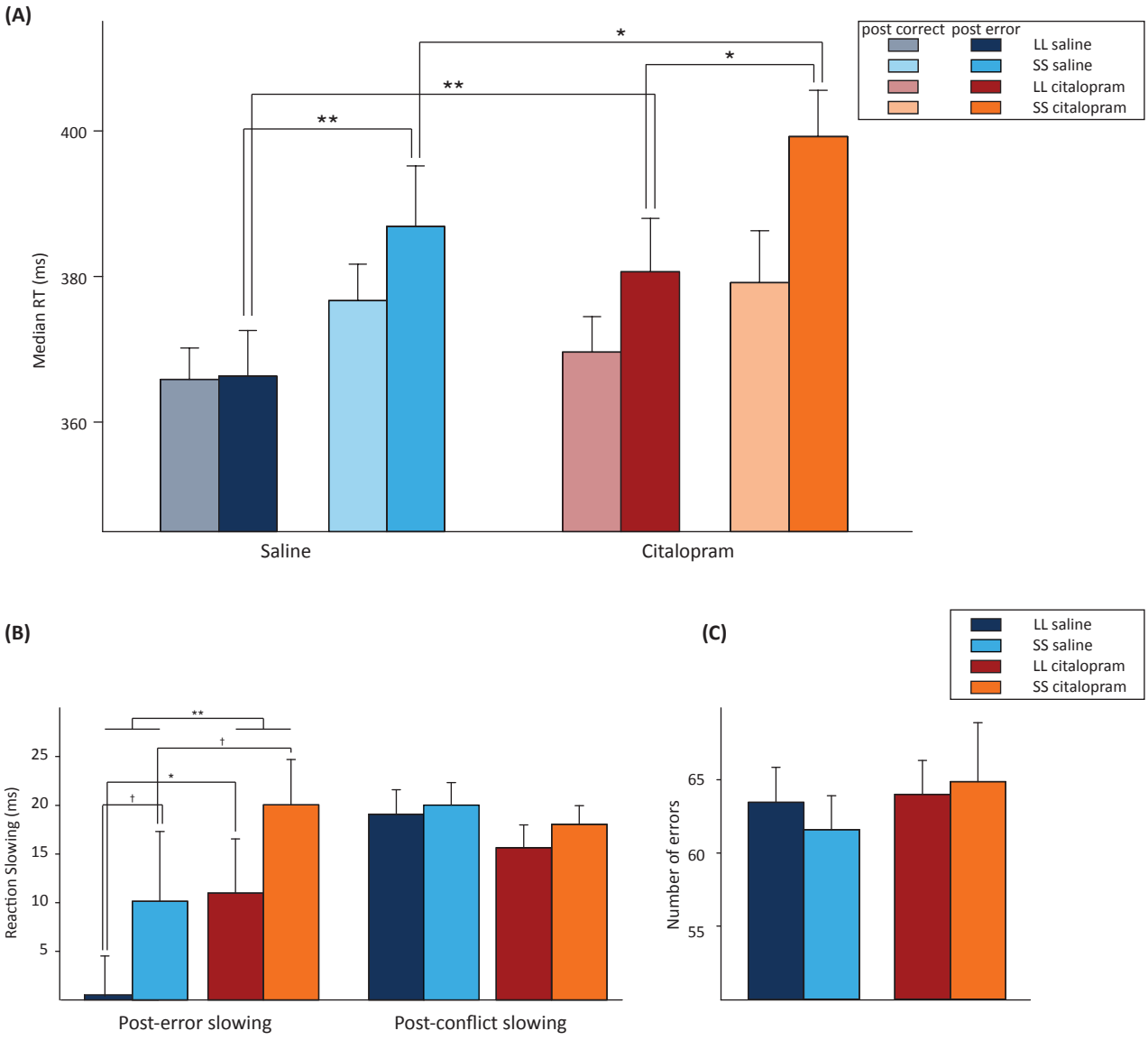

Figure 4-2. PES but not PCS is modulated by citalopram and 5-HTTLPR
(A) RT depending on the accuracy of the previous trial comparing post-error and post-correct trials. Genetic and drug effects were only present on trials that followed errors. (B) Post-error slowing is compared to post-conflict slowing. We found a modulation by 5-HT only for slowing induced by errors but not conflict itself. Citalopram increased PES (p = 0.003) and SS subjects showed overall higher PES than did LL subjects (p = 0.029). PCS was numerically but non-significantly reduced by citalopram. (C) Neither drug nor genotype effects were seen for the number of errors.
Significance is marked for post hoc tests of drug effects within each genotype and between genotypes as follows: † = p < 0.10, * = p < 0.05, ** = p < 0.01, all other comparisons in post-correct trials (A) and for PCS (B) are non-significant (all ps > 0.1).

ms, $F_{1,32}$ = 8.4, p = 0.006). On post-correct trials, no effects of *drug* (citalopram +2±4 ms, $F_{1,96}$ < 1, p = 0.501) or *genotype* (SS +11±7 ms, $F_{1,32}$ = 2.2, p = 0.14) were significant.

We calculated PES by subtracting median RTs on correct trials following errors from those that did not follow errors (**Figure 4-2B**). Trials that themselves preceded errors and double errors were excluded. Additionally, we did the same calculation for trials that followed incongruent events which are also known to induce slowing on the following trial (Ullsperger et al., 2005; Verguts et al., 2010) and submitted both to MLM analysis with factor *type of slowing* (PES or

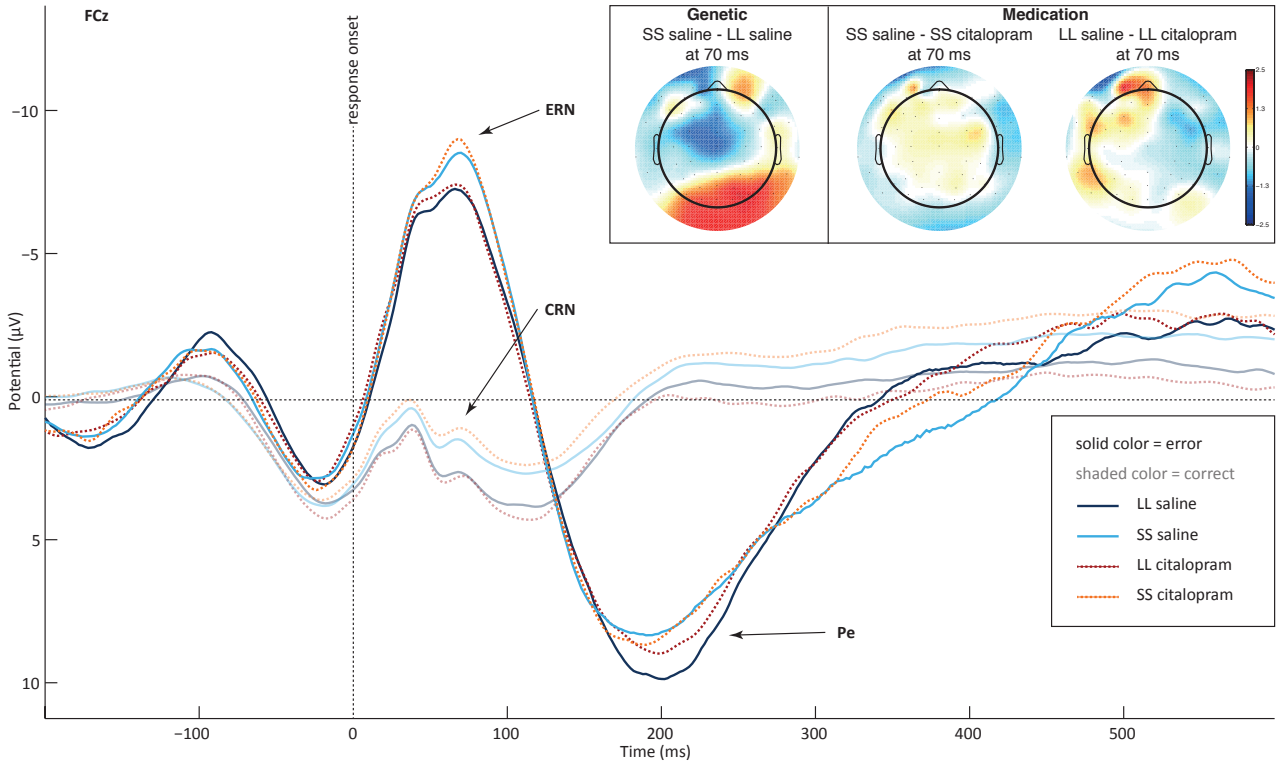

Figure 4-3. Citalopram and 5-HTTLPR do not affect ERN, CRN or Pe
Electrophysiological indices of performance monitoring were not different between genetic and pharmacological conditions. Topography maps show (nonsignificant) group differences between genotypes in the saline condition (left) and differences of medication within each genetic group (right) at the ERN peak around 70 ms post response onset.

PCS). This analysis revealed a main effect for factor *type of slowing* indicating higher slowing following incongruent events (+18±2 ms) compared to errors (+10±2 ms). Additionally, significant interactions for *type of slowing* with *drug* ($F_{1,96}$ = 7.8, p = 0.006) and *genotype* ($F_{1,32}$ = 4.2, p = 0.043) were seen. Post hoc tests confirmed a significant drug effect on PES where citalopram increased slowing by 9±3 ms ($F_{1,96}$ = 9.1, p = 0.003) while it led to a non-significant decrease of PCS (ΔRT -3±3 ms, $F_{1,96}$ = 0.9, p = 0.35). Subjects carrying the SS genotype showed higher PES (15±3 ms) than subjects with LL genotype (5±3 ms, $F_{1,55.8}$ = 5.0, p = 0.029). No difference between genetic groups was seen for PCS (ΔRT 0.6±4 ms, $F_{1,96}$ < 1, p = 0.88). The drug effect did not depend on the genotype (interaction *genotype x drug* and *genotype x drug x type of slowing* both F < 1 and p > 0.5). These results indicate that specifically slowing induced by errors but not conflict was increased by citalopram and that SS subjects show higher slowing following errors than do LL subjects.

Table 1. Exact ERP amplitudes.

	ERN (μV)	CRN (μV)	Pe (μV)
SS saline	-9.29±1.0	1.15±0.8	12.30+±1.0
SS citalopram	-9.75±1.3	1.05±0.9	11.59±1.0
LL saline	-9.15±0.7	0.79±0.7	13.21±0.9
LL citalopram	-9.69±0.8	1.49±0.6	12.73±1.4

ERN and CRN were measured as the individual grand-average minima between 0 and 100 ms following response onset at electrode FCz. Pe was quantified as the individual grand-average maxima between 100 and 350 ms following response onset at electrode Cz. Neither measure was modulated by acute SSRI administration, 5-HTTLPR genotype, or their interaction.

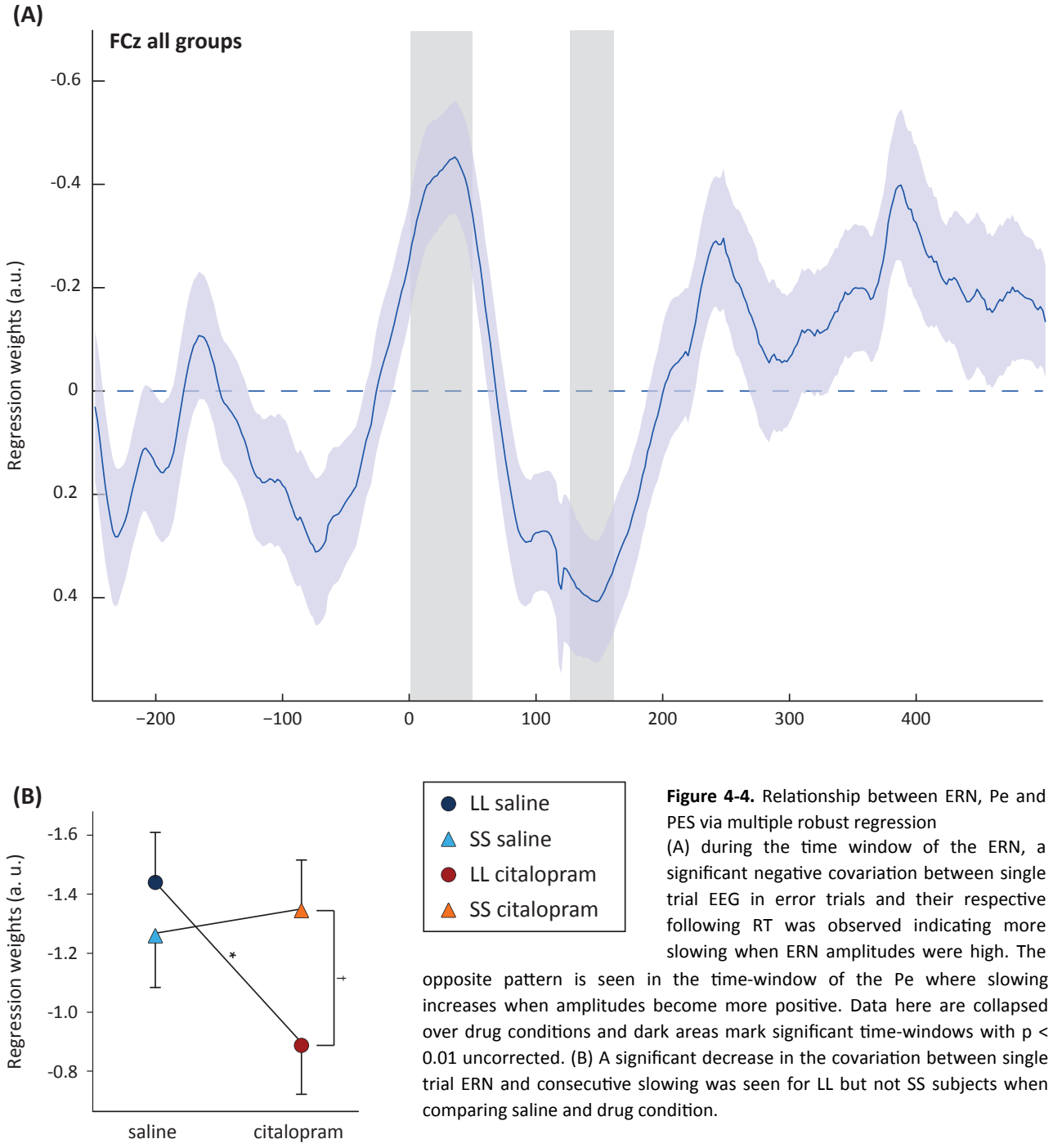

Figure 4-4. Relationship between ERN, Pe and PES via multiple robust regression
(A) during the time window of the ERN, a significant negative covariation between single trial EEG in error trials and their respective following RT was observed indicating more slowing when ERN amplitudes were high. The opposite pattern is seen in the time-window of the Pe where slowing increases when amplitudes become more positive. Data here are collapsed over drug conditions and dark areas mark significant time-windows with $p < 0.01$ uncorrected. (B) A significant decrease in the covariation between single trial ERN and consecutive slowing was seen for LL but not SS subjects when comparing saline and drug condition.

Across individuals, higher PES (but not PCS) correlated positively with a post-error increase in accuracy (PIA, Danielmeier et al., 2011) in both saline ($r = 0.45$, $p = 0.009$) and citalopram ($r = 0.48$, $p = 0.005$) conditions (PCS all ps > 0.18) suggesting that PES provided time for more task-specific post-error adjustments (Danielmeier and Ullsperger, 2011). However, accuracy following errors was not affected by any 5-HT related factor (all ps > 0.1). This can in part be explained by the high variability (range 1-21) and the low average number of double-errors (6±0.62) rendering this an insensitive measure. Additionally, we found no 5-HT related effects on post-error reduction of interference, PIA itself, or the conflict-adaptation effect (Gratton et al., 1992) (all ps > 0.1 for *drug*, *genotype*, and their interaction).

EEG Results

ERN amplitudes were measured as the negative peak between 0 - 100 ms at FCz comparable to the first study reporting an effect of 5-HTTLPR on ERN amplitudes (Fallgatter et al., 2004) (**Figure 4-3**). ERN amplitudes were not significantly altered by drug ($F_{1,31} < 1$, p = 0.37) or genotype ($F_{1,31} < 1$, p = 0.96) and no interaction was observed ($F_{1,31} < 1$, p = 0.90) (see Table 1 for exact values). CRN amplitudes, measured in the same way, again showed no effects of *drug* ($F_{1,31} < 1$, p = 0.42), *genotype* ($F_{1,31} < 1$, p = 0.92), and no interaction ($F_{1,31} < 1$, p = 0.35). Pe amplitudes, measured as the maximum peak between 100 and 350 ms at Cz, where amplitudes were highest, were again not modulated by genotype ($F_{1,31} < 1$, p = 0.44) or drug ($F_{1,31} < 1$, p = 0.29, interaction: $F_{1,31} < 1$, p = 0.88).
Furthermore, other measures of ERN amplitudes (difference wave between erroneous and correct trials, mean amplitudes, or trough-to-peak measures) did not lead to significant effects for any of the factors of interest (genotype, medication or their interaction) and neither were latencies of the ERPs modulated (all p > 0.1).

Single-Trial Regression Results

Using multiple robust regression analysis of single-trial ERN and following post-error RT, we first sought to establish the link between EEG data and PES across all subjects by testing regression coefficients collapsed over the repeated factor (drug) against zero. This revealed negative covariations in the ERN time window (ERN peak = 34 ms, $t_{30} = -4.33$, p = 0.00015, uncorrected, **Figure 4-4A**), indicating that the more negative the ERN on a single-trial level was, the higher was the following RT. This pattern is in accordance with other studies (Debener et al., 2005; Wessel and Ullsperger, 2011). Furthermore, the opposite effect was observed for Pe amplitudes, where a positive covariation was observed (Pe peak = 146 ms, $t_{30} = 3.66$, p = 0.00096, uncorrected). This suggests that ERN and Pe both are sensitive for single-trial variation and adjust subsequent slowing – likely to provide time for adjustment. No significant interaction depending on the following trials' congruency was found. We then established the effects of drug and genotype by submitting regression coefficients (measured as response-locked minima of regression coefficients between 0 and 100 ms) to MLM analysis. This revealed a significant *drug x genotype* interaction: for subjects homozygous for the L allele, citalopram significantly decreased the covariation between ERN and following RT (Δb = -0.55±0.19, $F_{1,31} = 7.82$, p = 0.009) which was not observed for subjects homozygous for the S allele (Δb = 0.09±0.20, $F_{1,31} < 1$, p = 0.638, interaction *drug x genotype*: $F_{1,31} = 5.22$, p = 0.029, **Figure 4-4B**). In accordance with the observed PES reduction in the second session while no ERN reduction was observed, we also found a session effect (see Supplements) in that covariation between EEG and RT was reduced when the task was repeated (Δb = -0.40±0.14, $F_{1,31} = 7.81$, p = 0.009). No other main effects or interactions were significant (all ps > 0.12) and no drug or genotype effects were observed for the following positive (Pe-related) covariation that was revealed in the initial analysis (measured as maximum regression coefficients between 100 and 200 ms, all ps > 0.14).

Discussion

The current study reports differential findings for behavioral and electrophysiological aspects of performance monitoring with regard to 5-HT manipulations. In accordance with theories linking a deficit in performance monitoring in S allele carriers to an increased risk of depression following aversive life events (Caspi et al., 2003; Karg et al., 2011), we found increased PES in the SS group. These subjects have also been reported to be more sensitive towards mood changes via 5-HT fluctuations following acute stress (Markus and Firk, 2009) which supposedly is mediated via a brain network including the aMCC (Holmes et al., 2010; Drabant et al., 2012). Similarly, increased PES has also been shown in depressed subjects (Compton et al., 2008). This is suggestive of a dysfunctioning PM network that attributes increased significance to errors. However, these findings also highlight that while the S allele may be a risk factor for mood disorders, under certain circumstances, greater sensitivity towards mistakes may also be beneficial, as it can serve to decrease the likelihood of repeating a mistake (Homberg and Lesch, 2011). In line with this, we found a clear correlation between PES and the increase of accuracy following errors suggesting that subjects in our task were more cautious in responding in post-error trials which effectively kept performance high (Danielmeier and Ullsperger, 2011).
Acute administration of the SSRI citalopram significantly increased PES in both genetic groups but did not affect conflict, or difficulty, induced RT slowing and general RT. This effect fits quite well to the idea that SS subjects may have increased extracellular 5-HT levels due to lower 5-HTT expression as has been demonstrated in mouse models of 5-HTTLPR (Mathews et al., 2004; Murphy et al., 2008). A further acute increase in extracellular 5-HT by blockade of these transporters then seems to further enhance this effect. Thus, higher 5-HT levels are associated with increased behavioral inhibition in the context of emotionally negative events when they are externally signaled or internally detected as errors (Crockett et al., 2009). Therefore, this finding is in line with models that tie serotonergic neurotransmission to behavioral inhibition in the face of punishment (Dayan and Huys, 2009; Cools et al., 2010; Boureau and Dayan, 2011), but serotonergic influences are usually minimal when measured in unrewarded context‘s both in humans and animal models alike (Winstanley, 2012). Furthermore, this finding is in accordance with studies reporting decreased behavioral slowing in punished contexts by ATD, a method that lowers serotonergic neurotransmission (Crockett et al., 2009), by showing the opposite pattern when 5-HT levels are increased.
Although the link between *in vitro* mRNA 5-HTT expression (Lesch et al., 1996; Hu et al., 2006) and *in vivo* measures of 5-HTT binding in humans (Kalbitzer et al., 2010; Murthy et al., 2010) and monkeys (Jedema et al., 2010) has been highly debated, many studies report differential responses to perturbations in 5-HT neurotransmission depending on 5-HTTLPR genotype (Roiser et al., 2006a; Markus and Firk, 2009). This includes differences in treatment responses and side-effects to SSRI administration in depressed patients (Murphy et al., 2003; Kato and Serretti, 2008). In line with this, the current study suggests that in fact higher extracellular 5-HT levels due genotypical variation and pharmacological manipulations or their consequence, decreased serotonergic neuron firing due to increased inhibitory autoreceptor activation (Gobbi et al., 2001; Cools et al., 2010), leads to increased behavioral slowing following affectively negative events.
In contrast to these behavioral findings, the current study questions the suggestion that alterations in aMCC dependent PM networks by 5-HT serve as a link between increased aversive processing and an increased risk for

depression in S allele carriers (Olvet and Hajcak, 2008; Holmes et al., 2010). So far, one study reported significant effects of 5-HTTLPR genotype on ERN amplitudes. Fallgatter and colleagues (Fallgatter et al., 2004) found increased ERN amplitudes at electrode Cz in a sample of 22 subjects comparing 11 homozygous L subjects to carriers of at least one S allele in a flanker paradigm not accounting for rs25531 (Hu et al., 2006). Another study compared 16 L_A homozygous to 28 heterozygous or homozygous S carriers without significant results (Olvet et al., 2010). An fMRI study found increased BOLD activity in the aMCC on error trials in S allele carriers (Holmes et al., 2010), but it is unclear if this BOLD signal truly reflects the same mechanisms as the ERN, that is error detection, or the Pe, that likely is error appraisal or awareness (Murphy et al., 2012; Ullsperger et al., 2014b). While certainly the current study and the other negative report do not seem sufficiently powerful to finally rule out an association between ERN and Pe with 5-HTTLPR polymorphisms, it is reasonable to assume that the effect of this genetic association is small. Independence of electrophysiological indices of error processing from the 5-HT system is further suggested by the absence of pharmacological effects in the current study despite a powerful repeated measures design accounting for session effects, well controlled intravenous drug application, and control for the genetic background. Furthermore, this is corroborated by other studies reporting that ERN and Pe are not affected by pharmacological alterations of the serotonergic system (de Bruijn et al., 2006). Additionally, it is unlikely that the drug dose used in the current study was insufficient to induce effects. Even lower intravenously administered citalopram doses have been demonstrated to block more than 80% of 5-HTTs as measured using PET (Meyer et al., 2004) and we observed clear behavioral as well as physiological effects on cardiovascular parameters (Supplements). Thus, it seems that the neurogenerators giving rise to ERN and Pe are insensitive to acute pharmacological perturbations and genetic variation in the serotonergic system.

In accordance with other studies, the behavioral effects on PES emerged in the absence of differences in subjective estimates of self-reported affect in response to errors (Hariri et al., 2002; Holmes et al., 2010). This suggests that 5-HT may mediate its effect on inhibition independent of subjective appreciation of the impact of errors. This is further supported by the absence of effects on ERN and especially Pe, which both are assumed to be sensitive towards the functional significance or salience of an error (Falkenstein et al., 2000).

One may wonder how the behavioral 5-HT effects then arise. When the link between error detection and translation into behavioral adaptation is investigated more directly, we found differential effects of the drug in both genetic groups. For LL subjects the strength of coupling between ERN and consecutive RT adjustments is decreased by SSRI administration while this is not the case for SS subjects. LL carriers showed no PES in the saline condition but this was induced by drug administration. This de-coupling of behavioral adaptation suggests an independent mechanism that increases behavioral inhibition and does not affect cortical EEG correlates. One may speculate that this is integrated in subcortical structures out of which especially the subthalamic nucleus (STN) seems a likely structure as it has been demonstrated to mediate PES (Cavanagh et al., 2014; Siegert et al., 2014). STN furthermore has long been known to receive strong serotonergic projections from raphe nuclei (Bobillier et al., 1976), and 5-HT injections into the STN increased firing rates (Xiang et al., 2005). At least a subpopulation of likely serotonergic raphe neurons furthermore code aversive signals (Nakamura et al., 2008), which may mediate the observed slowing effect.

In sum, the current study provides important new evidence for a role of 5-HT in mediating rapid behavioral adaptation following response errors in that higher 5-HT levels appear to increase RT slowing as evidenced by genetic and pharmacological effects. This mechanism may both be adaptive, as it allows to avoid repeating errors, or be a symptom of increased aversive processing seen in S allele carriers which has been linked to depression. Electrophysiological correlates of error processing appear unaffected by genetic and pharmacological serotonergic influences and the behavioral effect may be mediated subcortically. Clearly, more imaging as well as electrophysiological studies are needed to elucidate the neural mechanisms underlying 5-HT mediated inhibition, especially on the level of brain stem activity, and its association to aversive coding. This may then help clarify the underlying changes that mediate the link between genetic vulnerability and disease onset in depression, but also other disorders associated with the serotonergic system such as OCD.

CHAPTER

5

Interactive Effects of Citalopram and 5-HTTLPR on Inhibition and Attentional Orienting

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Fischer, A. G., Endrass, T., Goebel, I., Reuter, M., Montag, C., Kubisch, C., & Ullsperger, M. Interactive Effects of Citalopram and Serotonin Transporter Genotype on Neural Correlates of Response Inhibition and Attentional Orienting.

Abstract

Objective: The brain's serotonergic (5-HT) system has been implicated in controlling impulsive behavior and attentional orienting and linked to impulse control and anxiety related disorders. However, interactions between genotypical variation and responses to serotonergic drugs impede both treatment efficacy and neuropsychiatric research. We examine behavioral and electrophysiological responses to acute intravenous administration of a selective serotonin reuptake inhibitor (SSRI) while controlling for major genetic differences regarding 5-HT transporter (5-HTT) genotypes.

Method: Out of a genotyped sample of healthy Caucasian subjects (n=878) two extreme-groups regarding 5-HTT genotypes were selected (n=32). A presumed homozygous high-expressing group based on tri-allelic 5-HTTLPR and rs25532 (L_{AC}/L_{AC}=LL) was compared to homozygous S allele carriers (SS). Both groups were administered a low dose of citalopram (10mg) intravenously in a double blind crossover fashion and performed a novelty NoGo paradigm while high density EEG was recorded.

Results: Interactions between drug and genotype were seen on both behavioral and neurophysiological levels. Reaction slowing following inhibitory events was decreased by the administration of citalopram in the LL but not SS group. This was accompanied by decreases in the amplitude of EEG correlates of inhibitory processes (N2 and P3b) in the LL group which was not seen in the SS group. SS subjects showed an increase in P3a amplitudes following SSRI administration to any type of deviant stimulus possibly reflecting increased attentional capture.

Conclusions: The acute SSRI response on inhibitory processes and attentional orienting interacts with genotypes regulating 5-HTT gene expression. SS subjects may show increased attentional side effects reflected in increases in P3a amplitudes which could contribute to treatment discontinuation. Inhibitory processes and their neural correlates are affected only in LL subjects which might thus benefit from SSRI treatment in impulse control disorders. These findings may indicate an underlying mechanism that could relate genotypical differences to altered side effect profiles and drug responses both in experimental settings and clinical treatment.

Introduction

Genotypical differences are increasingly being recognized as risk factors for psychiatric disorders especially in interaction with the environment (Karg et al., 2011). These interactions are not limited to life events, but extend to treatment responses to drugs in various diseases (Mrazek et al., 2009; Thakur et al., 2010) and laboratory settings which complicates research in this field. Especially a functional promotor polymorphism (5-HTTLPR) in the serotonin (5-HT) transporter (5-HTT) gene (*SLC6A4*) has been well studied (Lesch et al., 1996). The short (S) genotype is associated with reduced 5-HTT mRNA expression (Hu et al., 2006), increased stress and startle responses (Drabant et al., 2012), and increased risk for depression following negative life events (Karg et al., 2011). Furthermore, this genotype shows increased side effects (Murphy et al., 2003) and poorer outcome (Kato and Serretti, 2008) when treated with antidepressant drugs of the selective serotonin reuptake inhibitor type (SSRI). The long (L) variant, on the other hand, has been associated with reduced response inhibition (Roiser et al., 2006b), characteristics of impulse control disorders (Curran et al., 2005), the onset of ADHD (Faraone et al., 2005; but see: Landaas et al., 2010), and possibly worse social and cognitive performance (Homberg and Lesch, 2011).

Electrophysiological correlates of inhibition and novelty processing have been shown to be sensitive for genetic variations in 5-HTTLPR. Specifically, an increased P3a, a correlate of attentional orienting (Polich, 2007), has been associated with the S genotype (Heitland et al., 2013). This has been interpreted as increased sensitivity to possibly, but not necessarily, threatening environmental changes (Homberg and Lesch, 2011). The N2 as an index of behavioral inhibition is thought to reflect activity of a brain network centered around the right inferior frontal cortex (IFC) (Aron et al., 2004; Enriquez-Geppert et al., 2010) and showed a decreased adaptive range in L allele carriers (Enge et al., 2011). Additionally, a more anterior inhibitory P3 scalp distribution has been seen in S compared to L allele carriers, possibly reflecting a deficit in inhibitory control in the latter ones (Fallgatter et al., 1999; Roiser et al., 2006b). However, acute pharmacological perturbations of the 5-HT system with regard to attentional orienting, inhibitory control, and their electrophysiological correlates have yielded mixed results (d'Ardhuy et al., 1999; Wienberg et al., 2010). A possible confounding factor could be the substantial degree in individual genetic variability that may interfere with the response to acute changes in serotonergic neurotransmission (Cools et al., 2008).

Behaviorally, 5-HT has since long been implicated in inhibitory processes (Soubrié, 1986), but across species an association of 5-HT with the ability to suppress a prepotent response, for example in stop signal paradigms or Go/ NoGo tasks, has repeatedly failed (Clark et al., 2005; Chamberlain et al., 2006; Winstanley, 2012). Rather, 5-HT seems to control the general initiation of responses (Eagle et al., 2008b; Walderhaug et al., 2010). It is well established that behavioral inhibition induces reaction time (RT) increases on the subsequent trial (Rieger and Gauggel, 1999), and thus delays the initiation of the next response, which may be influenced by the serotonergic system (Ahveninen et al., 2002; Crockett et al., 2009).

Identifying subjects that benefit from certain drug treatments based on their genetic background is of major interest (Crisafulli et al., 2011), but little is known about the interaction between the genetic profile and specific pharmacological challenges of the serotonergic system with regard to attentional processing, inhibitory control, and their underlying neural processes. To investigate differential responses to SSRI based on genotype, we used an

extreme-group approach. Caucasian subjects homozygous for the high expressing L allele and higher expressing variants of two other single nucleotide polymorphisms (SNPs) known to alter 5-HTT mRNA expression (Hu et al., 2006; Wendland et al., 2007) where compared to subjects homozygous for the low expressing S allele. These groups were selected out of a large sample of subjects (n=878) and reflected less than 30% and 18% of the sample, respectively. Both groups were administered a low intravenous dose of the SSRI citalopram in a double-blind cross-over design. We hypothesized that genotype may moderate the response to acute SSRI challenges. Furthermore, we expected P3a amplitudes as a measure of attentional orienting to be more strongly affected in the SS group. On the other hand, the N2 and P3b as measures of inhibitory control could be altered in the LL group and may be more similar to the SS group following acute blockade of the 5-HTT.

Materials and Methods

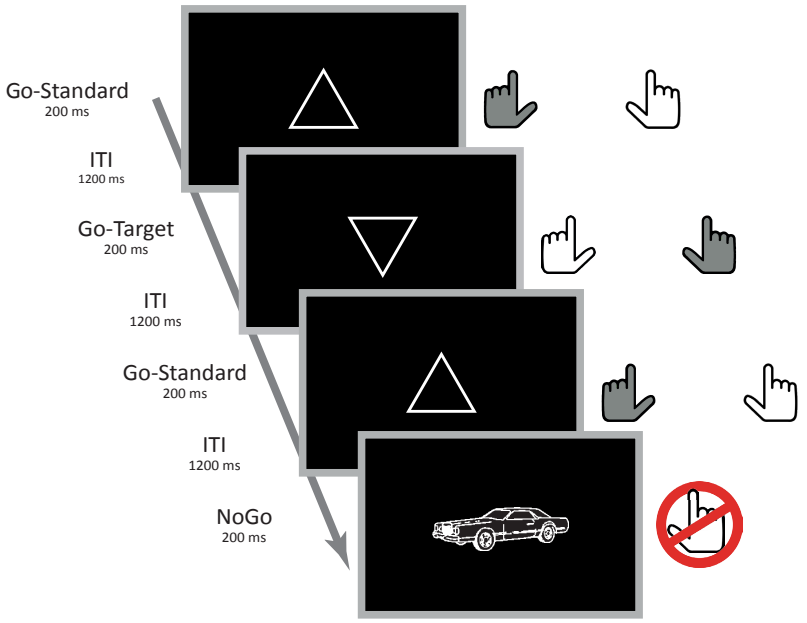

Figure 5-1. Go/NoGo Task Details.
In the NoGo paradigm, subjects had to respond by pressing a button with either the left or right hand following Go-Standard (upward triangle) and Go-Target trials (downward triangle), respectively. NoGo events were indicated by novel unexpected line drawings that were not repeated during the task. Go-Standards comprised 80% of the 500 total trials while Go-Target and NoGo each accounted for 10% of the trials. The assignment of stimulus types to responses was counterbalanced across sessions and drug conditions.

Go/NoGo Task

We used modified oddball task where subjects were instructed to respond as quickly as possible with one button press either with their left or right hand following presentation of Go-Standard or Go-Target stimuli (**Figure 5-1**), respectively. Additionally, on NoGo trials participants had to withhold responding. Stimuli consisted of triangles pointing up- and downward for Go-Standard and Go-Target trials and unique novel line-drawings for NoGo trials. Allocation of trial types and response hands were counterbalanced across both test sessions. The task comprised 500 trials out of which 50 were Go-Target and 50 were unexpected NoGo events whereby each stimulus onset was separated by 1200ms, irrespective of the response given.

EEG Data Processing

Data were recorded continuously at a 500Hz sampling rate with BrainAmp MR plus amplifiers (*Brain Products*) and analyzed offline using *EEGLAB 12.0* (Delorme and Makeig, 2004). Electrodes at the left and right outer canthus and above and below the left eye captured eye movements; the ground electrode was positioned at F2. Data were online referenced to CPz and offline re-referenced to common average. The signal was bandpass filtered from 0.5 to 42Hz and epochs spanning from 0.5s before to 2s after stimulus onset were extracted. Epochs that contained deviations greater than 5 SDs of the mean probability distribution were automatically rejected. Epoched data were then submitted to temporal infomax independent component analysis (ICA) integrated in *EEGLAB*. Independent components reflecting uniform artifacts such as eye blinks were removed from the data. Average and grandaverage waveforms were then calculated and ERP data was measured as described in the corresponding results. Note that in order to be comparable to the behavioral results, we only included successful response inhibitions and correct target responses in our analyses.

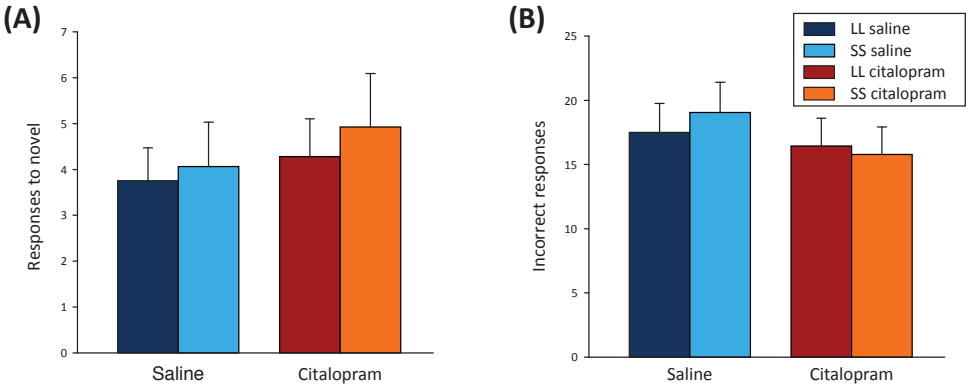

Figure 5-2. Commission and ommission errors.
Neither the number of failed response inhibitions on NoGo stimuli (A) nor the number of responses with the wrong hand to standard and target stimuli (B) showed an effect of *drug, genotype* or an interaction of both (MLM all p > 0.13).

Results

Behavioral Results

Subjects showed near optimal performance on standard trials (99.0±0.2% correct) and withheld responding to NoGo trials effectively (91.5±0.9% successful inhibition). Participants were less able to switch responses as required by Go-Target stimuli (73.6±1.7% correct responses, p<0.0001). However, neither the number of unsuccessful inhibitions (**Figure 5-2A**) nor the number of responses with the wrong hand to target and standard stimuli (**Figure 5-2B**), or target stimuli alone showed an effect of *drug, genotype* or an interaction of both (MLM all p>0.10). MLM analysis comparing RTs on Go-Standard and Go-Target trials revealed a significant main effect of *trial type*: subjects were 110±3ms slower on Go-Target trials ($F_{1,84}$=864.31, p<10^{-44}), but no effect of genotype, drug, or any higher order interactions were observed (all ps>0.28).

We then investigated the effect of NoGo and Go-Target stimuli on following RTs. Inhibitory events are known to slow down responses in immediately proceeding trials (Rieger and Gauggel, 1999), however under speeded conditions it is plausible that this effect outlasts the first trial. Thus, to determine RT effects induced by the previous events, we compared RT *differences* on trials immediately following deviant events to their respective succeeding trials in a MLM with the additional factors *previous trial type* (*NoGo*, *Go-Target*) and *trial number* (*1* to *3*). Only following NoGo events, subjects slowed down responding as indicated by the significant main effect for factor *previous trial type* ($F_{1,480}$=10.68, p=0.0001). Additionally, the RT decrease over the first trials was higher following NoGo compared to Go-Target trials as indicated by the interaction of *trial number x previous trial type* ($F_{3,480}$=19.26, p<10^{-10}). Direct contrasts revealed that after NoGo events, RT was increased by 31ms on the first compared to the second trial (p<10^{-10}), 18ms on the second compared to the third (p=0.0001), but no longer increased on the third compared to the fourth trial (-1ms, p=1). Thus, inhibitory events did not only slow down responses on the immediately proceeding trial but also on the trial thereafter. This effect was absent following Go-Target trials (increases of $\Delta RT_{t1/2}$=1ms, $\Delta RT_{t2/3}$=7ms, $\Delta RT_{t3/4}$=5ms, all p=1). As no slowing was seen following Go-Target events, we focused our MLM analysis on RTs to post NoGo trials.

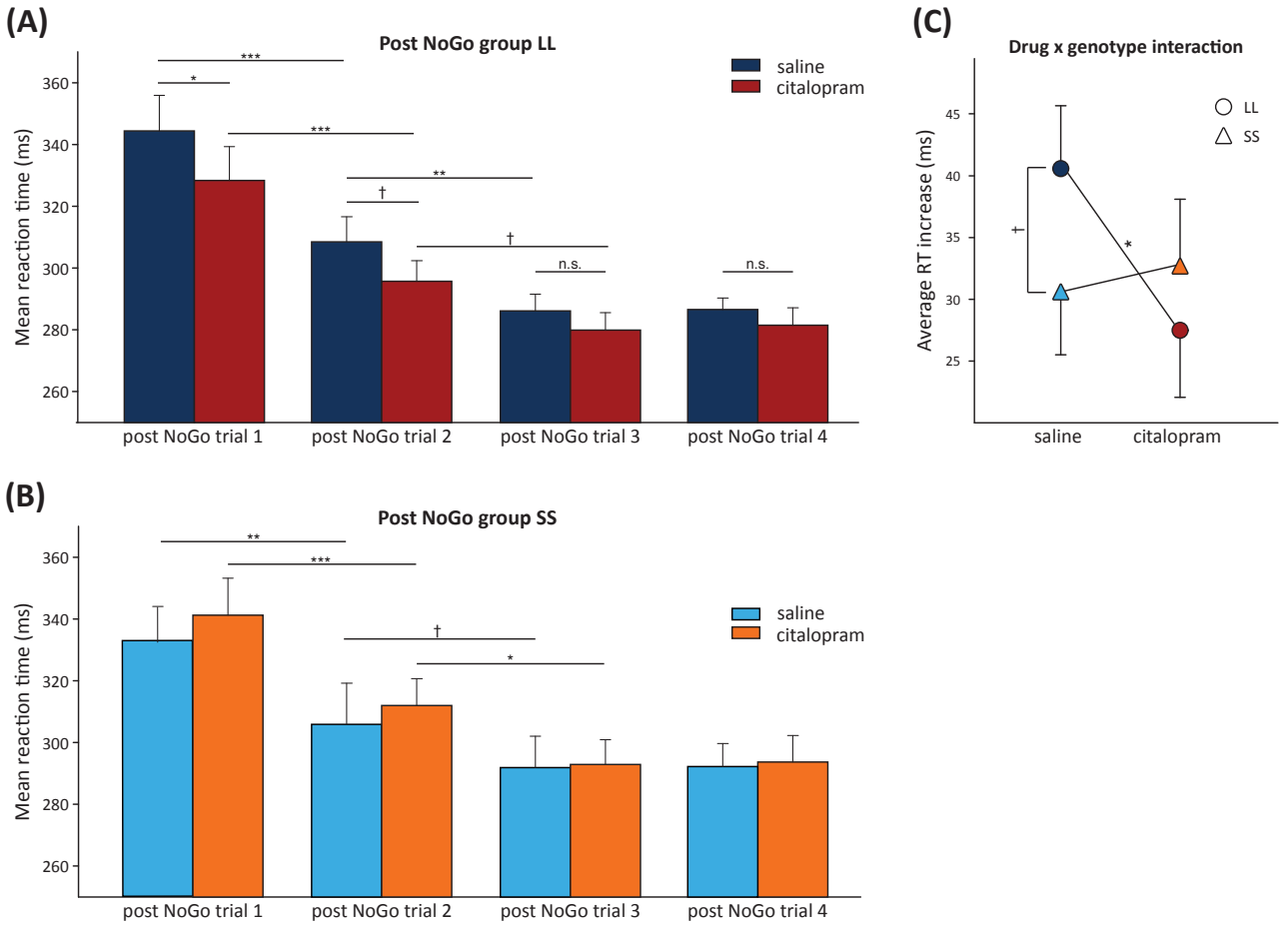

Figure 5-3. Behavioral Results.
(A,B) RTs following unexpected NoGo trials were increased in both groups and drug conditions in the first trial. Here, citalopram significantly decreased the RT for carriers of the LL genotype only (A), whereas even a slight, but non-significant, increase was observed in group SS (B). The slowing effect lasted until the second trial in group LL and here citalopram displayed a trend towards decreased RTs. In group SS, slowing in the second trial under saline was less pronounced and again, no significant drug effect was observed. Note that in none of the conditions a significant decrease of RTs following the third trial was observed (p>0.25). (C) When average RTs on the first two trials were compared to RTs on the third and fourth, a significant *drug x genotype* interaction was observed (p=0.028). Only for LL subjects, citalopram led to a decrease in slowing and additionally a trend towards a genotype difference was seen under saline (p=0.085) that was abolished by drug administration.
Significance for *trial number* comparisons is marked for uncorrected p values from comparison of marginal means derived from the MLM as follows: † < 0.1, * <= 0.05, ** <= 0.01, *** <= 0.001.

This MLM included mean RT in the first 4 trials after inhibitory events as factor *trial number* for which, as expected, a main effect was seen ($F_{3,224}$=59.47, p<10^{-26}). Additionally, we observed a significant *genotype x drug* interaction ($F_{1,224}$=6.144, p=0.014). While citalopram led to a significant RT reduction in group LL (-11±4ms, $F_{1,224}$=7.02, p=0.009, **Figure 5-2A**), a slight but non-significant increase was seen in group SS (4±4ms, $F_{1,224}$=0.83, p=0.36, **Figure 5-3B**). Although this drug effect in group LL was not strictly confined to the first trials, post hoc contrasts showed that RT was only significantly modulated in the first trial following an inhibitory NoGo event (-18ms, p=0.030). A trend was observed in the second trial (-14ms, p=0.091) but no effects in the third (-6ms, p=0.43) and fourth trial (-5ms, p=0.53) were seen. To account for this lack of specificity, we calculated an index of the amount of slowing as the difference between the average RT in the first two trials compared to the average RT of the third and fourth trial (**Figure 5-3C**).

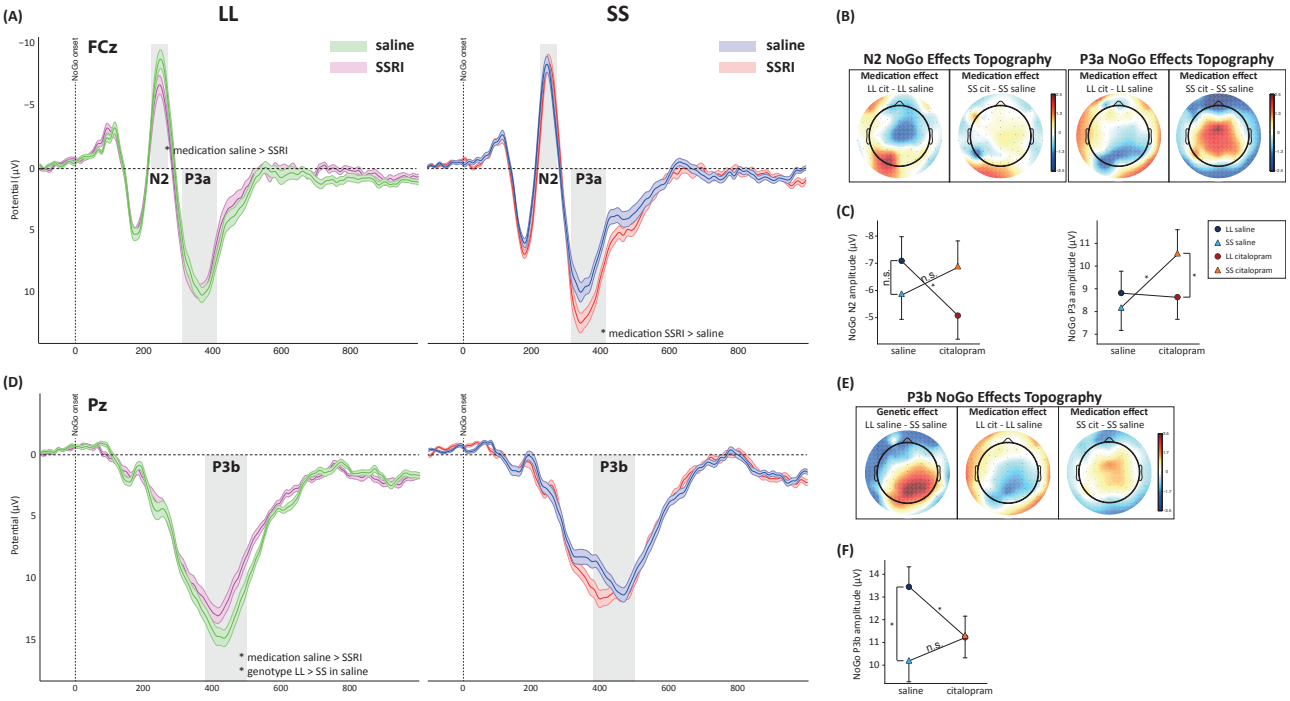

Figure 5-4. NoGo ERPs Across Genetic Groups and Medication.
(A-C) N2 amplitudes on NoGo events were significantly reduced by citalopram for homozygous LL but not SS subjects (genotype x drug p=0.015). Scalp topographies of the drug effect for N2 amplitudes in group LL show a lateralization to the right hemisphere while no effect is seen in group SS (B). P3a amplitudes were increased by SSRI administration in group SS but not LL (genotype x drug p=0.014) and this effect displayed a central, symmetrical scalp distribution (B).(D-F) When no drug was administered, group LL displayed higher P3b amplitudes compared to SS (p=0.013) and this effect was abolished by administration of citalopram (genotype x drug p=0.005) due to a significant decrease of P3b amplitudes in group LL (p=0.031). Scalp maps (E) show drug effects pronounced over the right hemisphere for between-genetic-group (left) and within-group medication effects in group LL (middle). No effect on P3b amplitudes was seen in group SS (right).
Significance is marked as * = p<0.05 derived from post hoc contrasts of the MLM.

Again, a significant *genotype x drug* interaction was seen ($F_{1,32}$=5.31, p=0.028). The drug effect in group LL remained significant (-10±5ms, $F_{1,32}$=4.91, p=0.034) and no effect was seen in group SS – although slowing numerically increased (+5±5ms, $F_{1,32}$=1.17, p=0.288). Additionally, a trend towards higher slowing in group LL under saline conditions was observed (+13±7ms, $F_{1,32}$=3.10, p=0.085) which was abolished by drug administration (-2±7ms, p=0.78).

EEG Results: Attentional Orienting

We analyzed mean P3a amplitudes from 320 to 420ms at electrode FCz in a MLM including factors *trial type (Go-Standard, Go-Target, NoGo*), *genotype, drug* and *session*. For P3a we found a main effect for *trial type* ($F_{2,160}$=90, $p<10^{-25}$) and amplitudes where higher for NoGo and Go-Target trials (both $p<10^{-10}$), but indifferent from each other (p=0.81, **Figure 5-4A**). A significant *drug x genotype* interaction was seen ($F_{1,160}$=6.1, p=0.014). P3a was increased in SS subjects by the SSRI (+1.97±0.64µV, $F_{1,160}$=9.5, p=0.002) and unchanged in group LL (-0.19±0.60µV, $F_{1,160}$=0.1, p=0.75). Post hoc contrast revealed that this effect was driven by significant drug effects in group SS on NoGo (-2.41±1.10µV, $F_{1,160}$=4.8, p=0.030) and Go-Target trials (-3.16±1.10µV, $F_{1,160}$=8.17, p=0.005), while no effect was seen on Go-Standard

trials (p=0.76). For LL subjects, no drug effect was seen in any condition (all ps>0.41). Genetic differences were seen in the SSRI condition only, where, due to the increase in group SS, amplitudes were higher on target trials in SS than LL carriers (+3.03±1.40μV, $F_{1,103}$=4.7, p=0.032).

EEG Results: Inhibitory Processes

Amplitudes of the N2 component were analyzed at electrode FCz. Amplitudes were measured as the mean amplitude in a 20ms time window surrounding the individual trial types grand average peak (for NoGo=261±2ms) (Enriquez-Geppert et al., 2010). N2 amplitudes depended on the trial type ($F_{2,160}$=77, $p<10^{-22}$) and were greater on NoGo than Go-Target trials (-3.29±0.50μV) and on Go-Target compared to Go-Standard trials (-2.97±0.50μV, $ps<10^{-6}$) confirming sensitivity of this measure to inhibitory events. We found a significant *drug x genotype* interaction ($F_{1,160}$=4.6, p=0.038): while a significant decrease of N2 amplitudes by citalopram was observed in group LL (-1.41±0.57μV, $F_{1,160}$=6.3, p=0.013), no effect was seen in group SS (+0.31±0.60μV, $F_{1,160}$=0.3, p=0.611). Post hoc analyses of the different conditions showed a significant N2 decrease by SSRI administration for LL subjects in NoGo (+1.99±0.98μV, $F_{1,160}$=4.2, p=0.043), a trend in Go-Target (+1.68±0.60μV, $F_{1,160}$=2.9, p=0.092), and no effect on Go-Standard trials. No medication effect was seen in group SS (ps>0.34). Additionally, no significant genetic differences were seen (ps>0.172) (see Supplements for an additional exploratory analysis that demonstrates pronounced genetic and pharmacological effects over the right hemisphere which is in line with the involvement of right inferior frontal cortex in motor inhibition (Aron et al., 2004)).

P3b was measured at Pz as the mean amplitude between 380 and 500ms. MLM analysis revealed a robust main effect for t*rial type* ($F_{2,216}$=216, $p<10^{-44}$) and amplitudes were higher for NoGo and Go-Target trials (both $p<10^{-38}$), and indifferent from each other (p=1). Again a significant *drug x genotype* interaction was seen ($F_{1,160}$=8.5, p=0.005): P3b was decreased by the SSRI in LL subjects (-1.35±0.59μV, $F_{1,160}$=5.1, p=0.025) and a trend towards an increase was seen in group SS (+1.13±0.63μV, $F_{1,160}$=3.2, p=0.077). Post hoc contrast indicated that this effect was driven by significant drug effects in LL subjects on NoGo trials (-2.24±1.03μV, $F_{1,160}$=4.7, p=0.031). A trend was seen on Go-Target trials for SS subjects (-2.11±1.10μV, $F_{1,160}$=3.7, p=0.057) and all other conditions were nonsignificant (all ps>0.11). Genetic differences were seen in the saline condition for NoGo trials where LL subjects showed higher P3b amplitudes (+3.24±1.29μV, $F_{1,160}$=6.3, p=0.013), and this effect was abolished by drug administration (under SSRI p=0.99). These findings indicate that N2 and P3b components were decreased by SSRI administration in LL carriers only. Furthermore, drug administration abolished naïve genetic group difference seen for P3b amplitudes at posterior electrodes.

Discussion

The genetic background in 5-HTTLPR was found to interact with effects of acute SSRI administration at different stages of the processing of unexpected, inhibitory events and their electrophysiological correlates.

Firstly, SSRI application increased fronto-central P3a amplitudes only in SS subjects on NoGo and Go-Target trials alike, which were thereafter also significantly higher compared to LL subjects. P3a has been suggested to reflect the rapid orientation response to novel and unexpected stimuli (Polich, 2007) and also been shown to be increased in S allele carriers in an auditory Oddball paradigm (Heitland et al., 2013). The increase of P3a in SS subjects suggests increased attentional orienting to unexpected stimuli by increasing 5-HT levels for these subjects. This fits to theories linking 5-HT levels to vigilance behavior (Homberg, 2012) and monitoring change in the environment. Such differential effects appear related to higher discontinuation rates of SSRI in depressed patients, where increased agitation and insomnia have been reported (Perlis et al., 2003). Increased P3a may represent the electrophysiological correlate of this differential drug response; however, without significant corresponding behavioral results, this interpretation has to remain speculative and additional research is clearly needed.

On the other hand, the LL group showed a decrease of frontal N2 and parietal P3b by SSRI application – which are both associated with inhibitory processes (Enriquez-Geppert et al., 2010). This translated into a corresponding behavioral effect where reaction slowing following NoGo events (Rieger and Gauggel, 1999) was reduced. Genetic differences were seen in the placebo condition where LL subjects showed higher NoGo P3b amplitudes and a trend towards more slowing thereafter, of which both effects were abolished by citalopram administration.

These findings have several consequences. Firstly, they provide evidence for 5-HT to play an important role in mediating response initiation after inhibitory or distracting events and modulating the saliency of these. In the task used here, NoGo events were always followed by Go-Standard trials. Even when participants were acquainted with the task in the second test session, significant post-inhibition slowing was observed (supplements), which indicates that subjects were unable to overcome the inhibitory aftereffects of these events (29). It seems that when tonic 5-HT is low, SSRI led to a decrease in exerted inhibition reflected in lower N2 and P3b amplitudes. Since both genetic groups showed no difference in error rates despite increases of inhibitory EEG correlates seen in group LL, it can be speculated that these subjects have to engage more inhibitory control which then could delay re-initiation of the inhibited response. Here, boosting 5-HT neurotransmission increased the ability to quickly reinitiate the dominant response tendency after it was inhibited in a plausible interaction between pharmacological and genetic factors. Consistent with that, lowering serotonergic neurotransmission by acute dietary Trp depletion showed the opposite effect: specifically following deviant stimuli RTs in an oddball paradigm were increased (Ahveninen et al., 2002). In line with this result, a recent study found that citalopram increased the tendency to perform active responses in a rewarded Go/NoGo task (Guitart-Masip et al., 2013). Both disturbed impulse inhibition as well as attentional deficits are core features of ADHD and it has been observed that phenotypical features commonly found in ADHD are associated with higher 5-HTT gene expression (Curran et al., 2005). Additionally, an association between the L allele and ADHD has been discussed (Faraone et al., 2005; but see: Landaas et al., 2010). Our data suggest that possibly LL subjects need to engage more inhibitory control to suppress responding – which here is reflected in increased P3b

amplitudes and a trend towards higher slowing following thereafter. Clearly, direct inhibition crucially depends on norepinephrine (Chamberlain et al., 2006) but interestingly the treatment response to stimulants in ADHD seems to interact with the 5-HT system (Thakur et al., 2010) and higher 5-HTT expressing subjects benefit more from stimulant therapy. It could be speculated that 5-HT is behaviorally especially relevant for re-initiating suppressed responses and re-directing attention to relevant task dimensions (Ahveninen et al., 2002). This effect seems specific to those events, because neither drug nor genotype effects were seen for RTs on Go-Target trials that require a switch away from the default response. This suggests a complex, modulatory role for 5-HT in the interplay between impulse inhibition, attentional orienting, and the pathology of ADHD where LL subjects may benefit from 5-HT targeting drugs.

Secondly, while differences in brain morphology between 5-HTTLPR genotypes have been shown in humans (Canli et al., 2005) and macaques (Jedema et al., 2010), which suggests a role for 5-HT as a growth factor during development, the clear overlap of genetic and pharmacological effects for P3b amplitudes (**Figure 5-4B**) shown here and the interaction effects between genotype and drug challenge, provide converging evidence for a direct link of these processes to 5-HT neurotransmission. However, it should be mentioned that another explanation of these findings could be an indirect effect via other neuromodulatory systems. Most prominently, P3 amplitudes have been suggested to be modulated by phasic activity of norepinephrinergic neurons (Polich, 2007). It is known that SSRI administration can down-regulate norepinephrinergic activity, but so far this has been only demonstrated for prolonged, and not acute, SSRI administration (West et al., 2008).

Thirdly, the absence of a main effect of *drug* in any of the dependent variables investigated here implies that it may be unlikely to find effects of 5-HT manipulations using an SSRI, and possibly dietary 5-HT manipulations as well (Roiser et al., 2006a), when these genotypic differences are not accounted for. Although sensitivity of the P3 complex to pharmacological 5-HT manipulations has been discussed (Soltani and Knight, 2000), results have been contradictory. While one study reported decreased P3 amplitudes for unexpected events following SSRI administration (d'Ardhuy et al., 1999), others could not replicate such an effect (Wienberg et al., 2010). However, as almost 50% of Caucasian and even more Asian subjects can be expected to have at least one S allele (Hu et al., 2006), this discrepancy may well be explained by the interaction effects and the absence of a drug main effect in the current study. When N2 was elicited in other tasks, like an auditory oddball (d'Ardhuy et al., 1999) or flanker (de Bruijn et al., 2006) task, no effects of serotonergic manipulations have been reported. Again, this would be expected based on the data reported here if the genetic background is not accounted for.

In sum, the current study reports a surprising degree of diversity of modulation of electrophysiological and behavioral variables following acute SSRI administration depending on the genetic background. While this highlights the importance of 5-HT for these functions, it also implicates that combining pharmacological and genetic approaches seems to be crucial in order to account for the complexity of neuromodulatory systems in order to better understand desirable and undesirable drug effects.

CHAPTER

6

A Task to Disentangle Real and Fictive Events

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Abstract

The ability to learn not only from experienced but also from merely fictive outcomes without direct rewarding or punishing consequences should improve learning and resulting value-guided choice. Using an instrumental learning task in combination with multiple single-trial regression of predictions derived from a computational reinforcement-learning model on human EEG, we found an early temporospatial double dissociation in the processing of fictive and real feedback. Thereafter, real and fictive feedback processing converged at a common final path, reflected in parietal EEG activity that was predictive of future choices. In the choice phase, similar parietal EEG activity related to certainty of the impending response was predictive for the decision on the next trial as well. These parietal EEG effects may reflect a common adaptive cortical mechanism of updating or strengthening of stimulus values by integrating outcomes, learning rate, and certainty, which is active during both decision making and evaluation. Neuronal processing of real (rewarding, punishing) and fictive action outcomes (which would have happened had one acted differently) differs for 400 ms and then converges on a common adaptive mechanism driving future decision making and learning.

Introduction

Wouldn't it be nice to know what would have happened if you had chosen differently? Imagine driving on a highway towards a traffic jam faced with two choices: bypass the highway or wait in the hold-up. Neither of the cases provides information about which decision really yields the better result. On the other hand, when choosing between two lanes in a traffic jam, you will always notice the progress you are making in your lane and the progress you could have been making in the other lane. Both humans (Burke et al., 2010) and monkeys (Subiaul et al., 2004) share the ability to learn complex rules and values from watching the actions of other conspecifics - termed vicarious or observational learning. This capability provides evolutionary benefits by reducing trial and error learning costs and can be speculated to be the progenitor of more abstract, counterfactual reasoning in humans. In reinforcement-learning models it has been theorized that learning can be based on results from unchosen options as well (Sutton and Barto, 1998). Although the neural implementation of counterfactual learning recently sparked considerable interest (Boorman et al., 2011), little is known about its exact timing – particularly with regard to the processing of fictive prediction errors (PEs) (Lohrenz et al., 2007; Chiu et al., 2008) and their neural realization in the absence of other actors (de Bruijn et al., 2009).

To study the temporospatial evolution of cortical brain activity during learning from real and fictive outcomes and behavioral choice based on the learned stimulus values, we used a probabilistic reinforcement-learning task while recording EEG. Subjects decided to either choose or avoid gambling following one centrally presented stimulus in every trial (**Figure 6-1A**). A chosen gamble resulted in a monetary gain or loss, depending on the reward contingency associated with that stimulus. In choosing not to gamble, subjects avoided financial consequences, yet still observed what would have happened if they had chosen to gamble. Although neither directly rewarding or punishing, fictive outcomes can be used in the same way as real outcomes to update learned estimated values of given stimuli and determine whether behavioral adjustments are needed. Notably, the subjective valence of the feedback reverses after avoiding a gamble: a fictive and thus foregone reward (reflected in a positive PE in our computational reinforcement learning model, see Experimental Procedures and further below) is unfavorable, and a fictive and thus avoided loss (reflected in a negative PE) is favorable (**Figure 6-1B**). Good, bad and neutral stimuli were presented; their valence was reflected in reward probabilities above, below or at chance level, respectively. By learning which symbols to choose and which to avoid, subjects could maximize their earnings.

Results and Discussion

Behavior and Computational Model

Subjects learned avoiding bad and choosing good stimuli comparably well: we observed no difference in the absolute number of correct decisions following good compared to bad stimuli (t30 = 1.31, p = 0.20). Additionally, median reaction times did not differ between conditions (p > 0.1). Learning of choice behavior for good and bad stimuli followed a logarithmic curve approaching an asymptote reflecting the probabilistic outcome of the respective stimuli (**Figure 6-1C**). This supports the notion that the weight of reward PEs in value updating decreases in an exponential fashion.

To derive single-trial estimates of individual PEs and subjective stimulus values, we fit a Q-learning model (see Experimental Procedures) (Watkins and Dayan, 1992; Sutton and Barto, 1998; Jocham et al., 2009) to each subject's sequence of choices. To account for the observed decrease in learning, we implemented an exponentially decreasing half-life time as a free model parameter which reduces the learning rate in later trials providing single-trial estimates of the learning rate (αt). Maximum likelihood estimated (MLE) learning parameters of the model did not differ for learning from real and fictive outcomes (Table 1), indicating that subjects could utilize both sources of information with similar efficiency. This is also supported by the fact that sensitivity to misleading probabilistic feedback did not differ significantly between real and fictive conditions (Supplementary Results). MLEs of the half-life time indicated an average decrease of αt of more than 90% in both conditions per block. Additionally, negative log-likelihood (-LL) did not differ when compared between good and bad stimuli.

Early Dissociation of Feedback Processing

Submitting feedback-locked EEG epochs to multiple robust regression analysis (O'Leary, 1990; Rousselet et al., 2008; Cohen and Cavanagh, 2011) revealed a double dissociation of cortical PE correlates between real and fictive outcomes in the first 400 ms following feedback. Intriguingly, the first significant covariation of feedback-locked EEG activity with PEs was found exclusively for fictive outcomes: a previously unknown negative early occipital effect

Table 1. MLE Parameters and Model Fit.

Parameter	Real	Standard error	Fictive	Standard error	*p* for difference
α_1	.484	.068	.421	.065	.497
Hl	9.781	2.606	13.467	3.309	.403
$\beta = 10.356$					
$-LL_{good} = 69.363$		$-LL_{bad} = 63.284$		$-LL_{neutral} = 116.641$	

Neither the initial learning rate α1 nor the half life time Hl differed significantly when estimated for real and fictive outcomes separately. The sensitivity parameter β was kept the same for both conditions to ensure that models were comparable. -LL showed significantly lower values for both good and bad compared to neutral stimuli (difference: $t_{30} > 8.7$, p < 10-7 for both) while no difference between good and bad was observed ($t_{30} = 1.23$, p = 0.229). Therefore, our model was equally effective in describing subjects' behavior for good and bad symbols, yet less effective for neutral ones.

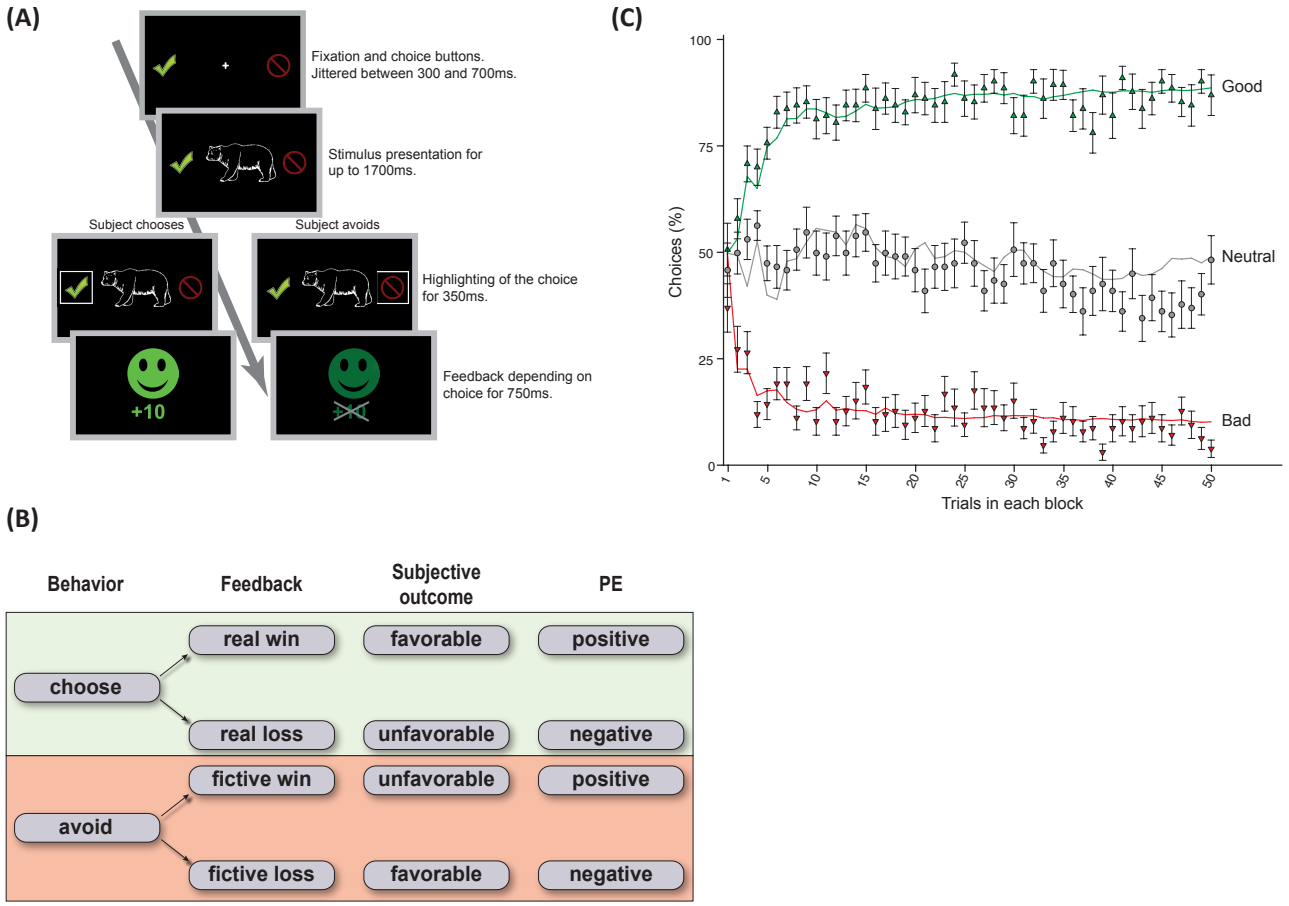


Figure 6-1. Experimental design, modeled and observed behavior.
(A) Time course of the learning task. Choosing to gamble following stimulus presentation leads to real feedback consisting of a win or loss of 0.10€. Avoiding the gamble leads to fictive feedback without financial consequences but still provides information about the outcome of the trial.
(B) Task structure separated by subjects' choices. Note that the sign of the PE reverses in relation to the subjective outcome depending on the choice made.
(C) Modeled and observed behavior. Learning curves for empiric behavior of all subjects (symbols ± s. e.) and predictions of the computational model (solid lines in the same color) for good, bad and neutral symbols. Learning curves were comparable in both conditions and approached asymptotically towards their final levels. See also **Figure 6-S1**.

occurred 192-238 ms after feedback (**Figure 6-2A** and **Supplemental Movie S1**) and was localized to extrastriate visual and posteromedial cortex (PMC, **Figure 6-S2A**). In contrast, only real outcomes were associated with a somewhat later positive early PE effect spanning from 236-294 ms and a subsequent negative mid-latency frontal PE covariation at 336-430 ms, which in the averaged event-related potentials (ERPs) give rise to the feedback-related negativity (FRN) and P3a components, respectively (**Figure 6-3**). Direct contrasts between both conditions showed significant differences at electrode Oz during the time window of the occipital PE effect (peak t_{30} = -4.18, 204 ms, $p < 0.0005$) and at electrode FCz during FRN (peak t_{30} = 4.95, 284 ms, $p < 10\text{-}4$) as well as P3a time windows (peak t_{30} = -7.95, 394 ms, $p < 10\text{-}8$) (**Figure 6-4B**). The temporospatial double dissociation in early processing of real and fictive feedback was statistically confirmed by a triple interaction of the factors electrode, time window, and condition in an ANOVA on the average regression weights of the early PE effects in significant time windows (190-240 ms and 250-300 ms, for

fictive and real feedback, respectively) at the most significant electrodes (Oz and FCz, for fictive and real feedback, respectively).

The FRN is usually found on unfavorable outcomes that violate expectancies (Miltner et al., 1997; Gehring and Willoughby, 2002). Our findings are consistent with an influential theory proposing that the FRN reflects PE signals (Holroyd and Coles, 2002; Nieuwenhuis et al., 2004). The negative polarity of the FRN is in accordance with a positive covariation, as unfavorable real outcomes cause negative PE values. It has been consistently localized to the posterior medial frontal cortex (pMFC) (Miltner et al., 1997; Gehring and Willoughby, 2002; Gruendler et al., 2011), which has been supported by fMRI findings on feedback processing (Ullsperger and Cramon, 2003; Ridderinkhof et al., 2004). The subsequent pronounced negative mid-latency frontal PE effect fits well with theories relating the P3a to the recruitment of attention (Polich, 2007), which is here caused by negative PEs leading to a negative covariation by instigating increased P3a amplitudes. Exploratory localization analysis suggest a source network in cingulate gyrus and orbitofrontal cortices (**Figure 6-S2B**).

In stark contrast to the real feedback condition associated with the well-known pattern reflecting FRN and P3a, following fictive feedback, these early and mid-latency frontal PE effects were conspicuously absent; the average ERP waveforms showed merely a small negative deflection in the FRN time window which was unmodulated by learning parameters (**Figures 6-3 and 6-4A**). Feedback-related pMFC activity has been proposed to reflect action value updating (Jocham et al., 2009). This suggests that a previous action is required in order to involve pMFC in the rapid processing of expectancy violations. The absence of an FRN-like PE effect on fictive outcomes could be explained in two ways: avoiding a stimulus is interpreted as abstaining from an action, or the neutral monetary outcome does not yield the necessary PE signal required for credit assignment to avoiding. The latter explanation seems very unlikely as other cortical PE correlates were found for fictive outcomes and MLE learning parameters in our task do not differ between conditions. It is also unlikely that the missing FRN results from reduced expectancy of and attention to fictive outcomes, because behavioral and modeling data as well as later EEG effects (see below) suggest similar utilization of fictive and real feedback. The absence of the FRN on fictive outcomes seems at odds with studies reporting FRN-like EEG deflections and pMFC activity on observed errors and feedback to others' actions (van Schie et al., 2004; Yu and Zhou, 2006; de Bruijn et al., 2009). Yet, in contrast to abstaining from choosing a stimulus in our experiment, observing actions could also lead to action simulation effects in motor-related areas via mirror systems (Rizzolatti et al., 2001) - permitting an update of action values. Taken together, it appears most likely that for motor-related areas, such as the pMFC, avoiding a stimulus in our learning task is equivalent to not performing any motor action.

However, the absence of the FRN and P3a modulation by fictive PEs does by no means indicate that outcomes are not processed in the fictive task condition. In sharp contrast to real feedback, we observed an early occipital PE-related EEG modulation following fictive feedbacks that even precedes the FRN time window, which has previously been interpreted as the fastest cortical correlate of feedback processing (Gehring and Willoughby, 2002; Philiastides et al., 2010). Its very short latency and localization to extrastriate visual areas and PMC (**Figure 6-S2A**) seem to suggest that fictive outcomes engage a specific mechanism that might ease counterfactual learning. Although EEG does not allow precise localization, the found source fits well with findings from fMRI studies where PMC has been associated with tracking values and PE signals of alternative unchosen options coding a counterfactual PE (Boorman et al., 2011). In

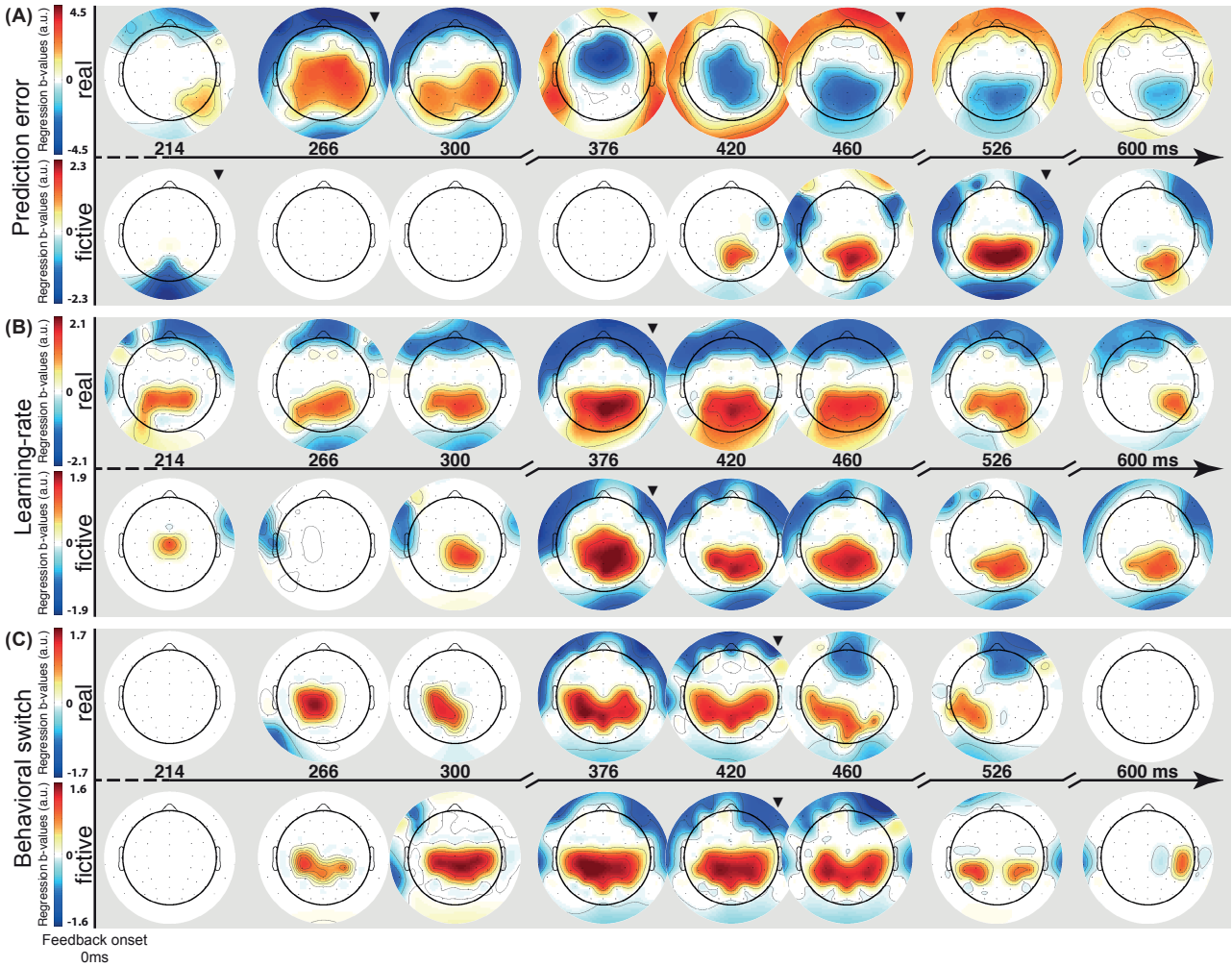


Figure 6-2. Multiple robust regression analysis of feedback locked epochs.
(A) Regression b-values for feedback-locked results for the predictor PE plotted separately for real (upper panels) and fictive feedback. Occipital electrodes showed the first significant effect only in the fictive condition (peak Oz 214 ms, t_{30} = -5.78, p < 10-5). A frontocentral positive (peak at FCz 266 ms, t_{30} = 5.17, p < 10-4) and negative (peak at FCz 382 ms, t_{30} = -8.70, p < 10-8) effect were present only in the real feedback condition. Both conditions showed later parietal covariations that were opposed in sign (real peak at Pz 460 ms, t_{30} = -7.86, p < 10-7 and fictive peak at Pz 526 ms, t_{30} = 6.02, p < 10-5).
(B) In both feedback conditions the learning rate showed a positive covariation with a centroparietal scalp distribution that peaked in a mid-latency time window around 376 ms (at Pz for chosen t_{30} = 8.38, p < 10-8 and for avoided t_{30} = 7.41, p < 10-7).
(C) The behavioral switch regressor yielded a peak at parietooccipital electrodes (at Pz for chosen at 408 ms, t_{30} = 7.12, p < 10-6; for avoided at 420 ms t_{30} = 5.67, p < 10-5).
Inverted triangles mark plots closest to peak amplitudes and nonsignificant (p > 0.00069) results are masked in white. See also **Figure 6-S2** for source localizations.

monkeys (Leichnetz, 2001) and humans (Mars et al., 2011) the PMC is intensely interconnected with the more lateral part of the parietal cortex that has been shown to code fictive PE signals defined as the value difference between outcomes that could have been attained by optimal investments and actually attained outcomes (Lohrenz et al., 2007; Chiu et al., 2008). Furthermore, afferent projections from the basal forebrain as well as reciprocal projections with the anterior cingulate cortex shown in macaques (Parvizi et al., 2006) permit a role of the PMC in value processing and it has been suggested as part of a network tracking evidence for future adaptations to pending options (Boorman et al.,

2011). Importantly, our results presented here differ from these previous findings since we describe how the same stimulus value representation is updated by different signals depending only on whether feedback was fictive or real. We suggest that this signal might reflect a process that converts fictive outcomes to subjective value signals (Gold and Shadlen, 2007), effectively facilitating counterfactual learning that can more easily guide subsequent decisions.

This fictive PE effect cannot be interpreted as a surprise signal (Ferdinand et al., 2012) as it was unaffected when outcome and surprise, measured as the absolute PE value, were included into the same regression model (**Figure 6-S4**). Additionally, the effect cannot be interpreted as a consequence of repetition suppression (Summerfield et al., 2008), as it would then be expected to occur also following real feedback. In order to further disentangle contributing factors of the different PE correlates, we decomposed the PE into its components - the outcome and the expected value - and submitted both to the same multiple regression analysis. This revealed that the FRN in fact codes a PE signal as both, outcome and expected value, showed significant effects with opposite signs indicating that the error signal increased when an unfavorable outcome was less expected (**Figure 6-S3**) thereby mimicking the response of dopaminergic neurons (Schultz et al., 1997). In contrast to this, the early fictive effect did not show significant influences by the expected value and thus may mainly code whether or not outcomes were favorable or not. Neither P3a nor later components showed a pattern that satisfies criteria for an axiomatic PE signal (Caplin and Dean, 2008), which is in line with other studies that found the FRN to be the only cortical PE correlate in accordance with axioms of reward PE models (Talmi et al., 2012). Thus, the data suggests that different cortical areas covary with PEs at different times between 190-400 ms after feedback depending on whether an outcome is directly experienced or fictive. Furthermore, the very early occipital PE correlate is mainly driven by the favorability of the outcome itself and not the expected value, suggesting a binary evaluation taking place here that may later on be converted into more fine scaled value updating.

Common Final Pathway

As feedback processing continues, the different streams appear to converge on a common late parietal PE correlate that coincides with the P3b ERP component (Polich, 2007). This PE covariation was evident in both conditions with reversed polarities that were negative for real and positive for fictive feedback (significant from 392-650 ms for real and 414-590 ms for fictive feedback, **Figures 6-2B** and **6-3B**). Notably, this polarity reversal results in the fact that unfavorable outcomes associated with negative PEs in real and positive PEs in fictive conditions always lead to positive-going deviations of parietal EEG activity. Thus, in order to compare the magnitude of the PE covariations, we multiplied the fictive feedback condition by -1 to account for the PE sign reversal in relation to the outcome's subjective valence before contrasting both conditions (**Figure 6-4B**). This is a logical consequence of the assumption that fictive feedback in which unfavorable outcomes are associated with positive PE signs engage counterfactual thinking. Contrasts did not show differences between conditions in this late time window which indicates that real and fictive outcomes have similar effects on P3b modulations although absolute P3b amplitudes are reduced following fictive feedback (**Figure 6-3**). This effect might reflect the updating of stimulus-response mappings or, similarly, of a stimulus' expected value. Interestingly, an early theory of the P3b suggested that it covaries with deviations from an

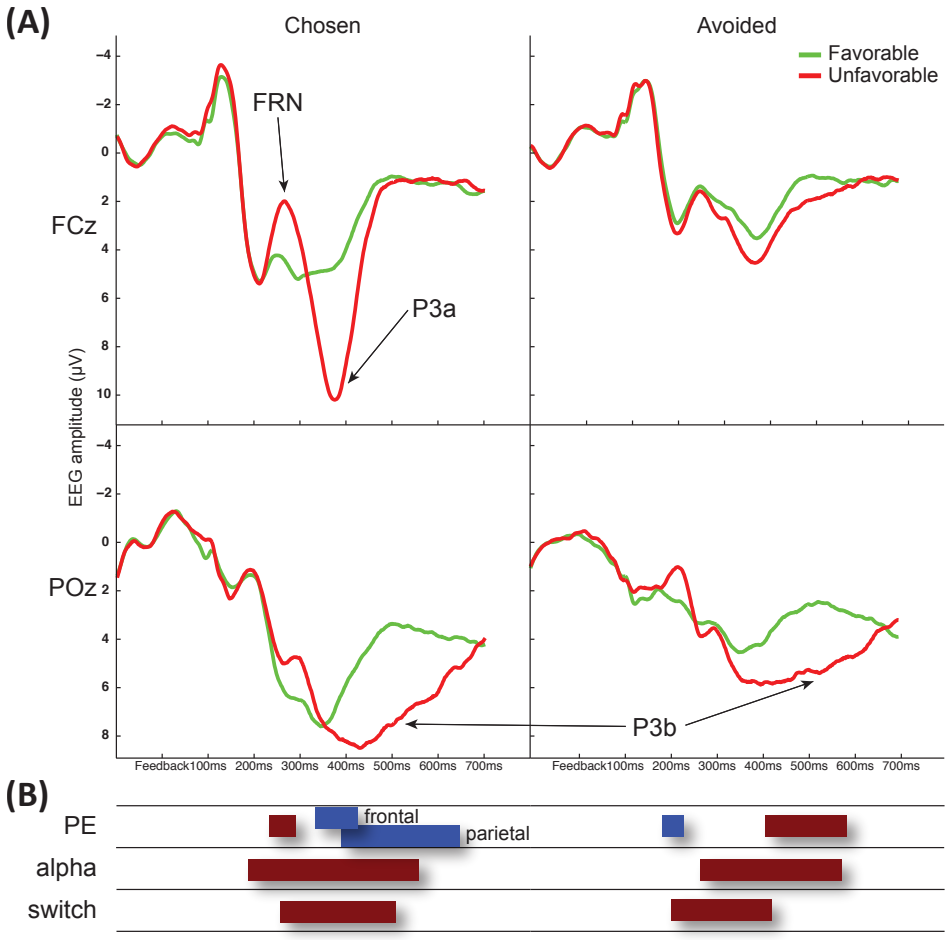


Figure 6-3. Comparison of event-related potentials (ERPs) and regression results.
(A) Grand average feedback locked ERP waveforms. Favorable (green) and unfavorable (red) outcomes are plotted separately for chosen and avoided feedbacks at electrode FCz and POz. A clear FRN component can be seen for real and fictive feedback at FCz but is not modulated by the valence of avoided feedback.
(B) Time windows of significant effects for regressors used in the regression analysis. Positive covariations are depicted in blocks in red and negative covariations in blue color. During the time window of the occipital early PE effect following fictive feedback, a negative deflection is present in the ERP waveform for fictive unfavorable feedback. Learning rate (alpha) and switch effects span over longer time windows that do not simply represent one single ERP component. See **Figures 6-S3** and 4 for a decomposition of the PE effects into contributing factors.

adaptation level (Ullsperger and Gille, 1988), a concept highly reminiscent of PEs, suggesting that the P3b is higher the stronger the necessary deviation from default behavior. In line with this, the P3b amplitude was increased before a behavioral switch in a reversal-learning task (Chase et al., 2011). The P3b has been shown to correlate well with surprise (Mars et al., 2008), but surprise alone is insufficient to explain the late EEG modulation and behavioral switching in the present study: even when surprise was included as a separate regressor, P3b still displayed significant covariation in both conditions with the outcome itself (**Figure 6-S4**). We thus suggest that the late parietal P3b effects modulated by PE represent a common pathway for adaptation based on the information extracted from the feedback. This view is strongly supported by the finding that an additional behavioral switch regressor (coding shift/stay behavior on next encounters with the same stimulus which happened on average on the third following trial) covaries positively with mid-latency and late parietal EEG amplitudes (**Figure 6-2C**), thus remarkably overlapping with the late

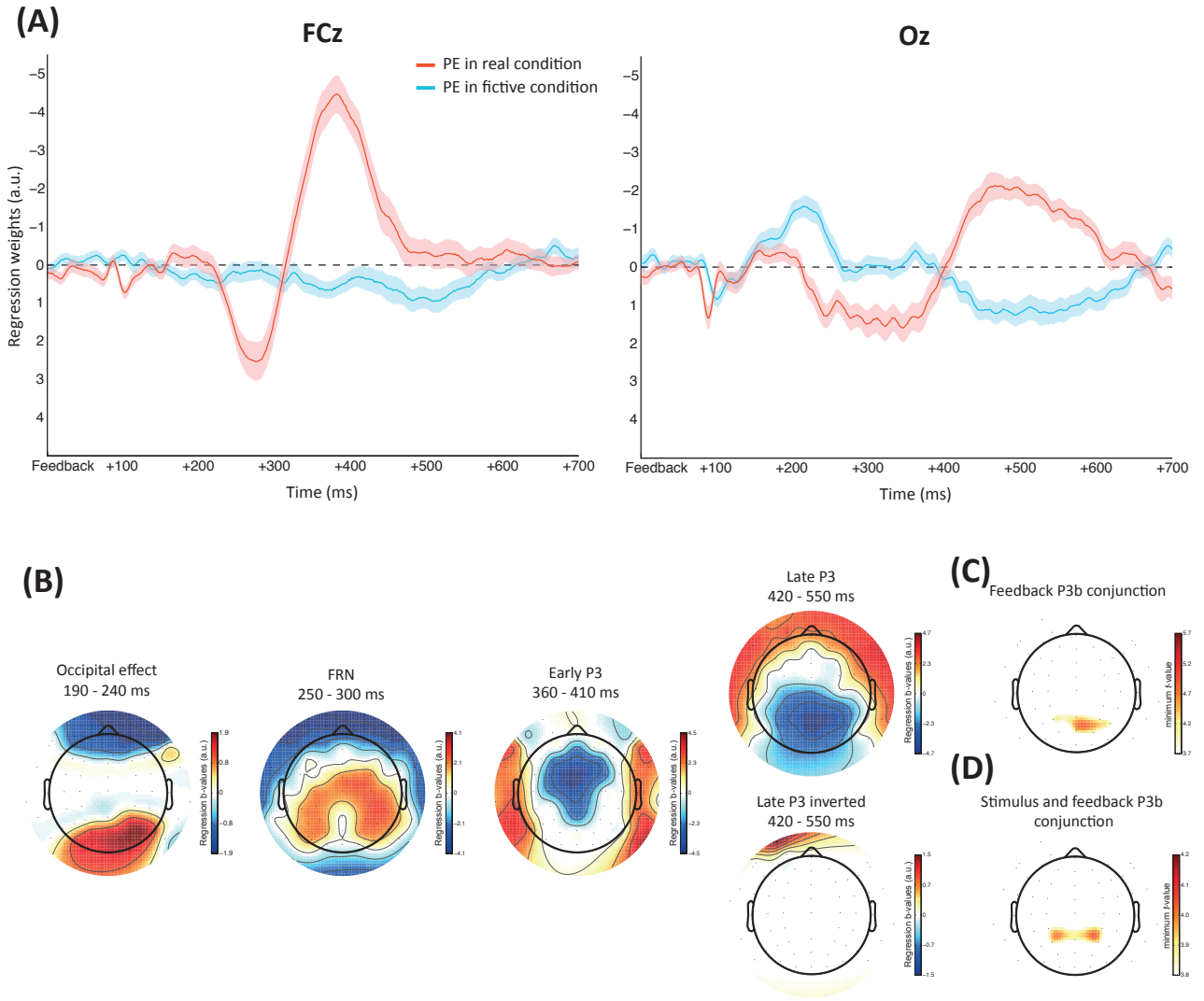


Figure 6-4. Time courses of regression weights, difference and conjunction maps.
(A) Time course of regression weights of the PE effects comparing processing of real (red) and fictive (blue) feedback. Shown at electrodes of maximal effects of early PE correlates (FCz and Oz). Thick lines: Mean regression weights, shadows: ± s. e. See **Figure 6-S6** for an across subjects correlation of regression weights with task performance.
(B) Difference-topography plots (fictive - real) in the time windows of the respective effects. Regression weights were collapsed over the effects' duration and average weights are plotted, while non-significant electrodes are masked in white ($p > 0.00069$). For the late parietal effect, because it occurred in both conditions, we also compared whether differences exist when the fictive condition was inverted (by multiplication with -1). This is based on the assumption that counterfactual thinking was employed following fictive outcomes, converting them to favorable or unfavorable events. Note that when inverted, both conditions did not differ significantly in the late P3b time window.
(C) Temporospatial conjunction map for regressors that showed effects in the late P3b time window in both real and fictive feedback conditions (learning-rate, PE and behavioral switch). Plotted are minimum t-statistics of significant coactivation of all regressors (Nichols et al., 2005) collapsed over time. Midline electrodes Pz and POz were significantly activated by all regressors in both conditions (see **Figure 6-S5** for details).
(D) Conjunction map for stimulus-locked SDC and feedback locked PE and learning rate effects in both conditions between 370 and 650 ms. Parietal electrodes were coactivated by feedback and stimulus-locked P3b effects for SDC in the decision making phase and by parameters critical for value updating in the feedback evaluation phase of the task.

PE effect in the temporal and spatial domain. Given previous findings that higher P3b amplitudes are associated with improved memory encoding (Paller et al., 1987; Fabiani et al., 1990), it is conceivable that the parietal EEG effects in

the P3b time range reflect update and storage of the stimulus value. Intriguingly, the PE correlate appears longer-lasting than the switch effect, suggesting that the late portions of the P3b may play further roles in addition to encoding the new stimulus value, speculatively autonomic responses and awareness (Wessel et al., 2011). In sum, the parietal P3b-like cortical activity seems to set the stage for future decisions.

As seen in the behavioral data and supported by the reinforcement learning model, with increasing learning success and thus increasing certainty of reward likelihood the impact of feedback on value representations and overt behavioral adaptation decreases exponentially towards an asymptote. This is reflected in a decreasing learning rate αt which, regressed against EEG activity, yielded comparable sustained positive centroparietal effects (significant at Pz from 192-562 ms for real and from 272-580 ms for fictive feedback, **Figure 6-2B**). The maximal learning rate effect fell in between the early and the late PE effects in both conditions thereby modulating the baseline of the FRN and the P3a and P3b amplitudes: the higher the learning rate the more positive the EEG signal. As the learning rate decreases the EEG amplitude decreases as well. We suggest that this effect indeed represents the weighting of the outcome in both conditions causing less value updating and behavioral adaptation in later trials within each block. This point is further corroborated by the observation that those subjects whose EEG signals more closely matched the reinforcement learning models' predictions made fewer bad decisions (**Figure 6-S6**). Our finding that the learning rate determines the baseline activity on which PE effects are modulated fits with fMRI results demonstrating that PE coding in pMFC is modulated by individual learning rates (Behrens et al., 2007; Jocham et al., 2009). Furthermore, it has been shown that functional connectivity of feedback processing brain areas is reduced in late phases of stable learning experiments (Klein et al., 2007).

Processing of Stimulus Information

When a stimulus value has been learned based on feedback, it needs to be retrieved and used to guide choice at the next encounter of the same stimulus. To investigate these processes, we submitted stimulus-locked EEG epochs to a multiple robust regression analysis. The signed Qt regressor - reflecting the individual's single-trial stimulus value estimates - showed a significant positive covariation at frontal electrodes 250-268 ms after stimulus onset with peak values at electrode AFz (**Figure 6-5**). Thus, stimuli with higher subjective values were associated with more positive EEG activity. Value-related activity has consistently been reported to correlate with activity of the vmPFC (Knutson et al., 2005; Plassmann et al., 2010; Wunderlich et al., 2010; Jocham et al., 2012). The anterior distribution of this frontal value effect fits with an origin in vmPFC and its timing is supported by a recent study reporting vmPFC magnetoencephalic correlates of overall value when different stimuli were presented simultaneously (Hunt et al., 2012) and single neuron activity in dlPFC and OFC in monkeys (Hayden et al., 2009). The translation of this value representation into action is indirect as indicated by an inverse relationship between EEG amplitude and reaction time for choosing compared to avoiding a stimulus (**Figure 6-S7B**). This EEG modulation reflects the intuitive observation that Q-values deviating further from 0 are associated with easier and quicker decisions about which option to choose (**Figure 6-S1A**). In other words, choice reaction time is driven rather by the certainty of the stimulus value than by the value representation and its early EEG correlate.

Following this early covariation with signed value, a prominent effect of subjective decision certainty (SDC) about which response to give was seen. Values for SDC were derived from the likelihood of the computational model to select one response over the other and rectified in order to range from maximal uncertainty (0) to absolute preference of one option (1) (see Experimental Procedures for details). SDC demonstrated clear positive covariance with EEG activity in a centro

-parietal scalp distribution, peaking at around 520 ms following stimulus onset (significant from 456-744 ms, **Figure 6-5**), which is close to median response time (539 ms). Therefore, response certainty was reflected by more positive single-trial parietal EEG activity at a much later time point than the frontal value effects. The timing of the observed covariation fits well to the latency of the stimulus-related P3b ERP component. This pattern of increased P3b with response certainty rules out an explanation of novelty or surprise, as newly occurring stimuli always lead to SDC values of zero. Note that since RTs were included as a separate regressor (**Figure 6-S7A**) in the multiple regression, neither the SDC nor the Q-value effect can be explained by an earlier onset of preparatory motor activity (**Figure 6-S8**).

Predicting Future Adaptations

Our analysis enabled us to study the entire time course of cortical processes underlying decision making, outcome evaluation and learning (i.e., updating) value representations. Upon stimulus presentation, retrieval of learnt values activates cortical value representations reflected in early midfrontal EEG activity. Decision certainty is reflected in P3b-like parietal EEG activity around response latency, and mapping of the selected action to the motor response is reflected in lateralized activity from (pre)motor cortices (**Figure 6-S8**). Following feedback, initially outcomes are processed separately depending on whether their consequences are real or fictive, presumably in order to convert feedback information into a common value currency allowing to efficiently learn stimulus values. Then the information about necessary value updates converges on common parietal P3b-like activity modulated by whether the action was successful or not. Given the probabilistic nature of the instrumental learning task, several parameters need to be used to weight the impact of single-trial outcomes. Over the course of multiple trials, learning rate indicates the learning success and downweights the single-feedback information at later learning stages. Moreover, when a choice is made with high certainty, perseveration of this behavior is favorable. This means that already at the time of the response (and thus before feedback), high certainty might be used to strengthen the current value representation thereby shielding it from potentially misleading feedback. Interestingly, the stimulus- and feedback-locked late parietal P3b-like activity is consistent with the notion of certainty- and learning-rate-weighted value strengthening and updates at different time points: high response certainty, which should be associated with re-encoding (strengthening) of the stimulus value to assure perseveration, is associated with high stimulus-locked P3b amplitudes. In contrast, after feedback high learning rates and unfavorable outcomes commonly give rise to high feedback-locked P3b amplitudes presumably reflecting value updating and storage, thereby increasing the likelihood to change future choice behavior. To put it briefly, lower stimulus-related P3b and higher feedback-related P3b amplitudes should be associated with an increased likelihood to switch choice on the next encounter with the same stimulus.

This notion that feedback- and stimulus-related P3b amplitudes are inversely related to switch behavior was tested at electrode Pz, which was identified via a conjunction analysis of all relevant stimulus- and feedback-locked effects in the P3b time window (**Figure 6-4D**). A discrimination threshold was iteratively estimated in one half of randomly chosen trials that was then used to predict switching in the second half of trials. This very simple algorithm predicted switches significantly above chance level, namely with average accuracy of 56.75% ± 1.18 (t_{30} = 5.67, p < 10-5) following real outcomes and 55.86% ± 0.72 (t_{30} = 8.26, p < 10-8) following fictive outcomes. When this algorithm was applied to the stimulus-related P3b, switches were predicted correctly with average accuracy of 53.17% ± 0.78 (t30 = 4.04, p = 0.0003) before choosing and 53.36% ± 0.77 (t_{30} = 4.34, p = 0.0001) before avoiding the stimulus. Note that the purpose of this analysis was not to predict future behavior as accurately as possible, but to demonstrate that the whole-brain regression reliably identified electrodes and time windows of importance for studying learning and decision making and that switches still refer to the next time the stimulus is shown again. Importantly, it was indeed the case that switches were predicted by increased feedback-related but decreased stimulus-related P3b amplitudes (see Experimental Procedures for details). This result demonstrates that simple attentional effects cannot account for the P3b effects: a global decrease of attention should lower stimulus- and feedback-related P3b amplitudes (Polich, 2007) and adaptive switches in parallel, which is inconsistent with our findings. To compare the importance of both factors in predicting future adaptations we used logistic regression on the switch behavior to determine the contributions of stimulus and feedback P3b. When let to compete for variance, feedback P3b was the better indicator of behavioral adaptation (p = 0.035 for chosen and p = 0.028 for avoided stimuli, two-sided t test of standardized regression weights), but both feedback and stimulus P3b had a significant effect (all p < 0.01). As is intuitively plausible, the actual feedback is more closely related to adaptation but already before feedback is presented, predictions about behavioral adaptation based solely on stimulus values are possible. Thus, with the mere knowledge of a short interval of raw stimulus- or feedback-related EEG at Pz and current behavior, predictions of future behavior can be made.

This strengthens the interpretation of feedback P3b representing value updating as P3b in both stages of decision making alludes to value coding and behavioral adaptation. It is tempting to assume that both processes are related and that in case of high certainty already before feedback is given the stimulus value is encoded. Although similarity in both processes is suggested by the conjunction analysis, these EEG results have to be interpreted cautiously as different generators may give rise to similar scalp topographies. The reversal of the relationship between P3b amplitudes and switch behavior, however, hints to a more specific mechanism than a mere reduction of attention or simple surprise. It therefore seems to be the case that PE correlates, processed in different cortical areas for real and fictive outcomes, modified by a weighting process serve as the basis for, and precede the timing of future decisions. After being exposed to an updated stimulus again, P3b covaries with the security of the selected action, possibly preventing switching away from a learned stimulus.

Being able to adapt behavior based on purely fictive events through counterfactual thinking may be a human ability that allows to learn from abstract information in the absence of any actor. Our results demonstrate through the whole time course of decision making, from value retrieval following stimulus presentation and its translation into action selection until the updating of these values following feedback, how real and fictive events can be utilized to enable

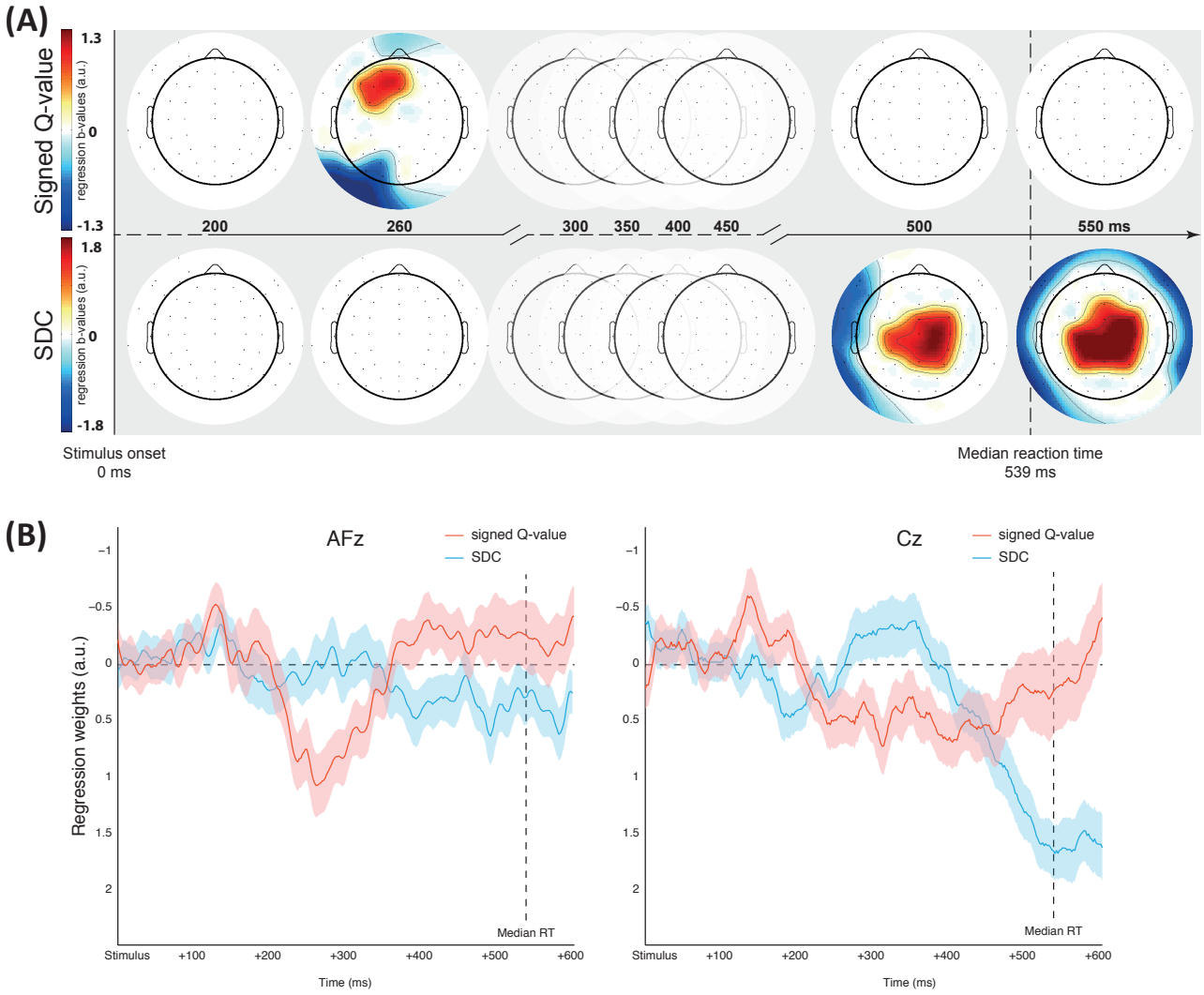


Figure 6-5. Stimulus-locked regression results.
(A) Top row: Signed Q-value showed a significant positive covariation at frontal electrodes (peak at Fz at 264 ms, t_{30} = 4.06, p < 0.0005, significant from 250-268 ms), lateralized to the left hemiscalp (AF3, F3/5, FC3). The scalp signal here was associated with reaction times in a condition dependent manner (**Figure 6-S7B**).
Bottom row: Results for the subjective decision certainty (SDC) regressor showed a positive mediocentral effect (peak at Cz at 520 ms, t_{30} = 7.71, p < 10-7, significant from 456-744 ms). As SDC is higher when subjects are more certain about the response to give (for both good and bad stimuli), these results imply increased stimulus P3b amplitudes after subjects established reasonable certainty about expected values and thus the optimal response. This effect is independent of whether or not the stimulus was estimated to be good or bad as in this time window no effect of the SQV was observed.
(B) Corresponding time courses of regression weights. SQV and SDC at the two electrodes of their respective peak values (AFz and Cz).
Thick lines: Mean regression weights, shadows: ± s. e. The vertical dashed line represents the group median reaction time (539 ± 16 ms) in both plots. Nonsignificant (p > 0.00044) time points in the topography plots are masked in white. See also **Figure 6-S8** for response-locked results.

adaptive behavior. Localization and timing of these fictive error signals suggest a distinct function that may have evolved by recruiting different cortical mechanisms than experiencing or observing real outcomes caused by an actor. The adaptation itself, however, seems to be based on a more general mechanism that can be employed by experienced and fictive outcomes.

Experimental Procedures and Supplemental Information

Participants

The sample was that described in **Chapter 3** and reported are effects from the placebo condition.

Task description

Subjects had to learn the associated reward probabilities of different stimuli in order to maximize their financial earnings in a probabilistic choice task. At each trial, subjects were presented with one stimulus where they had two options: they could either choose the stimulus and risk winning or losing €0.10 or avoid the stimulus and observe the outcome without financial consequences. The fictive feedback provided information about what would have happened if they had chosen that stimulus (fictive outcome). Subjects were informed that they would receive the money won in the task at the end of the session as a bonus to their expense allowance. The task was presented using Presentation 10.3 (*Neurobehavioral Systems*).

The experiment consisted of four blocks with a random series of three different stimuli, totaling 12 different stimuli over the time of the experiment. Four stimuli associated with high chances of reward (good stimuli, two with 80% and two with 70% win rate), four stimuli associated with low chances of reward (bad stimuli, with 20% and 30% win rate) and four stimuli with a random chance of winning (neutral stimuli, 50% win rate) were presented 50 times each and then replaced. Win rates and symbol sequences were pseudo-randomized. There were no pauses during the experiment, and trials where subjects failed to respond within the given deadline were discarded from analysis. In the last block of the experiment, until each stimulus had been shown 50 times, additional new filler stimuli were shown but not included in the analyses so that every subject concluded exactly 600 valid trials.

Each trial began with a central fixation cross that was shown for a random period between 300 and 700 ms accompanied by the two response options: choose (indicated by a green tick mark) and avoid (indicated by a red no parking sign, **Figure 6-1A**). The response options remained in place until feedback was shown and their sides were counterbalanced across subjects. Following the fixation cross, one central stimulus consisting of drawn animal pictures in white on a black background was presented until the subject responded or 1700 ms had elapsed. If subjects failed to respond in time, a message appeared asking them to respond faster. Subjects' choices were confirmed by a white rectangle surrounding the chosen option for 350 ms. Immediately thereafter, the outcome was presented for 750 ms depending on the subjects choice. If subjects bet money, they received either a green smiling face and a reward of €0.10 or a red frowning face and lost €0.10. When subjects did not bet on a symbol they received the same feedback but with a slightly paler color and the money that could have been received was crossed out to indicate that the feedback was fictive and had no monetary effect. Stimuli were kept as similar as possible between conditions to avoid introducing effects of stimulus salience. On average, subjects gained €6.36 ± 0.51 (range €0.50-€9.50) over the course of the experiment.

EEG-Data acquisition and analysis

Scalp voltages were recorded with 60 Ag/AgCl sintered electrodes from participants seated in a dimly lit electromagnetically and acoustically shielded chamber. Electrodes were mounted in an elastic cap (Easycap) in the extended 10-20 system with impedances kept below 5 kΩ. The ground electrode was positioned at F2 and data was online referenced to electrode CPz. Eye-movements were captured by electrodes positioned at the left and right outer canthus and above and below the left eye respectively. EEG-data was registered continuously at 500 Hz sampling frequency with BrainAmp MR plus amplifiers (Brain Products). Data were then offline analyzed using EEGLAB 7.2 (Delorme and Makeig, 2004) and custom routines in Matlab 7.8 (MathWorks). After filtering the signal from 0.5 to 52 Hz and re-referencing to common average reference, epochs spanning from -1.5 s before to 1.5 s after feedback and -1 s before to 1 s after stimulus onset were generated. Epochs containing deviations greater than 5 SD of the mean probability distribution on any single channel or the whole montage were automatically rejected. Epoched data were then submitted to temporal infomax independent component analysis (ICA) integrated in EEGLAB and manually corrected for artifacts such as eye-blinks. Hereafter, data were re-epoched to extract response-locked data with epochs spanning from -500 ms before until 100 ms after the response. The average EEG activity spanning from -250 to -50 ms before stimulus and feedback presentation and -500 to -400 ms before response onset were used as baseline and subtracted from each channel individually (see Supplemental Experimental Procedures for results of the stimulus- and response-locked data).

Computational model

We used a reinforcement Q-learning algorithm to model each subjects' sequence of choices (Sutton and Barto, 1998) that has been successfully adopted in reinforcement-learning paradigms (e.g., Jocham et al., 2009). For each stimulus and trial *t*, the model estimated the expected stimulus value Q_t based on that stimulus' previous reward and choice history. *Q*-values represent the expected reward (positive values) or punishment (negative values) and are updated according to the following rule:

$$Q_{t+1} = \begin{cases} Q_t + \alpha_{c,t}\delta_t & if\ chosen \\ Q_t + \alpha_{a,t}\delta_t & if\ avoided \end{cases} \quad (1)$$

δ_t represents the PE of the given trial, calculated as the difference between *Q*-value and reward magnitude (Rt):

$$\delta_t = R_t - Q_t \ (2)$$

To update the *Q*-value in equation (1), the amplitude of δ_t was scaled by exponentially decreasing learning rates $\alpha_{c,t}$ and $\alpha_{a,t}$ - respectively - depending on whether the subject had chosen or avoided the stimulus. This allowed assessment of differences in learning rates and behavioral flexibility on both conditions separately. The exponential decay was calculated by two half-life time parameters ($Hl_{c/a}$) depending on the subjects choice:

$$\alpha_{c,t} = \frac{\alpha_{c,1}}{2^{\left(\frac{t-1}{Hl_c}\right)}} \quad and \quad \alpha_{a,t} = \frac{\alpha_{a,1}}{2^{\left(\frac{t-1}{Hl_a}\right)}} \ (3)$$

$\alpha_{c,1}$ and $\alpha_{a,1}$ denote the two free parameters representing the initial learning rate in both conditions. A lower limit for $\alpha_{c,t}$ and $\alpha_{a,t}$ was set to 0.01 under which learning rates could not decrease. Note that our model additionally contained a constant learning rate ($Hl_{c/a} = \infty$) as part of the range of parameters in the fitted parameter set to account for the possibility of a time invariant learning rate.

The likelihood of the model to choose or avoid a given stimulus was calculated by the softmax rule of the associated *Q*-value (**Figure 6-1B**):

$$P_{c,t} = \frac{1}{1 + exp(-Q_t\beta)} \quad and \quad P_{a,t} = 1 - P_{c,t} \ (4)$$

The free sensitivity parameter β can be regarded as the inverted temperature (high values lead to predictable behavior and vice versa). For the first step, we determined parameter estimates for all five free parameters using a grid search minimizing -*LL* over all trials *T*:

$$-LL = \sum_{t=1}^{T} logP(c_t|\theta) \ (5)$$

P(ct|⊖) denotes the models' probability to choose in the same way as the subject did in each trial given the parameter-set theta. To determine reasonable parameter combinations, we applied the following constraints: $\alpha_{c/a,1} \geq 0.01$ and ≤ 1, $Hl_{c/a} \geq 1$ and ≤ 100 but separately including ∞ and $\beta \geq 0.01$ and ≤ 25 and step-sizes for β were logarithmized. The logarithmization reflects the assumption that the model is more strongly affected by differences at small β values. Secondly, the best fitting parameter combination was then used as the starting point for a nonlinear optimization algorithm (fmincon, *Matlab optimization toolbox*). Constraints for $\alpha_{c,1}$ and $\alpha_{a,1}$ were kept but no upper limits for β and $H_{lc/a}$ set. To obtain single-trial estimates of δ_t, Q_t and $\alpha_{c/a,t}$ the MLE parameters were reentered into the reinforcement-learning algorithm.

Model fit and parameters

The parameter combinations that led to the best fit were not significantly different between both conditions (Table 1). Best fits were obtained for slightly higher average initial learning rates in condition choose ($\alpha_{1,c}$ = 0.48 ± 0.07) than in avoid ($\alpha_{1,a}$ = 0.42 ± 0.07) that decreased slightly more rapidly (Hl_c = 9.78 ± 2.60 and Hl_a = 13.47 ± 3.30). For one subject the best fit was obtained with a constant learning rate (defined as a half life time > 100 trials which equals less than ~30% decrease per block) in condition choose and for four subjects in condition avoid. On average, learning rates decreased to 3% of their initial values in condition choose and to 8% in condition avoid, providing strong support for the assumption that the impact of PEs is reduced over time. To compare both learning rates between conditions, we conducted a repeated measures ANOVA with factors α_t (50) and condition (2) that showed no significant main effect of condition on the decaying learning rate (condition $F_{1,30}$ = 0.26, p = 0.613) and no interaction (*condition x* α_t $F_{1.8,54}$ = 0.553, p = 0.561).

Although we fit different sets of model parameters for both conditions (real and fictive), we did not account for possible differences in learning caused by the different reward contingencies. It is likely that this would influence the

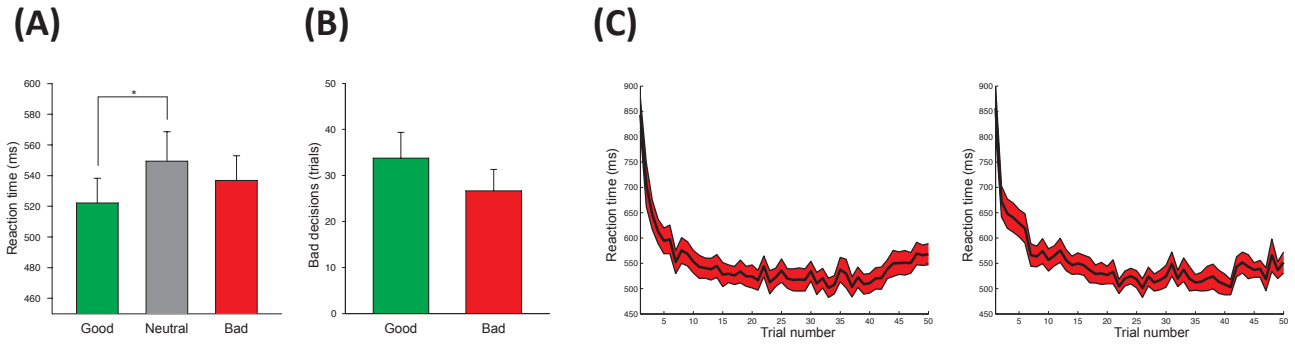


Figure 6-S1. Reaction time effects in the task.
(A) Median RTs of all subjects averaged over symbol categories (good, neutral, bad). There was a main effect of categories on RTs (ANOVA category $F_{2,60}$ = 6.57, p = 0.003) and post hoc comparisons revealed that subjects responded significantly faster to good stimuli compared to neutral ones (p = 0.002, corrected). No significant differences between the other categories were observed (always p > 0.10).
(B) Average number of suboptimal decisions defined as choosing bad and avoiding good symbols. The total number of suboptimal decisions did not differ significantly (paired *t*-test t_{30} = -1.31, p = 0.201).
(C) Decline of median RTs (± s. e.) averaged over each time the stimulus was shown in each block of 50 trials (12 blocks total) for chosen (left panel) and avoided stimuli (right panel). A repeated measures ANOVA with factors trial-number (50) and response (choose or avoid) showed a significant main effect of trial-number ($F_{10,260}$ = 26.91, p < 0.001) but no effect of the given response ($F_{1,30}$ = 0.020, p = 0.965) and no significant interaction ($F_{13,342}$ = 1.07, p = 0.304). As symbols were exchanged without prior notice, the high RT in the first trial reflects a subjects accommodation to a completely new stimulus.

results for parameter MLE, especially for the decaying learning rate. Notably, we did not observe a significant feedback-locked effect for the decaying learning rate when analysis was restricted to neutral stimuli alone, indicating that here no down-weighting of the PEs in later trials occurred (see Supplemental Experimental Procedures). However, we feel that fitting parameters separately even for different reward contingencies would lead to over-fitting and expand parameter space to unmanageable dimensions.

Multiple single-trial robust regression

To account for differences in the sensitivity parameter, z-scored results of the reinforcement-learning model were used to build a general linear model (GLM) and regress single trial EEG activity at each electrode and time point against model predictions and behavioral parameters. Robust regression that down-weights outliers by performing an iteratively reweighted least square method (O'Leary, 1990) was employed to determine parameters in the following linear equation:

$$Y = intercept + b_1 reg_1 + b_2 reg_2 ... + error (6)$$

Similar approaches have been successfully applied to EEG time- (Rousselet et al., 2008) and frequency-domain (Cohen and Cavanagh, 2011) data and allow the simultaneous investigation of multiple independent variables while preserving the high temporal resolution of the EEG. This mass univariate approach leads to individual b values for each electrode and time point for every subject. To ensure comparability between predictors within and between subjects and to penalize the model in case of multicollinearity of predictors, b values were standardized by their standard deviations before averaging across subjects.

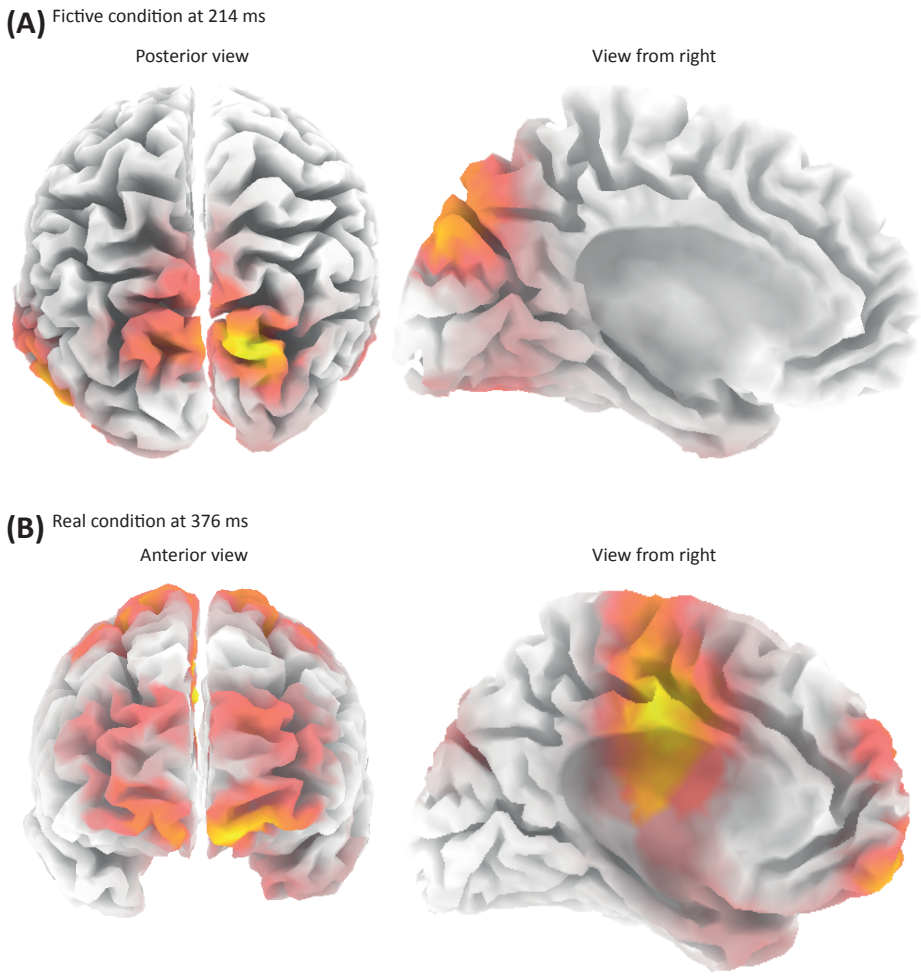


Figure 6-S2. Exploratory source analysis for PE effects.
(A) sLORETA (Pascual-Marqui, 2002) source localization at the peak latency of 214 ms of the early fictive PE correlate. b values averaged over all subjects were converted to sLORETA current source density maps. This method was chosen as it does not require a priori knowledge about possible sources and chooses the smoothest possible solution. Maximal activations (X = 15, Y = -90, Z = 35) rendered on the MNI brain are depicted. The resulting source network spans from extrastriate visual areas to the medial PPC.
(B) For the early P3a effect following real feedback, a source network centered around the cingulate gyrus (X = 5, Y = -5, Z = 45) but also including orbitofrontal cortex (X = 5, Y = -64, Z = -14) is found as the smoothest solution. Analyses of FRN and late P3b time-windows did not lead to clearly focused and stable localization results.

The stimulus-locked GLM included variable learning-rate (α_t), signed Q-value (sQ_t) and subjective decision certainty (*SDC*) plus the reaction time (*RT*) as a regressor of no interest. *SDC* was calculated from the models' softmax likelihood by equalizing $P_{c/a}$ for choosing and avoiding using the following equation: $SDC = abs(P_{c/a} - 0.5) * 2$. The result ranged from 0 (maximal insecurity) to 1 (absolute preference of one option). Feedback-locked data were analyzed separately for the categorical conditions fictive and real. Predictors included the PE (δ_t), variable learning rate (α_t), and a dichotomous regressor indicating a switch of response (coded as 1) or a stay (coded as 0) on the next trial that the same stimulus was shown again.

Standardized b values can be assumed to be gaussian due to the central limit theorem, and thus could be tested via two-tailed one-sample t-tests which were done separately at each datapoint in a whole brain approach across

subjects. Resulting p values were corrected for multiple comparisons using false discovery rate (FDR) following the method suggested in (Benjamini and Yekutieli, 2001) that has been shown to provide solid control of the family wise error rate (FWER) in EEG data (Groppe et al., 2011). However, as FDR in itself does not provide strong (local) control of the FWER, it was applied to all concatenated b value datasets per model. This ensured that all corrections were done with the same threshold value for each regressor in the models. H0 was rejected for all $p < 0.00070$ in feedback-locked model. Non-significant data-points are masked in white in the topography plots and Supplemental Movie S1. Both conditions in the feedback-locked epochs were contrasted via paired two-tailed t-tests thresholded at the same level as noted above.

Direct contrasts between real and fictive feedback evaluation

We compared both real and fictive feedback processing directly via paired two-sided t-tests of the regression b values, thresholded at the same level determined by FDR. This revealed that feedback processing indeed differed significantly for all PE effects. The late parietal effect did not differ significantly when it was inverted for fictive feedbacks assuming that counterfactual thinking was employed (by multiplication with -1) before contrasting. Contrasts for alpha and switch regressors did not reveal significant differences between both conditions.

Prediction of choice switches based on artifact-free raw EEG

Artifact free raw EEG was averaged from 370-430 ms at electrode (Pz) that showed the biggest overlap between effects of the switch, PE and learning rate predictors in the regression analysis (**Figures 6-4CD** and **6-S5**) and SDC effects locked to stimulus onset. As we observed a positive covariation in the regression analysis for switching behavior, we hypothesized that higher EEG amplitudes should be associated with a higher likelihood to switch. Additionally, because the absolute EEG amplitudes differed between both conditions (**Figure 6-3**), the analyses for real and fictive feedback were performed separately. For each subject, equally sized samples were randomly drawn from both conditions and split into two halves. One half was used to determine a discrimination threshold calculated as the simple arithmetic mean between the distributions of amplitudes for switches and stays. The predictions of this threshold were then tested in the other half of trials. 100 iterations were performed, and the results were averaged and tested against chance (i.e. 50% correct predictions) on group level using two-tailed one-sample t-tests.

Average amplitudes in the defined time window before switches were 6.29 µV ± 0.71 and 4.03 µV ± 0.62 before stays following chosen and 4.00 µV ± 0.61 before switches and 1.78 µV ± 0.53 before stays following fictive feedback. Predictions were equally valid in both conditions: the simple discrimination algorithm predicted switches correctly on an average of 56.75% ± 1.18 (t_{30} = 5.67, $p < 10^{-5}$) for real feedback and 55.86% ± 0.72 (t_{30} = 8.26, $p < 10^{-8}$) for fictive feedback (t_{30} = 0.68, p = 0.49 for difference in accuracy between conditions). In the real feedback condition out of the 31 participants, 27 had a prediction chance > 50% and 17 > 55% (maximum 70.88%). In the fictive feedback condition, 29 had a prediction chance > 50% and 17 > 55% (maximum 65.76%). Results remained significant when only neutral, good or bad stimuli were analyzed (always $p < 0.01$).

The same analysis was performed for stimulus-locked data, again separately for upcoming choose or avoid decisions to keep the results comparable with the feedback locked analysis. Average P3b amplitudes (from 520 to 580 ms,

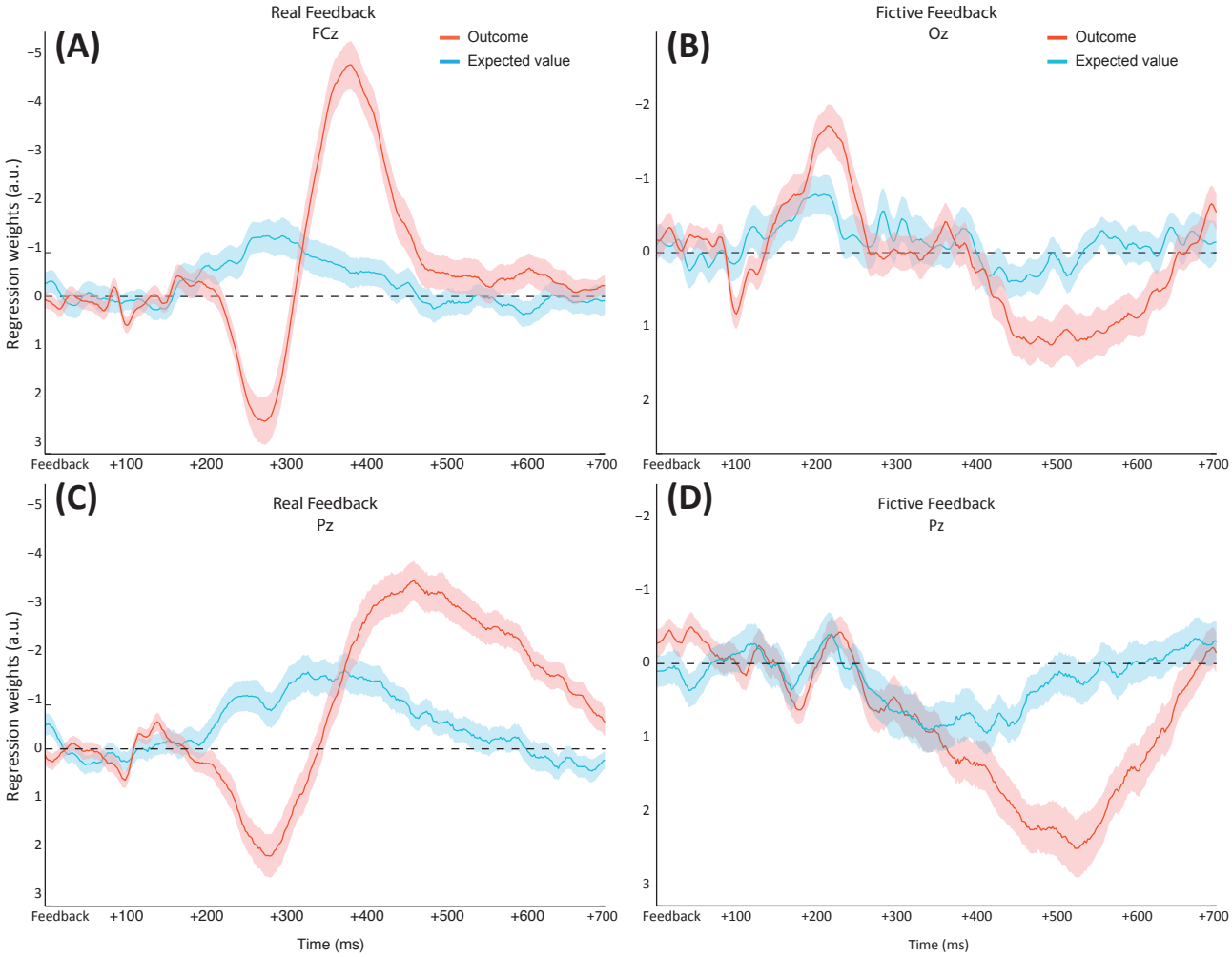


Figure 6-S3. Decomposition of PE effects into outcome and expected value.
Time-course of regression weights when the PE regressor is separated into outcome (win or loss, shown in red) and its expectancy (the signed stimulus Q-value, shown in blue) plotted at electrode FCz (A) and Pz (C) for real and at Oz (B) and Pz (D) for fictive feedback. All other regressors included in the GLM were the same as in the main analysis. In the real condition, the expected value displayed negative covariation with the EEG signal (peak at FCz 268 ms, t_{30} = -3.98, p = 0.0004) while the outcome showed positive covariation. Therefore, the time-window of the FRN satisfies necessary criteria of a PE signal (Caplin and Dean, 2008; Rutledge et al., 2010) and behaves similarly to dopaminergic neurons in that the error signal increases in magnitude when the expectancy of an error decreases (Schultz et al., 1997). The P3a component does not show this pattern of a true PE signal and does not show coding of signed expected values; therefore, it mostly reflects a pure valence and not a PE signal. When decomposed in this way, the early fictive PE correlate reveals negative value correlates (B) that are not significantly different from zero at FDR level (peak at Oz at 200 ms, t_{30} = -3.29, p = 0.0026). Value coding at parietal electrodes (C,D) reverses when stimuli are avoided compared to when they are chosen. This effect fits to an interpretation of policy rather than value coding itself (Li and Daw, 2011) in that it is the estimated likelihood of leading to a reward (avoiding punishment in the fictive condition) depending on the choice made that is encoded rather than the explicit Q-value.
Thick lines: Mean regression weights, shadows: ± s. e.

measured at Pz) before choosing were 4.81 µV ± 0.41 and 5.77 µV ± 0.48 for stimuli that led to switches and stays, respectively. Before avoiding average amplitudes were 4.78 µV ± 0.46 before switches and 5.99 µV ± 0.46 for stimuli

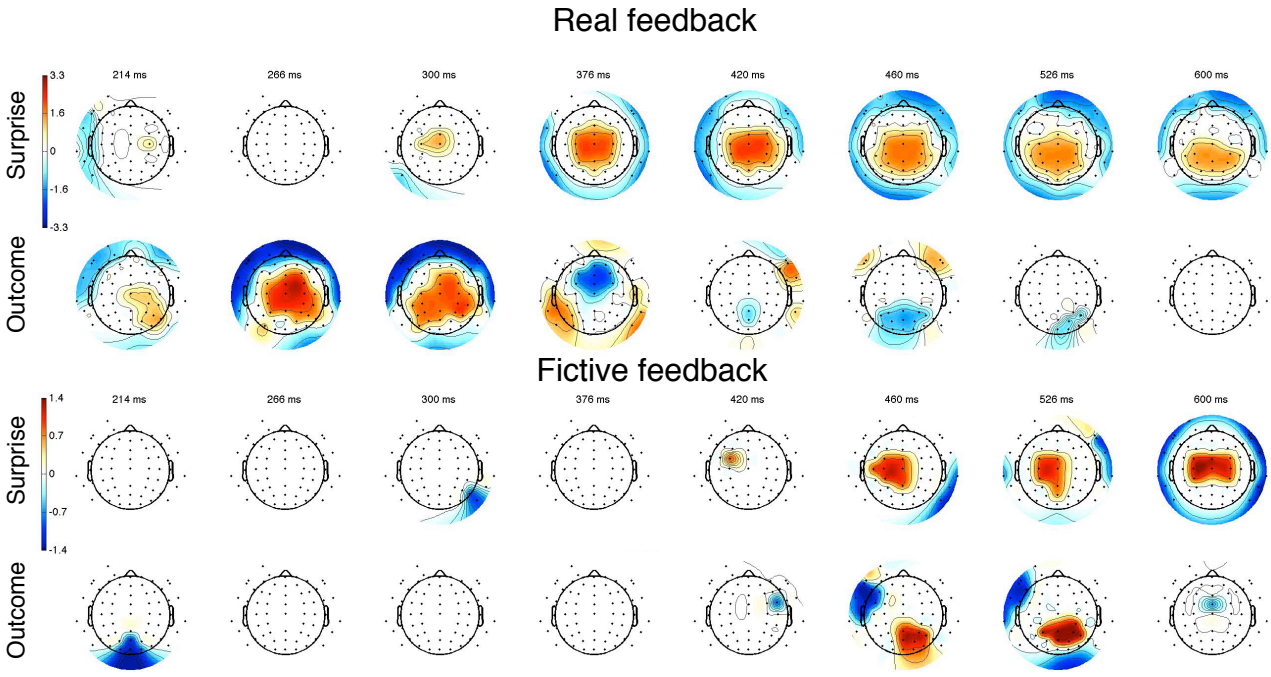


Figure 6-S4. Decomposition of PE effects into outcome and surprise.
In order to determine whether the P3b activity that was captured by the PE regressor in the main analysis reflects surprise (Yeung and Sanfey, 2004; Mars et al., 2008), outcome, or both, we included the absolute PE as a measure of surprise and the actual outcome (win or loss) into the same regression analysis. In both feedback conditions, surprise showed positive covariation with the P3b indicating that outcomes deviating further from expectation caused increased P3b amplitudes. However, the outcome also had a significant effect on P3b amplitudes in both conditions, located mainly more occipital on the scalp, which is the region that showed the highest overlap between regressors derived from the computational model and that was associated to future behavioral adaptations.
Results for P3b related activity are reported from Pz. Real feedback: *Outcome* t_{30} = -5.26, $p < 10^{-4}$ peak at 460 ms; *surprise* t_{30} = 7.04, $p < 10^{-7}$ peak at 486 ms, *t*-test of peak regression weights (inverted for outcome) for difference t_{30} = 0.45, p = 0.154. Fictive feedback: *Outcome* t_{30} = 4.23, p = 0.00019 peak at 524 ms; *surprise* t_{30} = 5.63, $p < 10^{-5}$ peak at 534 ms, *t*-test of peak regression weights for difference t_{30} = 1.21, p = 0.234. Plots are thresholded at p < 0.0001 for display purposes.

that led to switches and stays, respectively. Switches were predicted correctly on an average of 53.17% ± 0.78 (t_{30} = 4.04, p = 0.0003) for real feedback and 53.36% ± 0.77 (t_{30} = 4.34, p = 0.0001) for fictive feedback. Before choosing out of the 31 participants, 24 had a prediction chance > 50% and 8 > 55% (maximum 61.38%) and before avoiding 26 had a prediction chance > 50% and 11 > 55% (maximum 61.62%). Results remained significant when only good or bad stimuli were analyzed in both conditions (always p < 0.01) but not when only neutral stimuli were analyzed (both p > 0.39). The latter has to be expected as it is implausible that it would be possible to predict future adaptations for random outcomes if these affect switching. No differences were seen in the latency of the grand-average peak of the stimulus P3b amplitudes depending on high or low expected values or on following choices (ANOVA *choice (choose/avoid) x value (high/low)* p always > 0.1).

Logistic regression of switch behavior against stimulus and feedback P3b amplitudes was used to compare their respective predictive powers. Standardized b values for choices were 1.7 ± 0.26 (t_{30} = 6.55, $p < 10^{-6}$) for feedback and -0.66 ± 0.22 (t_{30} = -3.05, p = 0.005) for stimulus P3b amplitudes. For avoided trials b values were 1.72 ± 0.20 (t_{30} = 8.59, $p < 10^{-8}$) for feedback and -0.79 ± 0.23 (t_{30} = -3.45, p = 0.0016) for stimulus P3b amplitudes. Note the sign reversal of

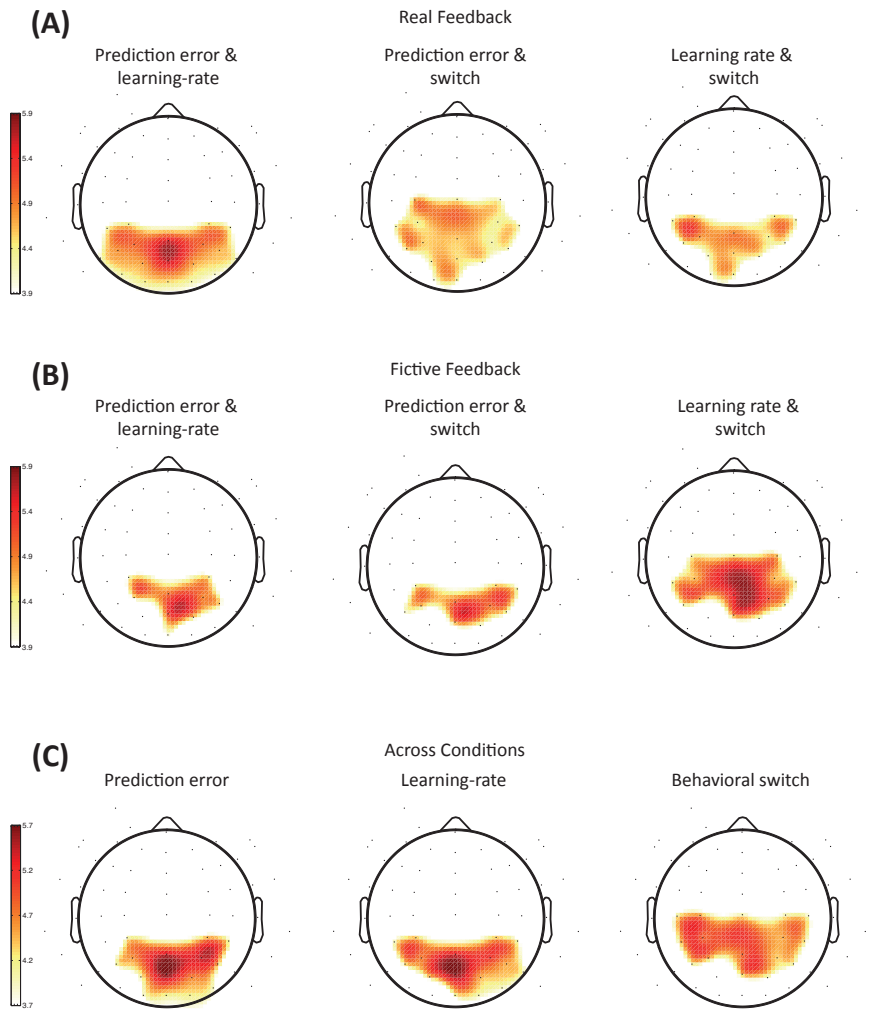


Figure 6-S5. Temporospatial conjunction maps over regressors and conditions.
The figure shows minimum t-statistics against the conjunction null hypothesis (Nichols et al., 2005) (that one or more effects are null) in the time window of the respective longest lasting effect. Results are collapsed over time of significant common activation. Conjunction maps are separated for real (A) and fictive (B) feedback. (C) shows coactivations across real and fictive conditions for the regressors affecting P3b. Electrodes Pz and POz can be seen to be activated by all regressors across conditions, which is confirmed by an overall conjunction analysis (displayed in **Figure 6-4**). Note that we rectified effects to be positive by multiplying negative group regression weights with -1 for the PE regressor in the fictive condition and only positive t-values are plotted for display purposes.

regression weights for stimulus and feedback P3b in relation to switch behavior. Combining feedback and stimulus-locked P3b amplitudes did not increase prediction accuracy for the logistic regression as measured by comparing summed *-LL* via likelihood-ratio tests between the model with only feedback P3b and the combined model (both $p > 0.59$).

Supporting behavioral analyses

To distinguish if subjects learned avoiding bad stimuli or choosing good ones more efficiently, we conducted a repeated measures ANOVA with factors *valence* (good vs bad) and trial-number (1-50) on the group average

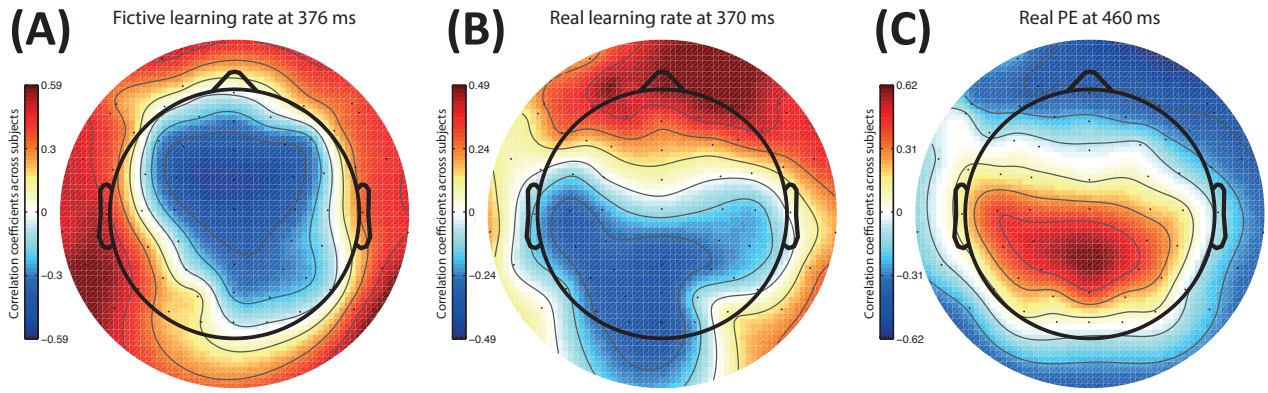


Figure 6-S6. Correlation of task performance and feedback locked regression weights.
Task performance defined as the number of suboptimal decisions (choosing a bad stimulus and avoiding a good one) was correlated across subjects with the feedback locked regression weights. Correlation coefficients between learning rate in the fictive (A) and real (B) condition revealed that the degree to which it covaried with the EEG signal at around 400 ms post feedback was associated with fewer bad decisions. Therefore, stronger learning rate coding in the EEG led to better performance. Additionally, in the real feedback condition a positive association between the PE related regression weights and task performance was seen in the time range of the P3b around 500 ms. Thus, more negative (i.e., stronger) covariation between PE and P3b was associated with less errors. No significant effects for the PE were found in the fictive condition which might be related to the somewhat lower number of bad decision made for bad stimuli (**Figure 6-S1B**). These interindividual difference effects suggest that those participants whose feedback-related P3b-like EEG activity best fit the reinforcement learning models' parameters (learning rate and prediction error) made fewest bad decisions.
In (A) values at Cz are r = -0.52 and p = 0.0023, in (B) values at Pz are r = -0.43 and p = 0.014 and in (C) values at Pz are r = 0.68 and p = 0.00002. The time point for the plots was chosen as the peak amplitude of the regression weights in the feedback locked analysis.

percentage of optimal choices. No significant difference in the learning efficacy between both types of stimuli was seen (*valence* $F_{1,30}$ = 1.22, p = 0.278) and subjects changed their behavior over time (*trial-number* $F_{49,411}$ = 10.04, p < 0.001). Additionally, no interaction between the factors was seen, indicating that the change in behavior over the blocks was comparable for both conditions (*valence x trial-number* $F_{11,357}$ = 1.50, p = 0.122). Furthermore, the total number of trials on which subjects chose compared to those where they avoided did not differ significantly ($trials_{chosen}$ = 292.8 ± 10.9 and $trials_{avoided}$ = 326.7 ± 11.3, t_{30} = 1.53, p = 0.14). This behavior seems to contradict loss aversion described in prospect theory (Kahneman and Tversky, 1979), although similar results have been described in gambling tasks (Erev et al., 2008) possibly because of the non-linearity of the value function down-weighting the impact of small losses such as those occurring in this task. As the efficacy of learning is not only determined by the total amount of optimal responses, but also by the time needed to process newly arriving information, we conducted the same analysis using median reaction times (*RT*) as the dependent variable analyzed again over blocks. Factor *valence* did not show a significant main effect ($F_{1,30}$ = 2.52, p = 0.123, median RT_{good} = 522 ± 16 ms and median RT_{bad} = 536 ± 16 ms, **Figure 6-S1A,B**), but a trend towards a faster decrease of RTs following good stimuli as indicated by the interaction term in the ANOVA analysis (*valence x trial-number* $F_{14,421}$ = 1.68, p = 0.055) was seen. This finding is in accordance with the literature (Pessiglione et al., 2006; Guitart-Masip et al., 2012b) yet does not alter interpretation of our task, as none of the results indicated a clear difference between both conditions. RT decreased as the task progressed during each block (*trial-number* $F_{11,343}$ = 53.05, p < 0.001). Additionally, RTs collapsed over stimuli and separated by choice behavior did not differ (**Figure 6-S1C**).

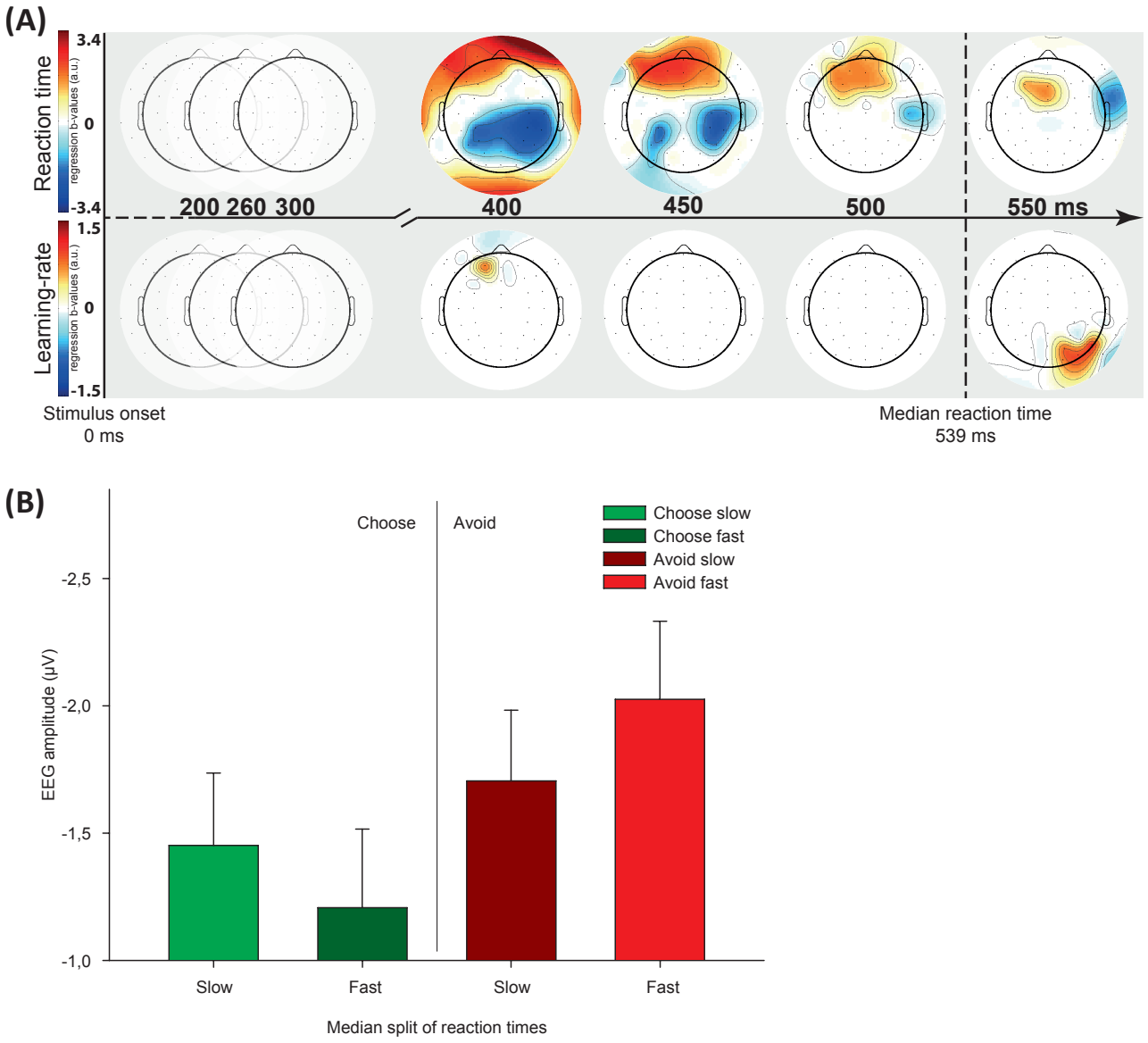


Figure 6-S7. Additional stimulus-locked analyses.
(A) Top row: Reaction time covaried with scalp activity pronounced at Pz significantly from 324-450 ms (peak at 392 ms b = -2.42, t_{30} = -5.804, p < 10^{-5}) and not during the time window observed as covarying with the signed *Q*-value in main **Figure 6-5**. Bottom-row: Learning rate did not show a consistent pattern of covariation.
(B) The mean EEG signal at electrodes identified in main **Figure 6-5** as covarying with the signed Q-value, separated by median-splitting reaction times for choosing (left) and avoiding (right) into fast and slow responses is displayed. Before choosing a stimulus, more positive EEG amplitudes lead to faster responses and this association reverses for avoided stimuli (interaction choice x speed $F_{1,30}$ = 10.8, p = 0.003). The significant main effect of choice ($F_{1,30}$ = 8.2, p = 0.007) indicates a generally more negative EEG amplitude for low value stimuli (main effect speed p > 0.1). Note that trials in which subjects did not choose according to model predictions were excluded in this analysis.
Nonsignificant (p > 0.00044) time points in the topography plots are masked in white. The vertical dashed line represents the group median reaction time (539 ± 16 ms).

To asses the impact of misleading feedback, we calculated a sensitivity measure defined as the number of trials in which a subject switched away from a correct response after probabilistic misleading feedback divided by the total number of misleading feedbacks. Again, no difference between conditions was seen ($sensitivity_{real}$ = 0.147 ± 0.029 and

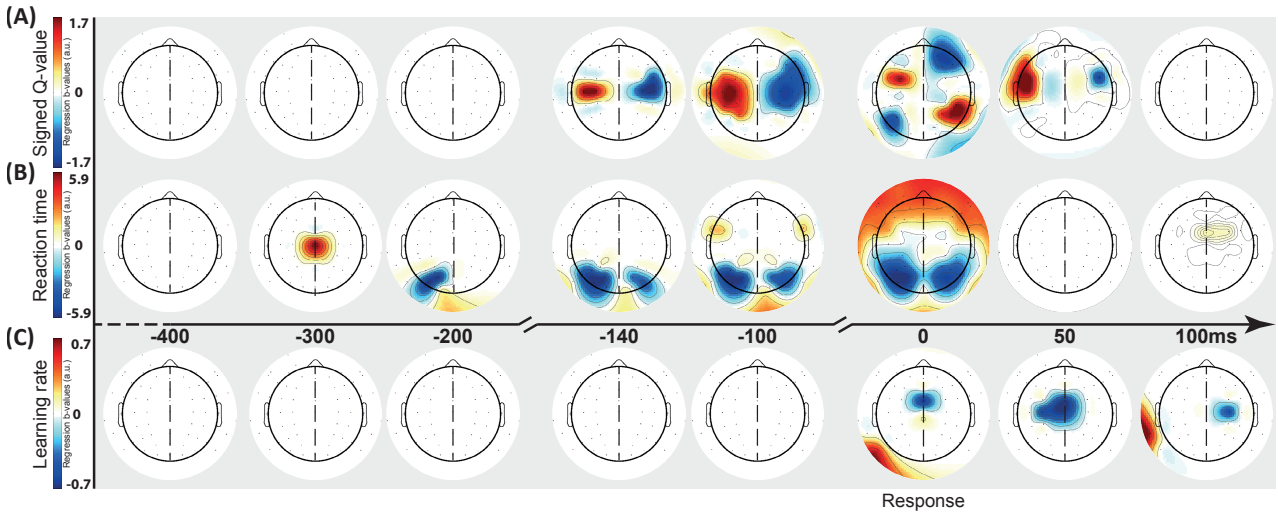


Figure 6-S8. Response-locked topography plots.
For this analysis, scalp topographies were flipped (indicated by the vertical dashed lines) for subjects that had to choose with their right hand so to ensure that the left hemiscalp always represented activity in preparation of avoiding and the right in preparation of choosing a stimulus. The response locked multiple robust regression analysis included the same regressors as the stimulus locked one except for RT and SDC. The latter can be assumed to be almost perfectly correlated with the signed Q-value after electrode flipping when no modulation over the inactive motor-cortex exists.
(A) The signed Q-value showed an up-ramping effect that was inverted over contralateral hemiscalps, likely representing preparatory (pre-)motor activity. Over the avoiding hemiscalp negative Q-values led to a more negative signal (positive covariation, left b = 2.92, t_{30} = 6.47, p < 10^{-6}, peak at -64 ms at C3) and vice versa over the choosing hemiscalp more positive Q-values led to more negative activity (negative covariation, right b = -2.13, t_{30} = -6.62, p < 10^{-6}, peak at 76 ms at C4).
(B) RT here likely reflected stimulus-locked occipital cortex activity or alpha power (Mazaheri and Jensen, 2008) that increases as the task progresses and reaction times decrease.
(C) Learning-rate did not lead to significant pre-response effects; a small effect around 50 ms post-response might reflect decreasing attentional resources in later trials.
The vertical dashed lines indicate the flipping of lateral electrodes. Nonsignificant (p > 0.0007) time points in the topography plots are masked in white.

sensitivity$_{fictive}$ = 0.144 ± 0.034, t_{30} = 0.05, p = 0.964). Thus, misleading feedback had comparable effects on subjects' behavior in both conditions and led to about 15% erroneous switches away from the optimal response. The effect of unfavorable feedback on choices of neutral stimuli was also not significantly different for real compared to fictive unfavorable outcomes (*sensitivity*$_{real}$ = 0.348 ± 0.024 and *sensitivity*$_{fictive}$ = 0.390 ± 0.045, difference: t_{30} = 0.74, p = 0.463).

After completing the experiment, subjects had to rate their emotional responses to the different feedback categories (condition) as experienced joy or anger having chosen correctly or incorrectly (result) on a scale from 1 to 10 (mean values for joy chosen = 6.6, avoided = 5.6, anger chosen = 5.6 and avoided = 5.3). A two factor repeated measures ANOVA revealed no significant effect of factors *condition* ($F_{1,30}$ = 2.98, p = 0.090) and result ($F_{1,30}$ = 3.34, p = 0.075, interaction *condition x result*: $F_{1,30}$ = 1.66, p = 0.208) thus it is unlikely that subjects did not appraise avoided outcomes emotionally. However, the trend towards an effect of factor condition is intuitively plausible as it would be expected that the difference in monetary reward (± €0.10) causes stronger emotional reactions.

Response locked analysis

The same regression model as in the stimulus locked analysis was used to evaluate EEG correlates related to the onset of the response and included the same regressors except for RT and SDC. A stimulus value correlate was found when regressing model predictions against response-locked data: significant covariations with the signed Q-value in preparation of responses over bilateral (pre-)motor-cortices which occurred 140 ms prior to the choice and lasted until 50 ms post response (**Figure 6-S8**). The direction of the correlation fit with the subsequent choice: more negative EEG amplitudes were seen over the hemisphere contralateral to the response hand. In other words, the lateralized readiness potential (LRP) (Kutas and Donchin, 1974) correlated with the signed Q-value. The scalp topographies identified in the regression analysis with maxima at C3 and C4 fit well with findings in the ERP literature confirming that the multiple regression analysis led to plausible results and topographies.

Frequency effects

As mentioned in the main text, one possible objection to our interpretation of the results might be that frequency effects in the form of unexpected events in good and bad stimuli simply cause an orienting reaction, classically associated with an increased P3 to target stimuli in oddball paradigms (Polich, 2007). Furthermore, it has recently been reported (Warren and Holroyd, 2012) that an N200 can be observed over occipital electrodes when stimuli consist of faces, presumably leading to an activation of the fusiform face area. This N200 component was sensitive to unexpected rare events in a passive viewing condition. However, restricting our analysis to only neutral stimuli associated with exactly a 50% chance of winning did not impair the early occipital PE effect for fictive feedback (t_{30} = -5.78 for all and t_{30} = -5.04 for neutral only stimuli). In fact even an increase in robustness of the effect in the FRN time window for real feedback (t_{30} = 5.17 vs t_{30} = 7.27 in neutral only) was observed. The mid-latency frontal PE effect, resembling P3a, was slightly less robust (t_{30} = -8.70 vs t_{30} = -6.20) and late parietal PE effects in both conditions were reduced, although remained significant (t_{30} = -7.86 vs t_{30} = -4.77 for chosen and t_{30} = 6.02 vs t_{30} = 3.94 for avoided). Results for the switch regressor remained significant for real feedback (t_{30} = 7.12 vs t_{30} = 5.48) but no longer passed FDR threshold for avoided feedback (t_{30} = 5.67 vs t_{30} = 3.58). Learning rate effects were markedly reduced when restricted to neutral stimuli, although this had to be expected if subjects continued to sample outcomes of stimuli associated with randomly distributed rewards (t_{30} = 7.12 vs t_{30} = 3.51 for real and t_{30} = 5.67 vs t_{30} = 3.42 for fictive feedback). Given the decrease in statistical power caused by reducing the trial number by two thirds and the persistence of the observed effects, we feel that this comparison renders an explanation based on sequence effects rather implausible.

CHAPTER

7

5-HT Effects on the Processing of Real and Fictive Events

Introduction

It is a matter of ongoing discussion whether 5-HT is especially important in order to tie expected aversive events to behavioral inhibition via Pavlovian mechanisms (Crockett et al., 2009; Boureau and Dayan, 2011). Additionally, 5-HT has been ascribed a major role in the etiology of depression, for which disorder recently a relationship to the experienced regret about unchosen options has been demonstrated (Brassen et al., 2012). Increased rumination about foregone life choices is a well known phenomenon in depressive patients but the processing of fictive events has never been studied in relation to depression or 5-HT (Howlett and Paulus, 2013). Additionally, despite clear evidence for a role of the 5-HT system in reward based learning (Chamberlain et al., 2006; Liu et al., 2014), results are far from unequivocal (Evers et al., 2005; Guitart-Masip et al., 2012a). We introduced the task described in **Chapter 6** which was designed to allow differentiating serotonergic effects on the processing of – and learning from – real and fictive events separately. One may speculate that SS subjects known to be more prone to developing depression (Karg et al., 2011), could show increased behavioral impact or EEG correlates of fictive outcome processing. Behaviorally, we measured the percentage of switches following unfavorable fictive feedback and the decay observed in the speed of learning rate decline in the fictive condition was derived from the computational model described in **Chapter 6**. As for the EEG correlates, we included the occipital early component, that was demonstrated to covary with fictive PE signals, the P3a which we found here to reflect the unexpectedness of fictive events, and the P3b which we found to reflect updating of stimulus values from fictive events, and compared these to their real counterparts.

This task furthermore should allow to delineate differences in decisions to approach or avoid a stimulus without introducing an actual difference in behavior, that is responding or withholding a response (Pessiglione et al., 2006). Differences here may be apparent in that increased avoidance may be reflected in an increased tendency to not choose to gamble in this task or higher RT on gambling decisions possibly reflecting decreased response vigor (Crockett et al., 2012). As one study found a general increase towards active behavior following SSRI application in a task that crossed action and valence in an instructed Go/NoGo paradigm (Guitart-Masip et al., 2013), a hypothesis regarding the drug effect may be more gambling decisions following SSRI application or decreased RT on gambling choices. Another prediction may be increased general RT following drug administration and possibly decreased learning success, as would be derived from the results of (Chamberlain et al., 2006). In the context of the dual signal theory introduced in **Chapter 2**, this may be due to a decrease of the glutamatergic component.

Materials and Methods

The task has been described in **Chapter 6**. We compared ERPs of real and fictive outcomes in a regular averaging approach. While we explored these potentials in the context of real and fictive events in the previous chapter, we did not chose a GLM approach for data analysis here. This was done to limit the number of multiple comparisons, avoid difficulties with correlations of brain data with predictors derived from computational models which are only comparable across subjects when the fit is identical in both groups, and to increase comparability with other studies that so far only employed averaging approaches combined with serotonergic factors. Analysis was focused on those components that could be clearly related to task relevant functions in the previous chapter. We assume the FRN to reflect prediction errors following real feedback and the early occipital component in the fictive condition to reflect the valence of fictive feedback. Furthermore, P3a is assumed to reflect surprise and valence of real feedback whereas P3b may reflect the updating of stimulus values independent of feedback type. The longer lasting positivity seen to be associated with the learning rate may not be reflected in a single ERP component, but rather emerge as a shift over multiple components.

Table 1. ERP Quantification Details

ERP	Electrode	Peak (ms)	Time Window	Method
P2	FCz	206	180 - 240	Max
N2	FCz	262	230 - 290	Min
P3a	FCz	370	330 - 430	Max
P3b	Pz	450	370 - 580	Max
FRN	FCz	-	-	N2 - P2
FRN-Diff	FCz	272	260 - 280	Mean of Difference
P3a-Diff	FCz	372	350 - 390	Mean of Difference
Fictive-Peak	Oz	216	190 - 240	Min
Fictive-Diff	Oz	216	200 - 220	Mean of Difference

Time windows were approximated as *Full Width at Half Maximum* (FWHM) of the amplitudes of the respective ERP in the condition where it was largest. For difference measures, a short time-window surrounding the peak for FRN and occipital peak (20 ms) and a broader time-window for P3a (40 ms) was chosen.

ERPs were measured as described in **Table 1** and entered into MLM analysis with the additional factors *factuality* (real, fictive) and *outcome* (win, loss). Due to the somewhat problematic quantification of the FRN, this component is measured in two ways (**Table 1**): as the mean of the difference between favorable and unfavorable outcomes (Dehaene et al., 1994) and, because a reward related positivity has recently been proposed (Holroyd et al., 2008), we also measured P2 (Potts et al., 2006) and following N2 separately and calculated the difference between both. For difference waves, mean amplitudes surrounding the peak were chosen rather than minima as this measure does not assume a certain directionality, i.e. one condition to be larger or smaller than the other. This is important as it may be plausible that FRN amplitudes could switch signs when comparing favorability in both conditions, e.g., in one genetic group and not the other. Amplitudes of the parietal component were determined as minima of regular amplitudes in all conditions and means of difference waves between fictive wins and losses. Due to the complexity of the analysis

and the number of ERPs involved, results are grouped by factors of interest beginning with overall task effects rather than individual component descriptions. In order to compare drug effects and their genetic dependence, post hoc contrasts comparing genetic differences without drug application and drug effects within both genetic groups will be reported even if these factors did not show significant interaction effects beforehand. This is justified in accordance with our a priori hypothesis of altered drug effects depending on genotype.

Behavioral Results

The amount of money won did not differ between genetic groups ($F_{1,31}$ = 0.5, p = 0.48) and SS subjects won on average 6.66€ and LL subjects 6.10€ (difference 0.56±0.80€). Subjects won significantly more money in the second test session (+0.89±0.32€, $F_{1,31}$ = 7.7, p = 0.009). The ratio between chosen and avoided trials as a broad measure of how conservative subjects were (higher = more risky), was not affected by any factor in the MLM (all ps > 0.14; SS = 0.98±0.09; LL = 1.02±0.09).

We submitted subject´s gambling choices collapsed over trials to MLM analysis with the additional factor *stimulus* (good, bad, neutral). This analysis did not reveal significant effects for factors *genotype* (p = 0.89), *drug* (p = 0.656), their interaction (p = 0.16), or an interaction with *stimulus* (all p > 0.65). To further account for the possibility of 5-HT effects only on specific intervals during the learning of stimulus values, we further subdivided the model by including a factor *bin* by averaging choices in 5 chunks of 10 trials for each stimulus. We found a main effects for *stimulus* ($F_{2,899}$ = 2062, p = 0) and an interaction between *stimulus* and *bin* ($F_{8,899}$ = 10.8, p < 10^{-12}) reflecting the change in choices over time. The 3-way interactions with *stimulus* (p = 0.172) and *bin* (p = 0.90), as well as the 4-way interaction (p = 0.99) were non-significant. Exploratory post hoc contrasts within factor *bin* revealed that the only significant effect was an increase of choices for LL subjects in the last bin (trials 41 to 50) of neutral stimuli (Δ+11.9 ± 4.9 %, $F_{1,899}$ = 8.9, p = 0.015, **Figure 7-1A**).

The same analysis was performed for mean *RT* as the dependent variable (**Figure 7-2A-C**). A main effect for *stimulus* ($F_{2,155}$ = 11.2, p < 10^{-4}) arose due to faster responses to good (Δ-32 ± 7 ms, p < 0.0001) and bad (Δ-21 ± 7 ms, p = 0.009) compared to neutral stimuli. Participants responded faster when the task was repeated (*session* Δ-48 ± 6 ms, $F_{1,155}$ = 70, p < 10^{-12}). Additionally, a main effect for *drug* ($F_{1,155}$ = 7.0, p = 0.009) that was further modulated by an interaction with *genotype* ($F_{1,155}$ = 7.6, p = 0.006) was seen. Administration of citalopram significantly increased RT for subjects with the LL (Δ+31 ± 8 ms, $F_{1,155}$ = 15.1, p < 0.0002) but not the SS genotype (Δ-1 ± 8 ms, $F_{1,155}$ < 0.01, p = 0.93). This effect was not modulated by *stimulus* type (3-way interaction p = .78) and further contrasts revealed significant drug effects in group LL for good and neutral stimuli (ps < 0.026, uncorrected) and a trend for bad stimuli (p = 0.06). No effect was seen for SS subjects (ps > 0.6). As in the analysis of choice behavior, a further division into bins of 10 trials did not lead to significant interactions with any 5-HT related factors and thus the RT increase seen for LL subjects is seen throughout the duration of each block of 50 trials. Therefore, citalopram increased RT for LL but not SS subjects independent of stimulus category and trial number within each block.

An influential study by Chamberlain et al. (Chamberlain et al., 2006) found a drastic increase of RT and much less effective learning following oral SSRI application in a reversal learning paradigm both for the initial learning of stimulus response associations and following their reversal. This paradigm, however, differed from the one used here in that it only employed two learning blocks with ~40 trials each. Subjects were thus much less able to consciously understand what the correct behavior may be. Thus, we also analyzed the data of the first block of this experiment when subjects had to learn the value of good, bad, and neutral stimuli for the first time. The reasoning behind this is that while learning in the absence of a clear conscious representation of a cognitive task may rely more on basic neurophysiologic functions, e.g. raphe reward signaling (Liu et al., 2014), this effect may vanish when subjects fully

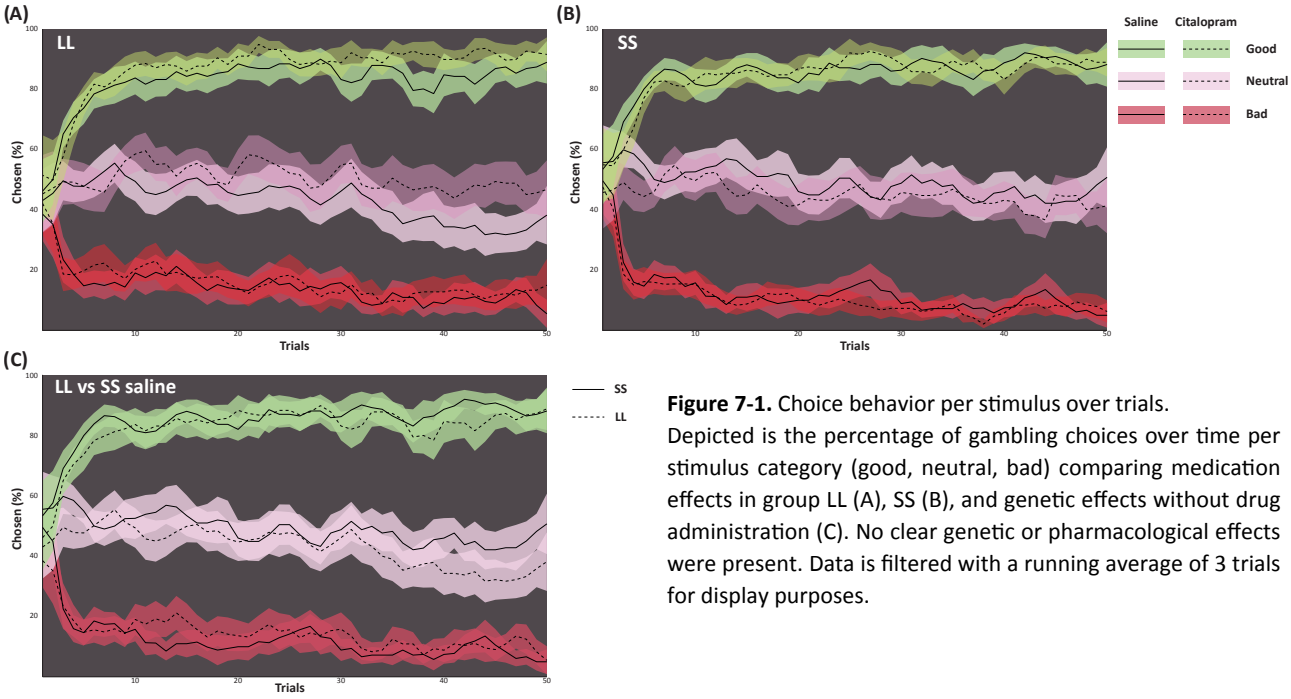


Figure 7-1. Choice behavior per stimulus over trials.
Depicted is the percentage of gambling choices over time per stimulus category (good, neutral, bad) comparing medication effects in group LL (A), SS (B), and genetic effects without drug administration (C). No clear genetic or pharmacological effects were present. Data is filtered with a running average of 3 trials for display purposes.

understood the task at hand. Therefore, we repeated both analyses described above confined to the first 50 trials each stimulus per category had to be learned.

For choice behavior, we found no additional effects and all results related to factors of interest remained non-significant (ps > 0.19). With regard to the 5-HT related effects seen on RT, however, we found that the *drug x genotype* interaction ($F_{1,155}$ = 18.4, p < 0.0001) was further pronounced and modified by the current *session* (3-way interaction $F_{1,31}$ = 7.2, p = 0.011) while the overall *drug* effect remained significant ($F_{1,155}$ = 7.3, p = 0.008). Within the first test session LL subjects that received citalopram showed strongly increased RT (Δ+143 ± 43 ms, $F_{1,37}$ = 11.0, p = 0.002) but when the task was repeated in the second session, this effect was absent (Δ-14 ± 43 ms, $F_{1,37}$ = 0.1, p = 0.75). On the contrary, SS subjects showed significantly reduced RT when citalopram was administered in the first session (Δ-95 ± 44 ms, $F_{1,37}$ = 4.6, p = 0.039) and a non-significant increase in the second session (Δ+66 ± 44 ms, $F_{1,37}$ = 2.2, p = 0.15). These effects were again not modulated by factors *stimulus* and *bin*.

We also investigated switch behavior depending on the favorability of the outcome in the real and fictive condition. Therefore, we submitted the likelihood to switch the response following an unfavorable outcome and to stay with the current response following a favorable outcome to separate MLM analyses including factor *factuality*. There were no 5-HT related effects. Unmedicated LL subjects were as likely to switch responses following real unfavorable feedback (29 ± 3 %) as were SS subjects (30 ± 3 %, p = 0.77) and no main *drug* effect was present (p = 0.60). Additionally, no significant interactions were observed (ps > 0.65). Similarly, there were no genetic influences on the likelihood to stay with the same response following a favorable real (LL: 77 ± 3 %; SS: 79 ± 3%; p = 0.68) or fictive outcome (LL: 85 ± 3 %; SS: 86 ± 3%; p = 0.74) and no other 5-HT related effects (ps > 0.25). Furthermore, constraining the analysis to the first learning blocks per stimulus as described above, did not alter results apart from showing session effects overall in that subjects tended to switch less following unfavorable outcomes (session 1: 35 ± 4 %; session 2: 31 ± 4 %; p = 0.057) and repeated a favorable decision more often (session 1: 78 ± 5 %; session 2: 83 ± 5 %; p = 0.011).

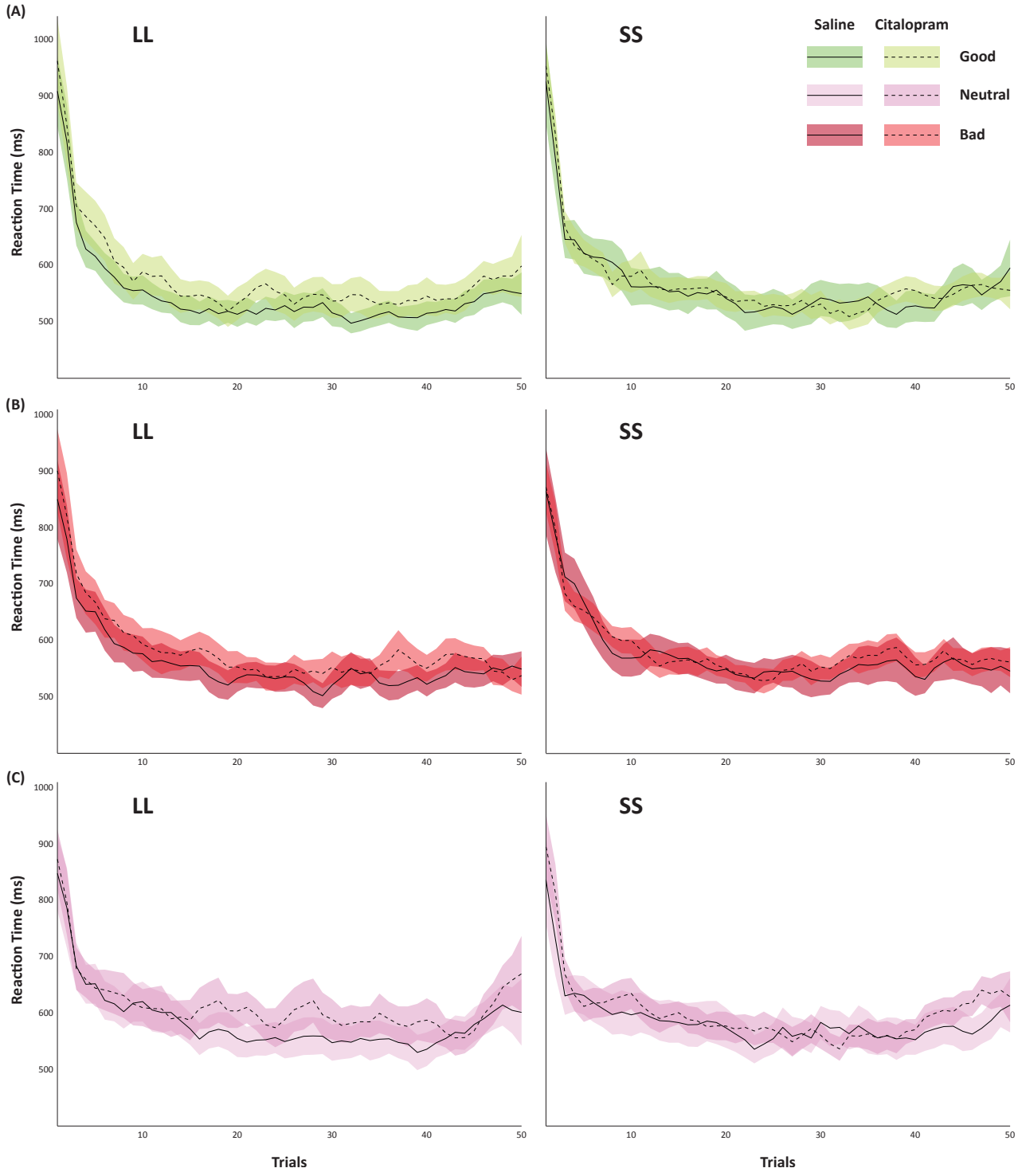


Figure 7-2. RT per stimulus over trials.
Depicted are mean reaction times for good (A), bad (B), and neutral (C) stimuli over trials separated by genetic groups (LL = left column, SS = right column). Citalopram caused an increase of RT only in the LL group that was present for all stimulus types. Data filtered with 3 trials running average.

This behavioral analysis, however, does not provide a comparison of how well subjects employed both types of feedback (real and fictive) as participants may, e.g., have learned to avoid bad stimuli, by implementing real unfavorable feedback. Thus, we ran the computational model introduced in **Chapter 6** again on the behavioral data of all subjects under both drug conditions. For this analysis, we fixed the initial learning rate (α_1) and the temperature parameter (β) to the average of the best fit of both conditions (i.e. $\alpha_1 = 0.45$ and $\beta = 10$). This was done as half-life and initial learning rate are negatively dependent and in the case of different temperature parameters, neither are directly comparable across subjects. MLM analysis of resulting half-life time parameters, however, did neither reveal significant *drug* ($p = 0.79$), *genotype* ($p = 0.37$), nor an interaction effect ($p = 0.34$). Additionally, as expected, no difference between the decay of learning rates for real and fictive conditions was seen across all subjects ($p = 0.99$).

EEG Results

Task Effects: Factuality and Outcome

First, we confirmed findings of the analysis reported in **Chapter 6** in the whole sample including the verum session. Means of FRN difference waves were significantly more negative for real (Δ-2.18 ± 0.27 µV) than fictive (Δ-0.50 ± 0.27 µV) outcomes ($F_{1,93}$ = 25.9, p < 10^{-5}). The same was seen in the peak-to-peak analysis when P2 was subtracted from N2: FRN amplitudes were significantly different between wins and losses in the real (Δ-2.23 ± 0.25 µV, $F_{1,217}$ = 78.5, p < 10^{-14}), but not fictive (Δ+0.30 ± 0.25 µV, $F_{1,217}$ = 1.40, p = 0.24) condition (interaction *factuality x outcome* $F_{1,217}$ = 50.4, p < 10^{-9}) and overall larger for real outcomes (Δ-0.97 ± 0.18 µV, $F_{1,217}$ = 30.0, p < 10^{-5}). Vice versa, peak amplitude analysis confirmed sensitivity of the parieto-occipital ERP component to fictive (contrast *outcome* in *fictive* $F_{1,217}$ = 37.1, p < 10^{-7}) but not real outcomes ($F_{1,217}$ = 1.1, p = 0.29, *factuality x outcome* $F_{1,217}$ = 25.5, p < 10^{-5}). This was also found in the difference wave analysis which showed significantly larger differences for the favorability of fictive (Δ1.59 ± 0.18 µV) than real outcomes (Δ-0.27 ± 0.18 µV, main effect *factuality* $F_{1,93}$ = 66.4, p < 10^{-10}).

P3a was significantly larger for unfavorable compared to favorable real (Δ+6.15 ± 0.38 µV, $F_{1,217}$ = 261.2, p < 10^{-37}) and fictive (Δ+1.46 ± 0.38 µV, $F_{1,217}$ = 14.7, p < 0.0005) outcomes. This was confirmed by a significant interaction between *factuality x outcome* ($F_{1,217}$ = 199.9, p < 10^{-30}). P3b, again, was larger for unfavorable real (Δ+3.46 ± 0.31 µV, $F_{1,217}$ = 118.3, p < 10^{-20}) and fictive events (Δ-2.39 ± 0.31 µV, $F_{1,217}$ = 56.4, p < 10^{-10}) and both factors interacted ($F_{1,217}$ = 169.0, p < 10^{-27}).

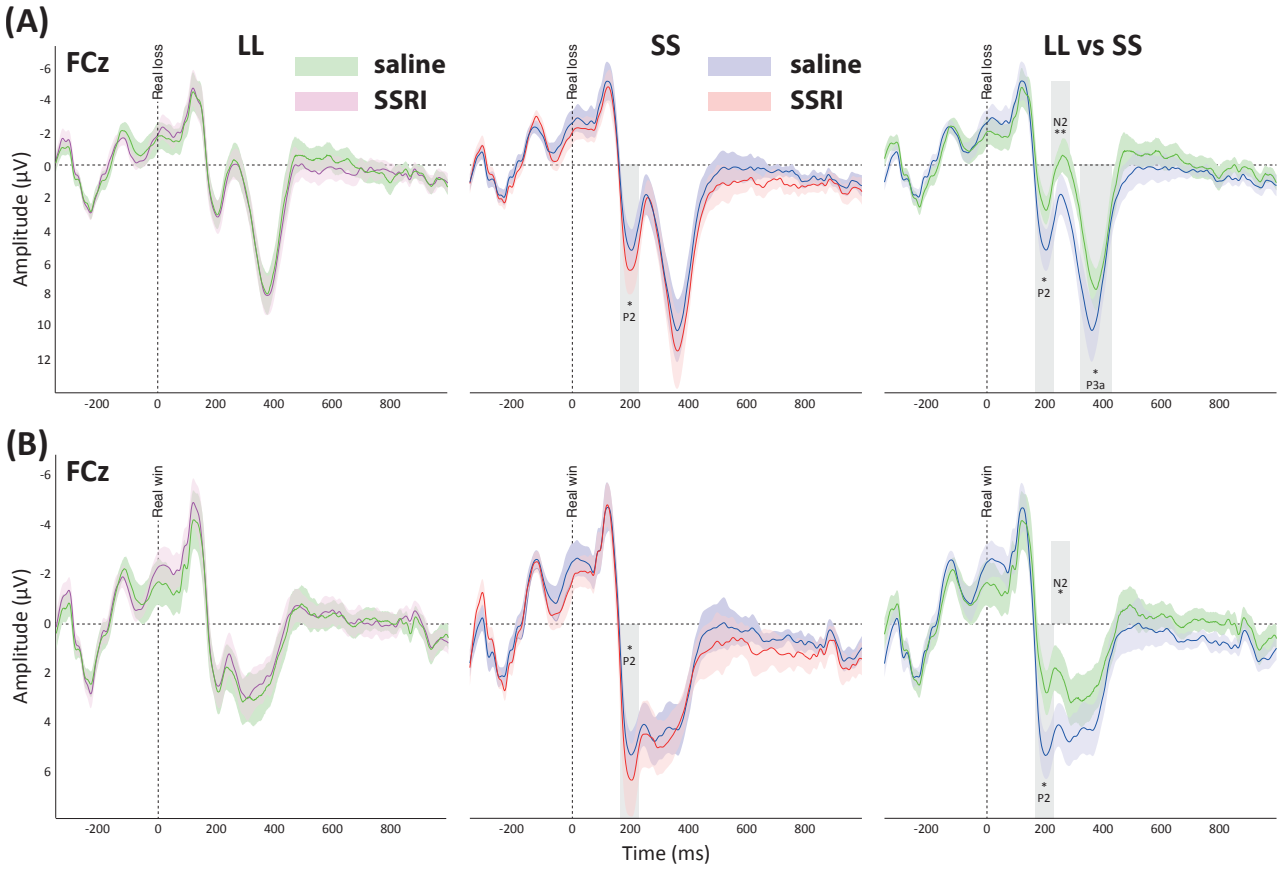
This would suggest that P3a and P3b are sensitive to the favorability of real and fictive outcomes. However, when surprise was controlled for by restricting the analysis to only neutral stimuli with an equal likelihood of favorable and unfavorable outcomes, only P3b was sensitive to the favorability of fictive outcomes (Δ+1.24 ± 0.41 µV, $F_{1,217}$ = 9.0, p = 0.003). P3a was insensitive to fictive favorability (Δ+0.23 ± 0.47 µV, $F_{1,217}$ = 0.24, p = 0.62) but remained sensitive to the favorability of real outcomes (Δ+4.16 ± 0.47 µV, $F_{1,217}$ = 79.4, p < 10^{-14}).

P2 amplitudes were insensitive to the favorability of real outcomes (on unfavorable: Δ+0.26 ± 0.30 µV, $F_{1,217}$ = 0.77, p = 0.38) and slightly higher for fictive unfavorable outcomes (Δ+0.72 ± 0.30 µV, $F_{1,217}$ = 5.99, p = 0.015).

These results replicate the more elaborate analysis of the previous chapter and confirm that P3a is still sensitive to the unexpectedness of a fictive event, but only P3b tracks favorability for both real and fictive outcomes. Thus, for the analysis of 5-HT effects it is concluded that FRN reflects the favorability of real and the parietal component of fictive outcomes, P3a reflects favorability of real and surprise of real and fictive events, whereas P3b also reflects the favorability of both and, thus, possibly the updating of stimulus values.

5-HT, Factuality and Outcomes

Inspection of the ERP waveforms (**Figures 7-3A,B** & **7-5A,B**) suggested that SS subjects generally show a pronounced positive antero-frontal potential following feedback which appears to encompass P2 up until P3a time-range. This was confirmed by significant *genotype* main effects on P2 ($F_{1,31}$ = 6.37, p = 0.017), N2 ($F_{1,31}$ = 5.59, p = 0.024), and a trend



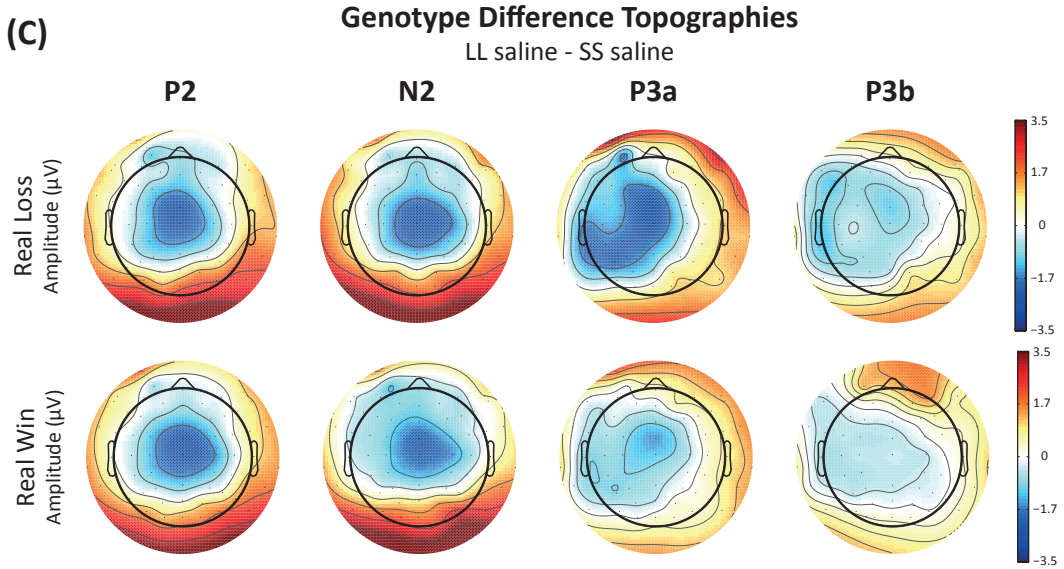


Figure 7-3. P2 until P3a are modulated by 5-HTTLPR genotype for real feedback.
(A, B) following both types of real feedback, beginning from the P2 onward, subjects carrying the SS genotype showed more positive EEG amplitudes (comparison LL vs SS saline, right plot). This was not the case for fictive outcomes (**Figure 7-4**). Additionally, a drug effect was seen for P2 amplitudes which were slightly increased by citalopram in SS carriers for real wins and losses (A & B, middle plots). (C) shows scalp topographies of the difference between LL and SS genotype in the saline condition at the time point of the peak of the respective ERPs (see Table 1). The main difference is a centro-medial increase in amplitudes which spans roughly 180 ms beginning with the P2 peak at 210 ms and is thus not restricted to one ERP component. For real losses, this extends to the P3a which it does less so for real wins. Note that these genetic effects were all seen, or increased, following citalopram administration (topographies not shown). Grey areas mark significant ($p < 0.05$) ERP time-windows from post-hoc contrasts.

for P3a amplitudes ($F_{1,31}$ = 3.45, p = 0.072). Furthermore, this effect was modulated by the factuality of the outcome for all three ERPs (interaction *genotype x factuality* all p < 0.001) and significant effects were only seen for real (all ps < 0.016) and not fictive (all ps > 0.183) outcomes. For P3a only, a trend for an interaction of *outcome x factuality x genotype* was seen ($F_{1,217}$ = 3.49, p = 0.063). Post hoc contrasts confirmed higher P3a amplitudes in SS compared to LL subjects only for real losses (Δ+3.00 ± 1.0 μV, $F_{1,49.8}$ = 8.92, p = 0.004), while the other comparisons were non-significant (all ps > 0.095). For P3b amplitudes we observed a significant *outcome x factuality* interaction ($F_{1,31}$ = 11.2, p = 0.001), but contrast between genetic groups were non-significant (all p > 0.13). None of the potentials was significantly altered by citalopram application (*drug* all ps > 0.38), but when contrasted within genetic groups, P2 amplitudes for real wins and losses were increased by drug application in SS subjects alone (both Δ>+1.2 ± 0.6 μV, $F_{1,217}$ > 4.0, p < 0.047).

When FRN amplitudes were analyzed as the difference between N2 and the preceding positive P2 peak, we found a significant *drug x factuality* interaction ($F_{1,217}$ = 4.18, p = 0.042), which was due to significant FRN increases seen in the real feedback condition ($F_{1,217}$ = 4.96, p = 0.027) which was not present in the fictive feedback condition ($F_{1,217}$ = 0.44, p = 0.5). When further split up by outcome and genetic groups, FRN was significantly increased in group SS for real wins (Δ-1.01 ± 0.51 μV, $F_{1,217}$ = 3.88, p = 0.05), a trend was seen for losses (Δ-0.92 ± 0.51 μV, $F_{1,217}$ = 3.24, p = 0.073), and all other conditions (fictive, and all comparisons in group LL) were non-significant (all ps > 0.52). However, this effect did not appear to be caused by the N2 peak, but rather the P2 peak itself which showed a similar effect when analyzed separately (see above). Thus, these marginally significant effects cannot be attributed to the FRN potential itself, but appear related to the P2. Furthermore, similar effects were seen for the P3a, as noted above.

We followed up the P3a effect with regard to the question whether it was rather reflective of the favorability of the outcome, or surprise – as we have found both to modulate P3a amplitudes in this task (**Chapter 6**). Thus, we repeated the MLM confined to neutral stimuli. In this analysis, none of the contrasts between genetic groups in the saline condition reached significance (ps > 0.13) and neither did the 3-way interaction (p = 0.87). This suggests that a longer lasting increased positivity beginning with the P2 component was seen for SS subjects following real outcomes which is not restricted to one single ERP. SS subjects displayed higher P3a following real losses, but when unexpectedness of these events was controlled for, this effect was no longer significant. This indicates that the genetic difference was mainly reflecting an orienting reaction and not evaluation of favorability. P3b amplitudes were not altered by genetic or pharmacological factors.

Difference wave FRN amplitudes (**Figure 7-5**) were unaltered by factors *drug*, *genotype* or their interaction (all ps > 0.82) and these did not interact with the factuality of the outcome (all ps > 0.16). P3a amplitudes of the differences revealed an interaction for *genotype x factuality* ($F_{1,93}$ = 6.27, p = 0.014) and a trend for *drug x factuality* ($F_{1,93}$ = 3.09, p = 0.082). When followed up by contrasts, the first interaction effect resulted from P3a amplitudes in the real condition trending to be more positive in SS subjects (Δ+1.38 ± 0.71 μV, $F_{1,65}$ = 3.83, p = 0.055) while they were non-significantly more negative in the fictive condition (Δ-0.67 ± 0.71 μV, $F_{1,65}$ = 0.89, p = 0.35). Additionally, there was a trend for a drug effect for increased P3a amplitudes on real feedback in the SS group ($F_{1,93}$ = 2.98, p = 0.088, LL p = 0.49) that was absent for P3a following fictive feedback ($F_{1,93}$ < 0.01, p = 0.95, LL p = 0.30). Like FRN amplitudes,

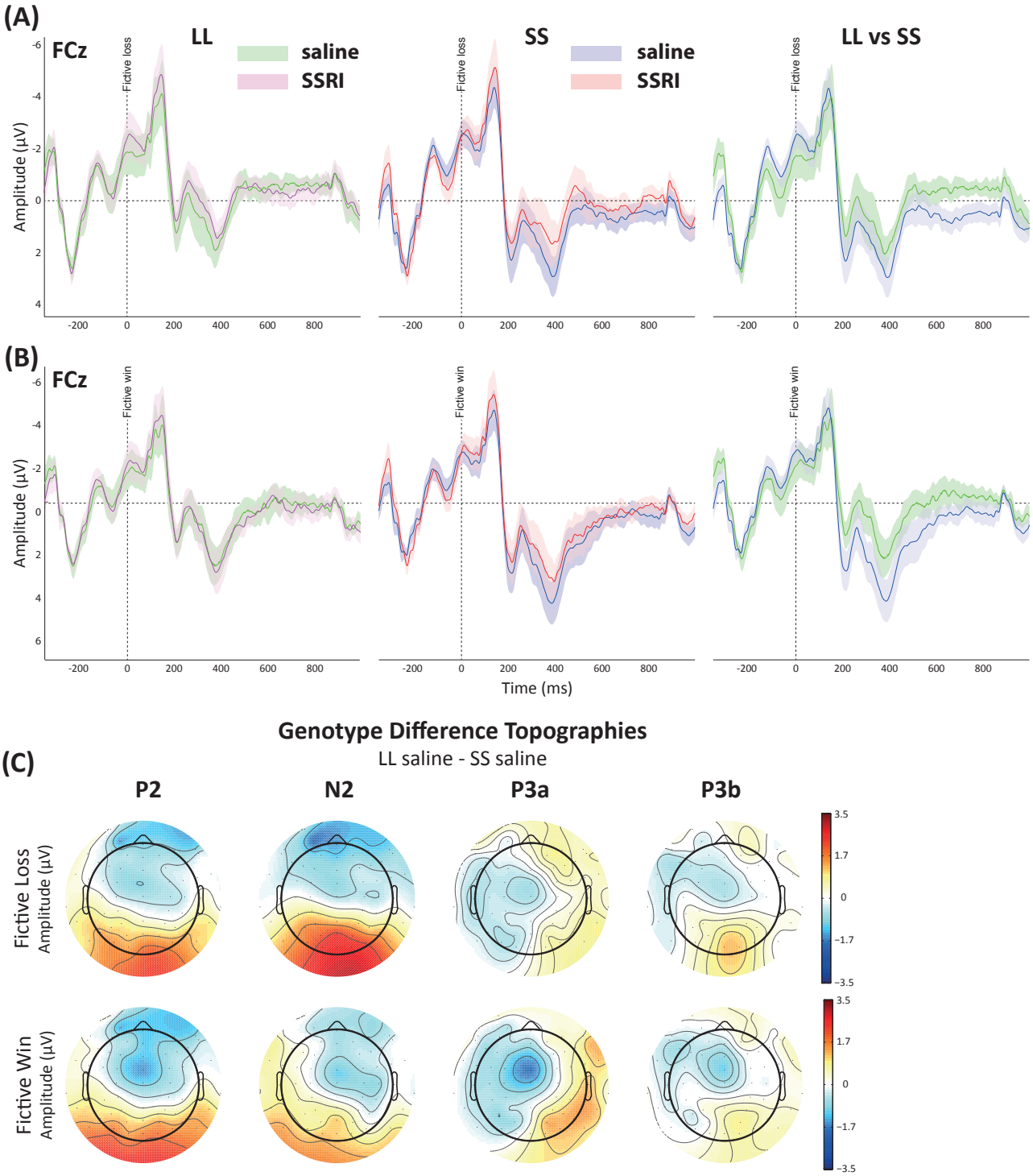


Figure 7-4. ERPs for fictive feedback at fronto-central electrodes are not modulated by 5-HT.
(A,B) ERPs following fictive favorable (A) and unfavorable (B) outcomes. Neither genetic differences nor medication effects were seen for P2, N2, and P3a. (C) scalp topographies indicate a somewhat more positive potential at posterior electrodes in early time-windows. However, the fictive occipital potential was not modulated by 5-HT (see **Figure 7-6** for details).

the occipital peak in the fictive condition was not modulated by factors *drug*, *genotype* or their interaction (all ps > 0.23) and these also did not interact with the factuality of the outcome (all ps > 0.21, **Figure 7-6**).

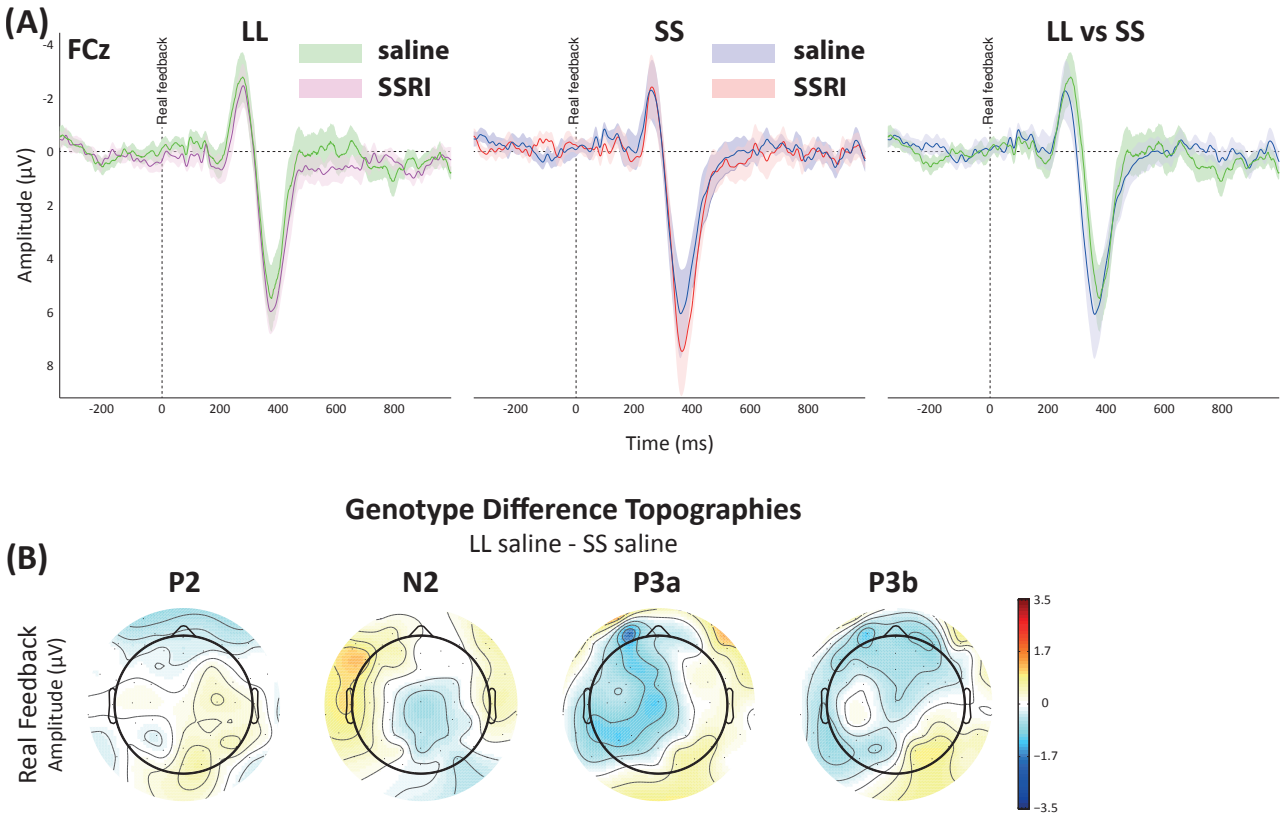


Figure 7-5. Difference wave analysis of ERPs for real feedback.
(A) We calculated difference waves for real wins and losses by subtracting the ERP following wins from the ERP following losses. FRN was not modulated by 5-HT related factors. P3a trended to be increased in the SS group ($p = 0.055$) where citalopram also trended to increase amplitudes ($p = 0.088$). (B) In the saline condition, no difference is seen between both genetic groups in the scalp topographies.

Thus, both early correlates of real and fictive outcome processing appear unmodulated by 5-HT. The increase in positive amplitudes seen in the SS group in the peak measure analysis does not appear to be pronounced on unfavorable outcomes. However, difference wave measures of P3a indicate a trend towards increases in group SS which is further pronounced by a trend towards a drug effect in this group.

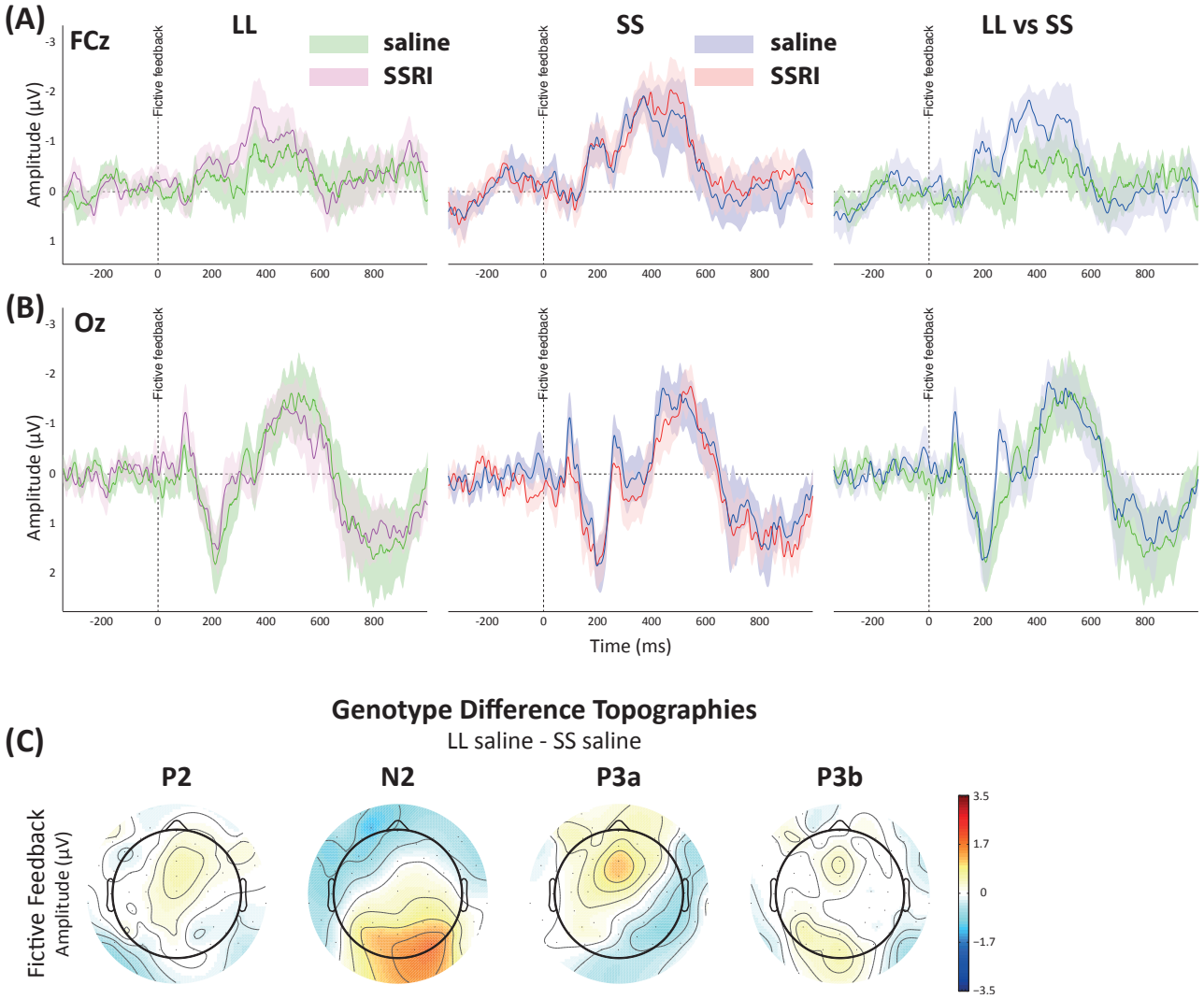


Figure 7-6. Difference wave analysis of ERPs for fictive feedback.
(A) At FCz, difference waves of fictive events for P3a switched signs reflecting mainly the surprise associated with rare events. (B) the fictive peak is clearly present in all drug and genotype groups, but not modulated by 5-HT. (C) Scalp topographies for genetic differences indicate a somewhat more negative (i.e., larger) P3a amplitude in SS subjects with a frontal topography. However, this difference did not reach significance.

Discussion

Before discussing the results of this chapter, it should be noted that this analysis has a highly exploratory character and due to the multitude of analyzed ERP components and behavioral variables, no straightforward correction for multiple comparisons can be implemented. Furthermore, thus far no studies have analyzed the modulation of the processing of real and fictive events by 5-HT and results are thus difficult to relate to other findings. Behaviorally, results of 5-HT manipulations on regular, real learning have revealed somewhat mixed results (Chamberlain et al., 2006; Cools et al., 2008; Guitart-Masip et al., 2012a; Ouden et al., 2013), which may be due to the proposed dual signal of 5-HT signaling (**Chapter 2**). Conclusions will therefore be drawn with utmost care and formulated as hypotheses that appear to be in accordance with what is known about the 5-HT system.

Overall, the analysis of 5-HT effects on learning from, and the processing of, real and fictive events yielded no differential behavioral modulation by pharmacological manipulation or genetic association. It was found that SSRI application increased RT independent of stimulus type and learning interval, or trial number bin, for subjects of the LL group alone, which however would not have survived correction for multiple comparisons of the factor stimulus type. As we neither observed such an effect in the trail-making-task nor the two other tasks that required speeded responding, and furthermore the VA scales did not indicate increases on sedation related items (**Chapter 3**), it is rather unlikely that this increase of RT reflected general sedatory effects in the LL group. Although independent overall from learning time per stimulus, the RT increase was especially pronounced during the first learning blocks and when the task had to be performed for the first time, suggesting presence of a ceiling effect. As we observed no effects on learning or choice performance of these subjects, one may speculate that SSRI administration impaired initial learning of the task, as would be the consequence of disrupting the glutamatergic part of the dual 5-HT signal (Liu et al., 2014) in that LL subjects after citalopram administration took longer time to match the performance of SS subjects or the unmedicated state. An even more pronounced RT increase (> ~200 ms) by a higher dose of oral citalopram (30 mg) has been reported in a reversal learning paradigm (Chamberlain et al., 2006) which was also accompanied by learning impairments. Subjects in our task performed many trials in two sessions and it is likely that even if such 5-HT effects exist, participants learned to overcome them after understanding the task structure. Note that this is not a failure of task design but rather a consequence of electrophysiological (or any neural) measures that require averaging across trials to identify neural signals within unrelated brain activity. Therefore, the absence of behavioral effects in this analysis clearly cannot be interpreted against the influences of serotonergic signaling on learning.

An additional caveat may be the drug dose used in the current experiment. While behavioral and electrophysiological pharmacological effects have been observed in the flanker and oddball paradigm, this was not the case here. As pointed out in **Chapter 2**, recent evidence suggests that 5-HT neurotransmission displays characteristics of a dual signal and reward guided learning appears to rely on the glutamatergic component of this signal. The fact that only higher acute SSRI doses disrupt raphe neuron firing (Hajós et al., 1995), which in turn is speculated to restrain the glutamatergic signal, suggests that the dose used here may not have been high enough to cause such a disruption. On the other hand it did cause significant peripheral cardiovascular effect which are clearly mediated via 5-HT itself. If

true, this would furthermore suggest that the effects observed in both the oddball (**Chapter 5**) and flanker paradigm (**Chapter 4**) rely more on the serotonergic part of the 5-HT dual signal.

The RT effect was present only in the LL group and appeared even reversed in the SS group which in turn depended on the current session. Such an effect of serotonergic drug administration order has been described and it was speculated that SSRIs interfere with the acquisition of a novel task but may even enhance performance of familiar tasks (Drueke et al., 2009). However, this unexpected 3-way interaction cannot easily be explained and additionally the small number of subjects per cell for these within session contrasts limit interpretability. The presence of a drug main effect, despite interaction effects, however, are well compatible with the finding reported of Chamberlain et al. (Chamberlain et al., 2006) especially when considering that the L allele is the more common one in Caucasian populations (Hu et al., 2006).

One genetic association study using the same task as Chamberlain et al. (2006), found increased switching behavior following losses for LL compared to LS or SS carriers. Although these results were statistically significant ($p = 0.003$) in a sample of more than 700 subjects, the reported effect sizes ($\eta^2 = 0.011$) would render it highly unlikely to replicate this finding in the current sample.

The analysis of the electrophysiological response to real and fictive outcomes depending on 5-HTTLPR genotype and administration of an SSRI revealed a generally increased frontal positivity in SS compared to LL subjects that lasted roughly from P2 until P3a. P3a was especially pronounced following real losses in SS subjects as was indicated by a trend for an interaction between *factuality, genotype*, and *outcome* and follow up contrasts. This effect furthermore was clearly reduced when analyzed in neutral stimuli with equal likelihoods of favorable and unfavorable outcomes suggesting that it was driven rather by the unexpectedness of the outcome than its subjective favorability. On the other hand, the absence of such an effect following fictive feedback, which was equally unexpected, would suggest that at least in part the actually experienced valence of the feedback influences possible serotonergic modulation of this ERP.

A similar P3a increase in SS subjects has been observed before in an auditory oddball paradigm (Heitland et al., 2013) and it may reflect increased attentional orienting to external cues for SS subjects which may indicate possible threat or the need for adjustment (Homberg and Lesch, 2011). Although it is known that 5-HTTLPR genotype affects brain morphology in human (Canli et al., 2005) and non-human primates (Jedema et al., 2010), the data here cannot easily be explained by morphological differences in brain structure between genetic groups. The further increase of the P2 and trends for difference wave P3a amplitude increases following SSRI administration in the SS group clearly suggest that these ERPs, or the longer lasting potential spanning P2 until P3a, are sensitive to actual serotonergic neurotransmission. Furthermore, a significant P3a increase by SSRI administration in the SS group was also seen in the analysis of the novelty Go/NoGo task (**Chapter 5**), although in that task no clear P3a increase when comparing groups was seen in the saline cognition and appeared only after SSRI application.

P2, or a similar potential, has been suggested to reflect a so called reward positivity (Holroyd et al., 2008), with the idea being that the FRN reflects a superposed N2 that is cancelled out by the reward positivity on trials with a positive outcome (Hewig et al., 2010). These data do not speak for such an interpretation. P2 itself was not sensitive towards the favorability of real outcomes, and was even increased on fictive unfavorable outcomes (which should not be

rewarding at all) and numerically also on real unfavorable outcomes. SSRI increased the P2 in the SS group in similar ways for favorable and unfavorable real outcomes and was also larger compared to the LL group both under saline and citalopram conditions. It appears unlikely that S allele carriers show higher reward-positivities, given reports of increased risk for depression (Caspi et al., 2003) and sensitivity towards errors (**Chapter 4**, Holmes et al., 2010). Thus, this potential here appears to rather reflect attentional capture, as suggested by the attentional capture theory of the P2 (or P2a) (Potts et al., 1996).

Like ERN amplitudes in the flanker task, FRN amplitudes as well as the fictive occipital ERP component sensitive towards fictive outcome favorability, were neither associated with genotypes studied here, nor sensitive to acute pharmacological manipulation of 5-HT levels. The effect seen on peak-to-peak FRN was driven by the preceding P2 and when FRN was quantified as the difference between favorable and unfavorable outcomes, no effect was seen. This was found despite very clear cut results of the involvement of these EEG components in the task (**Chapter 6**). Surely these negative findings cannot rule out serotonergic involvement in the generation of these ERPs, but these serotonergic effects are likely small – if present at all (Fallgatter et al., 2004; Althaus et al., 2009) (see also **Chapter 4**).

In sum, suggestive evidence is found that citalopram increases RT especially during the initial stage of learning stimulus values when speculatively no clearcut understanding of the task has been established. Neither real nor fictive learning was found to be modulated by serotonergic factors investigated in the current study. However, the studied sample may have been overtrained in the task which could impede detection of more subtle effects. Additionally, when it is assumed that acute SSRI administration disrupts reward signaling, the dose used in this study might have been too low to show clear effects. Still, SS subjects exhibited a pronounced frontal positive-going ERP shift especially following real losses which appears to mainly reflect detection of immediately relevant, affectively negative, unexpected outcomes.

CHAPTER

8

Summary and Discussion

Summary

This thesis was conducted with the goal to increase the understanding of the role of 5-HT in PM, a topic for which thus far many contradictory results have been reported. An essential assumption was that acute responses to SSRI challenges may interact with genotypic factors known to influence the expression of 5-HTTs, which are the target of SSRI. Such an interaction may help to explain the poor replication rate of studies with PM paradigms on the 5-HT system if indeed responses to SSRI depended on the genotype of the studied population. Additionally, the combination of acute manipulations of neurotransmission with genetic analyses offers the possibility to distinguish morphological effects of genes from acute shifts in neurotransmission, which is a major problem for genetic association studies. Therefore, an extreme group approach was employed and participants were pre-selected to be homozygous for three different genetic variants that are associated with high (LL) and low (SS) 5-HTT mRNA expression. This theoretically based genetic approach circumvents some of the problems of association studies by eliminating the possibility of post-hoc selection of genetic loci and offering a theoretical background to interprete findings with regard to 5-HT levels.

Chapter 1 provides an update of recent theories of PM and their neurobiological underpinnings which are reviewed in detail. The method of model based analysis of single-trial EEG data is discussed which was applied for the analyses presented in **Chapters 4** and **6**. The scope of this part includes findings of other monoaminergic systems, especially DA, which appear to share many features with the serotonergic system. However, especially in the case of DA, these are both empirically and theoretically far better understood in their functioning and which could serve as a paradigm for future 5-HT studies.

Following a broad introduction of the brain´s serotonergic system and essential properties of 5-HT, effects of genetic variation in functional genes that control 5-HTT mRNA expression and the interaction of these polymorphisms with other perturbations of 5-HT neurotransmission are discussed in **Chapter 2**. Furthermore, recent evidence is reviewed that suggests co-release of glutamate by raphe neurons which thereby constitutes a dual signal. From this, a novel hypothesis is deducted which appears to explain the so far puzzling differences of acute versus long-term SSRI administration both in neuroscientific research and clinical treatment. This is based on recent findings that both components of the dual signal serve different functions, namely reward based learning and hedonic reward signaling (glutamate) and, e.g., waiting for delayed rewards or sustained motivation (5-HT). It is argued that these components are likely to be affected differentially by acute SSRI administration which should reduce the glutamatergic but enhance the serotonergic component of the dual signal. Following desensitization of 5-HT autoreceptors, however, the dual signal should be restored while 5-HT levels remain elevated and in fact this appears to be the actual time when SSRI treatment shows remedial effects in depressed patients.

Thereafter, the design of the study presented here, the sample, and a careful analysis of possible side effects induced by drug administration is reported in **Chapter 3**. This analysis confirmed that both genetic groups were well matched in their demographic characteristics. Furthermore, trends in differences regarding neuroticism scores were found that are compatible with previous studies. A drug effect was found on blood pressure, which, consistent with the physiological characteristics of 5-HT, showed a mild but robust increase after SSRI infusion over the course of the

session. No detrimental drug effects were seen on visuomotor-coordination and neither were measures of state anxiety altered, nor self-reports indicative of sedatory effects were found. Therefore, it is concluded that analyses of the pharmacological challenge as well as genetic group comparisons can be performed reliably as both groups are well matched and effectivity of the pharmacological challenge is confirmed but no severe side-effects are seen that could lead to a general reduction of attention in the drug condition.

Chapter 4 tackles the question of the specificity of serotonergic action with regard to its suggested role in linking an overactive PM system to depression. Therefore, a flanker paradigm that allows to measure the ERP components of error processing as well as behavioral effects introduced by erroneous responses was employed. A critical hypothesis was that affectively negative events, such as errors, but less so relatively valence free events, such as more difficult trials, should be more sensitive to factors that affect serotonergic neurotransmission. We found that following both difficult and erroneous trials, RT was reliably increased. However, this RT increase was only modulated by genetic background and pharmacological manipulation when its cause was the commitment of an error. Presumably increased extracellular 5-HT concentrations due to genetic factors in group SS and acute SSRI administration increased post-error slowing. ERN and Pe as electrophysiological markers of error processing, on the other hand, were not significantly modulated by genetic and pharmacological factors investigated in this study. This null effect is in accordance with the majority of other studies and thus far no replicable effects of serotonergic factors, be it genetically and pharmacologically, on ERP indices of error processing exist. Furthermore, the current study extends the scope of previous studies by demonstrating single-trial relationships between ERN and Pe amplitudes and post-error slowing which were significantly modulated by SSRI application in the LL group only. LL subjects demonstrated a decoupling between ERN and behavioral adaptation under citalopram. Thus, it is concluded that, due to the absence of cortical electrophysiological effects but the presence of behavioral alterations, a mechanism that mediates this slowing effect is likely to be found in subcortical regions. Especially the subthalamic nucleus (STN) appears to be a candidate structure as it receives strong serotonergic projections which control neuron firing rates and furthermore the STN has been found to mediate post-error slowing.

In **Chapter 5** the results of a novelty Go/NoGo paradigm are reported which asseses inhibitory control and the orienting response to unexpected events and their electrophysiological correlates. The behavioral analysis is focussed on the cost of inhibition of the previous trial which delays initiation of the default response even when this response is always the same. Here, differential drug effects on the ERP correlates of inhibitory processes (N2 and P3b) and RT slowing induced by inhibition are seen. Only the LL group showed a facilitation of responding following unexpected inhibitory events by SSRI administration which was corroborated by significant decreases of N2 and P3b by the drug restricted to that genetic group. Subjects with the SS genotype showed increased P3a amplitudes following unexpected events which may be related to an increased startle responses. This seems related to increased side effects when depression is treated in patients with this genetic background, in part due to increased agitation. In accordance with the hypothesis that acute drug effects may depend on the genetic background, these findings are able to explain why so far equivocal results have been found in studies of inhibitory control that employed pharmacological or dietary 5-HT manipulations and did not control for the genetic background. Furthermore, in the light of a known association of the 5-HTTLPR L allele with ADHD and reports of interaction of methylphenidate

responsiveness in ADHD dependent on the L allele, these data suggest interactions between noradrenergic and serotonergic factors in the etiology and possibly treatment of ADHD.

In **Chapter 6** a novel task that allows to disentangle learning from real and fictive outcomes is introduced and analyzed in depth employing computational modeling of the behavioral data and single-trial multiple robust regression analysis. Therefore, a reinforcement learning algorithm was used that down-weighted the impact of events after learning of a stimulus' value. From this, estimates of real and fictive PEs and single-trial learning rates were derived and regressed onto the EEG signal to reveal the exact cortical time-course of outcome processing for real and fictive feedbacks. It was found that while subjects are behaviorally equally well able to learn from both outcome types, their neural processing displays a spatio-temporal double dissociation in early time-windows. The FRN was modulated by real but insensitive to fictive outcomes whereas an early occipital correlate was sensitive to fictive PE signals only. However, a common pathway was apparent in parietal EEG amplitudes which predicted future choice behavior on a trial-to-trial basis. Furthermore, during the decision phase, it was found that parietal EEG amplitudes in the P3 time-window increased with subjective decision certainty, potentially shielding the value against later perturbations. This observation also fits well to the proposal of the P3 to reflect a decision signal as in the face of unambiguous decisions P3 was increased.

Despite being generally informative towards the question whether differences in processing real and fictive events are manifest in the brain, a further motivation in introducing this novel task was that regret may be an important factor in the etiology of depressive symptoms (Brassen et al., 2012). Given the prominent role of 5-HT in depression, one might speculate serotonergic influences on the processing of fictive events that reflect what could have happened if one had made different decisions which could be related to subjective regret. However, the results of the analysis of serotonergic variables on this processing of both real and fictive outcomes and learning from it, described in **Chapter 7,** did not show a consistent pattern. It was found that especially when the task was not well trained, L allele carriers showed increased RTs following citalopram administration, but no clear effect was seen on learning from real or fictive feedback. Given the dual signal theory introduced in **Chapter 2,** which puts special emphasis to the importance of the glutamatergic component for learning, it is concluded that a possibility for these null results could be the relatively low drug dose used in the study at hand. Such a low dose may not be sufficient to reduce 5-HT neuron firing rates to a level that disrupts the glutamatergic component of the dual signal. EEG results showed that the SS group displayed an increased frontal positivity spanning the P2 until the P3a time-window. P2 following real feedback was further increased in the SS group in the citalopram condition, but all of these effects were absent when the unexpectedness of the event was controlled for and analysis was confined to feedback following neutral stimuli. This suggests that a positive-going ERP deflection not confined to one single ERP is sensitive to 5-HT levels and increases with them.

Discussion

5-HT and Aversive Events

Major theories regarding the role of 5-HT suggest special importance in mediating behavioral inhibition in the context of expected punishment (Crockett et al., 2009; Cools et al., 2010). In accordance with this view, we found combined genetic and pharmacological evidence that higher 5-HT levels are associated with increased behavioral inhibition following errors. PES was higher in subjects homozygous for the S allele, for which it is assumed that lower 5-HTT activity leads to slower 5-HT reuptake and consecutively increased 5-HT levels. A previous study found that ATD reduced punishment induced slowing on trial-to-trial basis (Crockett et al., 2012), and here SSRI administration increased PES. Furthermore, slowing following more difficult trials, which served as a control condition, showed no genetic or pharmacological effects. Thus, there seems converging evidence that higher 5-HT levels increase motor-inhibition both in the face of expected punishment and following response errors.

A previous study reported that ATD abolished a bias against responding with a previously punished button (Crockett et al., 2012), thus abolishing a Pavlovian bias against punished actions. In the learning task, we found no clear behavioral effect, neither in the time to choose or avoid a stimulus nor in the speed of learning. One could speculate that a Pavlovian effect could not arise in the current study design, as it was the case that only real feedback could lead to actual punishment, but when subjects learned the task, choosing in sum led to more positive than negative events, as was the case for avoiding to chose when subjective favorability is taken into account. Thus, a Pavlovian explanation is not applicable for the task used here as both responses resulted in more favorable than unfavorable outcomes. Thus, results obtained here are not directly comparable to the previous studies with that regard. It could be possible that instrumental learning is less sensitive to serotonergic modulation than Pavlovian biases (Crockett et al., 2012; Geurts et al., 2013). When related to the proposed dual signal theory (**Chapter 2**), it appears likely that this effect is mediated by the serotonergic component of the dual signal as ATD and SSRI application show opposing effects and the optogenetic data suggests a role for the glutamatergic component especially in appetitive, hedonic aspects of behavior (Liu et al., 2014). However, a direct investigation of effects of dual serotonergic signals with regards to affectively mediated inhibition has not been conducted so far but would be highly desirable.

Another study that employed acute SSRI administration found reduced learning speed, impaired reversal learning, and prolonged RTs following drug administration (Chamberlain et al., 2006). Such an effect was not present in the data presented in **Chapter 7**, and it could be speculated that this can be explained by the rather low drug dose used here which may not interfere with the proposed glutamatergic component of the dual serotonergic signal (**Chapter 2**) (Mestikawy et al., 2011; Liu et al., 2014). Furthermore, subjects in our task were well informed about the task, completed a test session, repeated the task in two sessions, and had to perform many trials in order to enable analysis of EEG signals. In the mentioned study, subjects only performed two learning blocks and a parallel group design was employed (Chamberlain et al., 2006). One may speculate that the importance of midbrain monoaminergic systems for learning (Schultz et al., 1997; Pessiglione et al., 2006; Liu et al., 2014) lies in the first establishment of stimulus-outcome associations but can be overcome following exhaustive learning of the task structure.

ERP correlates of response errors, namely ERN and Pe, had previously been associated with 5-HTTLPR genotype (Fallgatter et al., 2004), while the FRN has not been investigated with regard to genetic variants controlling 5-HTT expression. In previous studies, increases in ERN and trends for increases of Pe amplitudes were found in S allele carriers, which led to the suggestion of an overactive PM system that may link differences in PM functions to an increased risk for depression given multiple negative life events (Caspi et al., 2003; Fallgatter et al., 2004; Karg et al., 2011). Furthermore, this finding was replicated in a sample of 10 year old children employing a feedback based learning task, which however elicited only an ERN and no FRN and thus appears difficult to interpret (Althaus et al., 2009). Another study found increased BOLD responses for S allele carriers (Holmes et al., 2010) following response errors in a region in aMCC, compatible with the suggested neurogenerators of the ERN (Dehaene et al., 1994). On the other hand, previous pharmacological studies of these ERPs did not reveal significant effects of serotonergic agents (de Bruijn et al., 2006; Barnes et al., 2013) and an attempt to replicate the findings of Fallgatter et al. (2004) failed in a larger sample (Olvet and Hajcak, 2008). Both flanker (**Chapter 4**) and learning tasks (**Chapter 7**) analyzed in this thesis elicited reliable ERP components that were sensitive to response and feedback errors, respectively. However, none of these error-related ERPs was modulated by serotonergic variables investigated in the study. The ERN was completely insensitive to citalopram administration in both genetic groups and the same was the case for the FRN when it was analyzed in a difference wave approach, which appeared more suited here given genetic and pharmacological effects for surrounding potentials. Furthermore, we could not replicate the ERN effects reported in previous studies despite controlling for additional variance in 5-HTTLPR genotypes by means of genotyping SNPs 25531 and 25532 which should help to separate low- und high-expressing genotypes more efficiently. Thus, the currently available data suggests independence of ERN, Pe, and FRN of genes that influence 5-HTT expression and pharmacological agents that target serotonergic neurotransmission.

Attention and Inhibition

A recent proposal for a unifying function of 5-HT is that it might mediate a shift between habitual stimulus-bound and goal-directed behavior by controlling vigilance (Homberg, 2012). In this view, higher 5-HT levels both in 5-HTTLPR S genotypes and following SSRI administration should thus be associated with increased attention towards external and internal stimuli and improved behavioral inhibition. This interpretation is quite well compatible with the behavioral findings reported in the Go/NoGo task and electrophysiological findings of the learning task and the Go/NoGo task when it is assumed that an ideal intermediate level of serotonergic tone follows the characteristics of an inverted U-shape (**Figure 8-1**). Such a relationship has been suggested by computational modeling as a consistent pattern of serotonergic effects across different cognitive functions (Cano-Colino et al., 2014). Furthermore, general serotonergic tone determined by genotypical features, for example 5-HTTLPR L genotype, may be beneficial when low for some, but detrimental for other functions (Homberg and Lesch, 2011).

First, we found that LL allele carriers, with presumably low extracellular 5-HT levels, tend to show an increased cost of inhibitory control by increased delays on responses, although trial structure predicts Go responses in 100%, such that optimal responses should be given at maximal speed especially after an inhibitory event. This effect could be

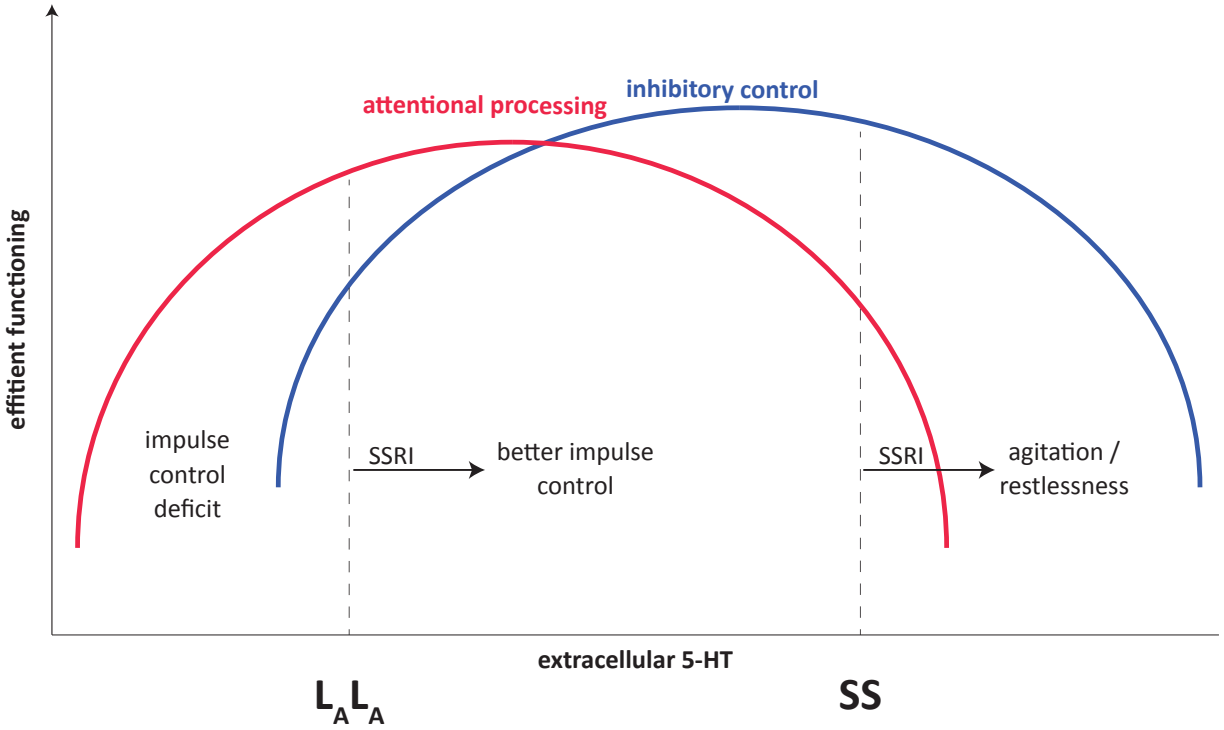


Figure 8-1. Possible link between genetically determined 5-HT levels, SSRI effects and efficiency of functioning. If an inverted U-shape function is assumed when relating 5-HT levels (right y-axis, dashed black line) to efficiency of functioning (left y-axis, red and blue solid lines for attentional processing and inhibitory control, respectively), one could derive predictions of the effects SSRI exert. The LL group is supposed to show lower extracellular 5-HT levels which could render them more susceptible for impulse control deficits (blue curve), such as those seen in ADHD (Curran et al., 2005). Increasing 5-HT levels by means of SSRI could be beneficial for this genotype, as indicated by the results found in **Chapter 5**, whereas it has no effect for the SS genotype which remains in an almost optimal level of 5-HT. On the other hand, attentional processing may show a different relationship between 5-HT levels and optimal functioning. Here, SSRI could lead to super-optimal levels in the SS group (red curve, right side), causing side-effects such as restlessness or agitation (Murphy et al., 2003), whereas no effect is seen for the LL genotype. Such an explanation is compatible with the increased incidence of such adverse events in the SS genotype and the electrophysiological effects of increased frontal positivities seen in the Go/NoGo and learning task (**Chapters 5** and **7**).

counteracted by increasing 5-HT levels via SSRI application for the LL group whereas the SS group did not benefit from this intervention, possibly because they already are closer to an optimal serotonergic tone for inhibitor control. Such an observation fits well to results of genetic association studies that reported phenotypical features commonly found in ADHD to be associated with higher 5-HTT gene expression (Curran et al., 2005) and would put the LL allele carriers at the lower bound of optimal 5-HT levels which could be more likely to be affected by impulse control disorders. Further support for this idea comes from the electrophysiological data. Firstly, we found that in the saline condition, LL allele carriers displayed increased inhibitory P3b amplitudes on NoGo trials compared to SS allele carriers (**Chapter 4**). Secondly, we found that SSRI application had an effect well compatible with more efficient inhibition, namely it significantly reduced P3b amplitudes of LL allele carriers to an amplitude indifferent from that seen in the SS group and furthermore it reduced the amplitude of the inhibitory N2 as well. Importantly, this effect appeared quite specific for inhibitory events. When a P3b with similar scalp topography and latency (**Chapter 7**) was elicited by feedback events that lead to updating of stimulus values, no such genetic difference or pharmacological effect was

seen. This rules out an explanation based on morphological factors (Jedema et al., 2010), as otherwise one would expect to see the genetic effect present in all instances of P3b amplitudes measured in the same subjects. Additionally, this suggests that P3b amplitudes here are in fact modulated by the actual serotonergic tone, with higher levels associated with lower P3b amplitudes and more efficient behavioral control. Furthermore, seemingly the same ERP, P3b like phenomena may be caused by different neural processes (Nieuwenhuis et al., 2005; Polich, 2007; O'Connell et al., 2012; Ullsperger et al., 2014b) or at least its sensitivity to changes in neuromodulatory levels is context specific. In a similar vein, it has been suggested that N2, ERN, and FRN are actually the same phenomenon elicited under different circumstances (Holroyd et al., 2008). However, we found a modulation of the inhibition-related N2 by SSRI (**Chapter 4**) while FRN and especially the ERN were completely insensitive to the pharmacological manipulation (compare time-courses SSRI vs placebo in **Chapter 4** which demonstrate almost identical ERPs). While certainly this does not rule out the proposed identity of these ERP phenomena, it suggests differential sensitivity towards neuromodulatory systems and appears to be better compatible with accounts that consider these ERPs (or especially the N2) to be separable entities (Folstein and Van Petten, 2007).

In sum, these data suggest an inhibitory deficit in LL subjects compared to SS subjects that is manifest in increased EEG correlates during inhibition of a prepotent response and behaviorally as a trend for delayed re-initiation of the default response. Increasing 5-HT levels, which should render LL subjects more similar to drug naïve SS subjects, abolishes genetic differences on electrophysiological and behavioral levels. Thus, higher 5-HT levels appear associated with more optimal inhibitory control as it enables efficient control of responding as no group difference was seen for the number of failed inhibitions, but flexible and rapid re-initiation of another response.

For P3a amplitudes as an electrophysiological correlate of attentive processes, we found a context sensitive influence of 5-HT. Consistent with another study that found increased P3a amplitudes in S allele carriers in an auditory oddball paradigm (Heitland et al., 2013), which was interpreted as evidence towards increased vigilance in these subjects (Homberg and Lesch, 2011), we found a difference in P3a amplitudes between SS and LL subjects in the real and fictive learning task (**Chapter 7**) that was especially pronounced following real losses. When controlling for the unexpectedness of these events, the effect was abolished, suggesting that this genetic effect was dependent mainly on a surprise driven modulation and less the valence aspect that we demonstrated to account for variation in P3a amplitudes as well in **Chapter 6**. In the novelty Go/NoGo paradigm (**Chapter 5**), we found that furthermore P3a amplitude is sensitive towards SSRI application and increased thereafter for subjects of the SS group. Similarly, P3a difference wave analysis of the feedback related P3a (**Chapter 7**) showed the same trend for an increase in group SS in the citalopram session. Although the genetic factor was significant in the Go/NoGo paradigm only in the drug and not naïve comparison, these results suggest that P3a amplitudes are affected by genetic and pharmacological factors that influence acute 5-HT neurotransmission. Higher 5-HT levels appear to be associated with increased P3a amplitudes possibly reflecting increased vigilance. The increase seen in group SS, but not LL, may be related to the increased susceptibility of subjects that carry an S allele to side-effects associated with SSRI treatments which may push extracellular 5-HT levels to supra-optimal levels resulting in an increased likelihood of experiencing restlessness or agitation. VAS also showed a trend towards a reduction of calmness, which was numerically larger in the SS group,

although we did not observe an effect on subjective alertness. This may be due to electrophysiological measurements being more sensitive than self report scales.

Interestingly, in the learning task, the increase of frontal positivities in group SS and was not restricted to a classical ERP, but spanned the range from P2 to P3a including the N2 component. Furthermore, the SSRI effect in group SS also appeared to be similar for P2 and P3a, although it was significant only for the P2. However, this could suggests that P2 and P3a with the N2 or FRN framed by both positivities, reside on a baseline that is to a certain degree sensitive to common serotonergic modulatory input. Interestingly, this baseline was also found to be modulated in the analysis of the task in **Chapter 6** where it positively covaried with the learning rate – which is closely related to the relevance attributed to an event. The degree of neural representation of this learning rate also correlated with task performance across subjects. However, this notion is limited by the absence of behavioral effects for the learning rate when directly tested for serotonergic influences.

Outlook and Future Perspectives

In sum, it appears that 5-HT and the effects of agents interfering with the serotonergic system itself, display complex effects dependent on the function studied. For inhibitory control, we found interactions between SSRI effects and genotype which can explain difficulties of previous studies. This highlights the importance of pharmacogenetic studies both in cognitive neuroscience and clinical research alike. These results further suggest that developing more targeted therapies should be a promising research area. For example treating ADHD patients with the L allele with SSRI, which in our task led to more effective inhibitory control, might be a useful (co-)treatment for some patients. Vice versa, evidence for less efficacy and possibly increased side-effects for SSRI in the treatment of depression in S allele carriers is accumulated and corroborated by increases of attention related brain responses in the current study, which appear to reflect heightened vigilance. However, the implications of pharmacogenetic effects also raise great challenges. If certain drugs interact with genes, it appears incredibly difficult to define the interactions more precisely given the vast diversity of possibly interacting genes (in promotor regions of monoamine transporters or structural regions of receptors to name just a few). Nonetheless, at least in part, these findings demonstrate the usefulness of using a combined pharmacogenetic approach in the study of monoaminergic functions. This approach is by no means limited to the serotonergic system and should be applied in the study of other neuromodulators, for example by controlling polymorphisms in the dopamine receptor D2 gene such as Taq1A in studies targeting D2 receptors, such as when using for example D2 receptor antagonists.

Another approach to reduce the complexity of expected effects of a pharmacological challenge could be to target receptor subtypes directly instead of manipulating serotonin levels. More precisely, for future studies it remains an open question if agents that target specific 5-HT receptor subtypes, such as mCPP for 5-HT_{2C} receptors (Anderson et al., 2002), e.g., could unveil additional serotonergic influences on PM functions. For example in the rat model, it has been shown that 5-HT activation of 5-HT_{2A} receptors increased, but activation of 5-HT_{2C} receptors decreased impulsive choice (Winstanley et al., 2004; Homberg, 2012). Therefore, future studies should carefully outweigh the possibly increased specificity of using receptor agonists against the increased physiological validity of manipulating 5-HT levels

in general with methods like ATD, SSRI application or Trp loading. In this regard, appreciation of the well established co-release of other neurotransmitters, especially glutamate (**Chapter 2**), could be a valuable new line of research (for further research questions arising from the proposed dual serotonergic signaling of raphe neurons, please refer to the Box *Outstanding Questions* in **Chapter 2**).

Unless a precise, formalized, unifying theory for serotonin´s essential role in the homeostasis of an organism emerges, it will be difficult to derive hypotheses regarding certain PM functions modulated by 5-HT (Cools et al., 2010; Dayan, 2012). However, the results of the current investigation appear mostly compatible with 5-HT effects that are mediated via vigilance (Homberg, 2012). However, the diversity of the serotonergic system and its involvement in such a wide variety of functions still poses a great challenge for computational models of 5-HT functions, but also empirical and clinical research alike. Paying closer attention to the differences in the applied methods across species, for example 5,7-DHT application in animal research which severely impairs the 5-HT system and likely both components of the dual serotonergic signal, SSRI which likely acutely dissect the dual signal, or ATD which possibly reduces only the serotonergic component of the dual signal, could help to better translate results between methods and species. Evidently, the difficulties in reproducing results of serotonergic studies may have led researchers to shy away from investing resources into laborious neurophysiological or -imaging studies in combination with pharmacological or genetic analyses. However, as more evidence for the role of 5-HT in the interplay of inhibition especially in the context of aversive events, and possibly the role in reward learning, mediated by glutamate, emerges, this may resolve and open promising new avenues for scientific research.

GLOSSARY

5-HT transporter (5-HTT): the serotonergic signal at the synapse is primarily terminated by the re-uptake of 5-HT into the presynaptic neuron mediated by this protein. From there, 5-HT is either catabolized (preferentially via monoamine oxidase type A) or translocated back into storage vesicles by VMAT2 (Hensler, 2006).

5,7-dihydroxytryptamine (5,7-DHT): the neurotoxin 5,7-DHT can be used to selectively destroy serotonergic raphe neurons in animal studies. This complete obliteration unselectively impairs both components of the dual signal. Thus, animal studies on cognitive impairments following the use of this technique can be used to infer the functional significance of the intact serotonergic dual signal.

Acute tryptophan depletion (ATD): a method to temporarily lower serotonin levels in the brain that is widely used in cognitive neuroscience and can be applied in human and animal studies alike. The amino acid tryptophan (Trp) is essential for the synthesis of 5-HT and by ingestion of a solution that lacks Trp but contains other amino acids with similar properties, the transport of Trp through the blood brain barrier is reduced. This consequently leads to lowered brain 5-HT levels on a time scale of approximately one to six hours (Fadda et al., 2000). The expected increase in raphe neuron firing rates due to less extracellular 5-HT has not yet been confirmed (Trulson, 1985) and thus ATD may decrease the serotonergic component of the dual signal but appears to leave the glutamatergic component unaffected. However, the specificity of this technique is not undisputed (van Donkelaar et al., 2011).

Autoreceptor: a receptor sensitive to the transmitter released by the same neuron it is found on. Autoreceptors in turn reduce the release of this transmitter which constitutes a negative feedback loop.

Error positivity (Pe): error-related positive ERP deflections following the ERN in reaction time tasks. The sharp frontocentral early Pe occurs immediately after the ERN and appears to be related to the P3a. The more sustained centroparietal late Pe occurs about 200-400ms after the error. It seems related to the P3b and is modulated by conscious error perception (Wessel et al., 2011).

Error-related negativity (ERN): large frontocentral negative ERP deflection peaking 50-100ms after errors when stimulus-response mappings are known (Ullsperger et al., 2014a). The ERNs is independent of stimulus and effector modality but modulated by subjective error significance. Trial-by-trial variations of ERN and early Pe predict post-error slowing of reactions (Debener et al., 2005).

Event-related potential (ERP): EEG amplitude changes time-locked to stimulus events that are compared to a baseline. To increase signal-to-noise ratio, ERPs are averaged over multiple repetitions of the event.

Feedback-related negativity (FRN): frontocentral negative ERP deflection peaking 200-300ms after sensory feedback disambiguating the action outcome (Walsh and Anderson, 2012; Ullsperger et al., 2014a). Modulated by RPE and surprise, with different weighting of the two factors across tasks.

Heteroceptor: a receptor sensitive to a transmitter released by another neuron. Hetero- and autoreceptors can be structurally identical and differ only with regard to their location.

Learning rate: in reinforcement learning, the amount of updating the RPE on the expected reward exerts is scaled by a weighting factor termed learning rate (Ouden et al., 2012). It is likely not stationary but depends on many factors like the volatility of the environment and the reliability of feedback.

Microdialysis: a method to estimate the extracellular concentrations of neurotransmitters. A probe with a membrane permeable to low-molecular weight substances is implanted into a target region which is perfused ("dialysed") with a physiological solution. Because of the concentration gradient, neurotransmitters like 5-HT will diffuse into the probe and proportionally reflect concentrations in the extracellular space. Neurotransmitter concentrations in the dialysate can then be measured by liquid chromatography combined with electrochemical detection for transmitters like serotonin or dopamine.

Model-based analyses: a computational model reflecting a theory of a brain function is used to predict parametric variations of data reflecting neuronal activity. The model is usually fitted to behavioral data and reveals latent variables varying dynamically over time. Covariations with neuronal data may reflect representations of these (or covarying) variables in the brain and are interpreted as support for the theoretical model (Mars et al., 2012). Goodness of data fit allows comparing different models.

N2 (or N200): Frontocentral negative ERP deflection peaking around 200-300ms after stimulus presentation. Modulated by response conflict, task engagement, and surprise (Folstein and Van Petten, 2007; Eichele et al., 2010; Wessel et al., 2012).

Optogenetics: a technique in which a gene encoding a light sensitive ion channel is introduced into specific neuronal populations and consecutively stimulated with light of a certain spectrum. Light stimulation can be used either to excite or inhibit a neuron depending on the ion channel expressed. In the case of excitation, activation will correspond to the frequency of optical stimulation (Boyden et al., 2005; Zhang et al., 2007). Neuronal specificity can be obtained by introducing a promoter that is activated only in certain neuronal populations, e.g. tryptophan hydroxylase 2 which is almost exclusively expressed in 5-HT neurons. When either combined with pharmacological inhibition of serotonergic or glutamatergic receptors or experimental animals that lack one of these receptor types, the dual signal can be dissected into both components (Liu et al., 2014).

P3 (or P300): refers to a family of positive ERP deflections elicited by (potentially) action-related stimuli. The earlier, sharp frontocentral P3a is associated with fast orienting and attentional processes; the later, more sustained parietal P3b with updating in memory and behavioral inhibition (Polich, 2007). P3a sources have been localized to frontal cortex including pMFC. P3b sources appear widely distributed across cortex, particularly parietal and temporal areas.

Polymorphism: A gene sequence variant that is found in more than 1% of the population.

Prediction error (PE): Difference between prediction and outcome (Holroyd and Coles, 2002; Ouden et al., 2012). In Rescorla-Wagner-type reinforcement learning the signed reward PE (RPE) varies along its valence axis from better than expected (reward, positive RPE) to worse than expected (punishment, negative RPE). Unsigned PEs, closely related to surprise, are implicated in sensory predictive coding and Pierce-Hall learning (Ouden et al., 2012; Roesch et al., 2012).

Raphe nuclei: a midbrain region classically subdivided into a dorsal and median part, where about half a million serotonergic cells reside. These form the vast majority of all serotonergic cells and project to almost the entire forebrain (Hornung, 2003).

Selective Serotonin Reuptake Inhibitor (SSRI): a class of drugs with high affinity for blocking the 5-HTT. This leads to increased extracellular serotonin levels and its long term administration is the primary drug treatment in different diseases such as depression and obsessive compulsive disorder.

Serotonin Receptors: More than 14 receptor subtypes are currently classified based on different characteristics such as drug response, intracellular signaling, and structural properties which is still in ongoing development (Hoyer et al., 1994). Receptors are mainly grouped into seven classes. Out of these, the $5\text{-HT}_{1,2,4,6,7}$ are G-protein coupled receptors and the 5-HT_3 represents a separate class as it is the only receptor known to be a ligand-gated ion channel with mainly depolarizing properties (Hensler, 2006). The distribution of receptor subtypes differs between brain regions, and some receptors function both as auto- and heteroceptors (Celada et al., 2013).

Surprise: The unexpectedness of an event independent of its valence can be formulated as the absolute amplitude of the PE and termed surprise. More general measures of surprise for any kind of event can be derived from Information Theory (Barceló et al., 2007; Mars et al., 2008).

Variable number tandem repeat (VNTR): Locations in a genome were a short sequence of nucleotides is repeated adjacent to each other. The length of such a repeat is inherited and is termed *functional* when it influences gene expression.

Vesicular glutamate transporter 3 (VGLUT3): a transport protein that translocates glutamate from cytosol into presynaptic storage vesicles. It is mainly located in neurons that do not primarily release glutamate (Mestikawy et al., 2011).

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Declaration of Originality / Eigenständigkeitserklärung

I herewith declare that I am aware of the docotral degree regulations of the Otto-von-Guericke Universitiy Magdeburg. Furthermore, that this work entitled *Serotonergic Modulation of Action Monitoring and Cognitive Control* has been written and conducted by myself and that all means used are explicitly mentioned and all sources thoroughly cited. Additionally, this work has neither by myself nor someone else been used to attain any academic degree.

Hiermit erkläre ich, dass mir die Promotionsordnung der Otto-von-Guericke Universität Magdeburg bekannt ist. Weiterhin, dass die vorliegende Arbeit *Serotonergic Modulation of Action Monitoring and Cognitive Control* von mir selbständig verfasst wurde und alle verwendeten Hilfsmittel benannt, so wie alle Quellen vollständig zitiert sind. Ebenfalls wurde diese Arbeit weder von mir noch einer anderen Person zur Erlangung eines akademischen Grades an anderer Stelle eingereicht.

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2010 - Exchange scholarship for the Harvard Medical School from the German National Academic Foundation

2004-2010 - Full stipend of the German National Academic Foundation

2003 - Award for the best Abitur (German general qualification for university entrance) of the year

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International Journal articles

Fischer, A. G., Jocham, G. & Ullsperger, M. *Dual Serotonergic Signals: A Key to Paradoxical Effects?*. **Trends in Cognitive Sciences** 19, 21-26 (2015).

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