From the Cell to the Brain –Fear and Anxiety across the Levels of Neuroscience

Cumulus zur

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

(Dr. rer. nat.)

dem Fachbereich Psychologie

der Philipps-Universität Marburg

vorgelegt von

Erik M. Mueller

aus Offenbach am Main

Marburg, 2011

When you suffer an attack of nerves you're being attacked by the nervous system.

What chance has a man got against a system?

~Russell Hoban

Acknowledgements

I would like to express my gratitude to a number of people who have provided invaluable support throughout the course of my research and without whom I would not have been able to compose this thesis.

First of all, my „Doktorvater“ Jan Wacker, for being a terrific mentor in all respects. The degrees of freedom you provided on the one hand and the support you gave whenever I asked for it on the other lead to a really pleasant and productive work atmosphere. Gerhard Stemmler, my „Doktorgrossvater“, for being a highly inspirational work group leader and for providing a lot of substantial support for the herein presented studies (including the laboratory and the scientific expertise on psychophysiological measurements).

I would like to express particular thanks to Prof. Dr. Nater (Biological Cinical Psychology, Marburg University) who agreed to evaluate this thesis together with Jan Wacker. I am thankful that you take this time although you certainly have a plethora of far ranging other duties and commitments. In this context I further like to thank Prof. Dr. Gollwitzer (Statistical Methods, Marburg University) and Prof. Dr. Schubö (Cognitive Psychophysiology, Marburg University) for taking their time to be part of my dissertation committee.

The work presented herein would not have been possible without a variety of collaborators and supervisors overseas. First of all I would like to thank Tom Borkovec (Pennsylvania State University), a pioneer in the field of experimental research on Generalized Anxiety Disorder, for helping with Study 3 but even more for his very authentic expression of respect for each and everyone that keeps inspiring me today. Without the help of Stefan Hofmann, who is an expert on Social Phobia at Boston University, and the trust he put into me, neither my diploma thesis nor Study 4 would have been feasible and I am very looking forward to future collaborations. Thank you very much, Stefan! I further have to express my gratitude to Scott Makeig (University of California, San Diego) who is among the leading experts in computational EEG research, and who kindly invited me to visit his laboratory for the spring and

summer of 2010 where the foundations for Study 2 were laid. The work atmosphere you provided was so inspirational that I ended up being in San Diego for several months and only went surfing once! In this context, I obviously have to thank the Society for Psychophysiological Research for funding my stay at Scott's laboratory with the 2009 SPR Research Fellowship Award. With regard to collaborators and helpers from abroad I would also like to mention Arnaud Delorme and Tim Mullen (UCSD), William Ray and Jennifer Nguyen (PSU), James Cherry (BU), Diane Santesso (Brock University), and Diego Pizzagalli (Harvard University) who played a substantial role in the studies I have conducted on my way to the PhD.

When I started my work as a PhD student my office mate Mira Chavanon welcomed me with a small plant. Although I occasionally forget to water it, this plant is still alive and reminds me of the beginnings. Throughout all the time Mira provided terrific support with whatever questions there were. Particularly at the end she had a close look at this thesis and helped me with some great remarks. Leslie, you did the same and proof-read the thesis as a native speaker, which was a great help! Sebastian and Frank: thank you so much for also having a closer look at the thesis and pointing out some important aspects.

I wish to thank my friends and my family for providing the social support that made my PhD years a time with very precious memories. My father, my mother and my brother, my very close friends Annika, Sebastian, Stefan, Eva and her son Leo. Thank you for being there! Last but not least and most importantly of all I thank you, Verena, for being so encouraging when it comes to my work and for many more things that are far beyond the scope of this thesis...

Content

List of Abbreviations

AMC	**Anterior Midcingulate Cortex**
BIS	**Behavioral Inhibition System**
BOLD	**Blood Oxygen Level Dependent**
cAMP	**Cyclic Adenosine Monophosphate**
CECT	**Cardio-Electroencephalographic Covariance-Trace**
CNS	**Central Nervous System**
CR	**Conditioned Response**
CREB	**cAMP-Responsive Element Binding Protein**
CS	**Conditioned Stimulus**
COMT	**Catechol-O-Methyltransferase**
DA	**Dopamine**
DSM – IV	**Diagnostic Statistical Manual of Mental Disorders, IV**
EEG	**Electroencephalography**
ERN	**Error-Related Negativity**
FPS	**Fear Potentiated Startle**
FRN	**Feedback Related Negativity**
GAD	**Generalized Anxiety Disorder**
HA	**Harm Avoidance**
ICA	**Independent Component Analysis**
IGT	**Iowa Gambling Task**
PDE-4	**Type IV Phosphodiesterase**
PES	**Post-Error Slowing**
PFC	**Prefrontal Cortex**
PKA	**Protein Kinase A**
PNS	**Peripheral Nervous System**
SS	**Sensation Seeking**
US	**Unconditioned Stimulus**

Introduction

Fear and anxiety from genes to behavior – a dynamic multilevel perspective

From the perspective of a genome, danger is something bad. Danger means that the probability of primary or secondary needs of an organism being violated is larger than zero (and smaller than one). Eventually, such violations may have negative consequences for the survival and/or reproduction of an organism and its genes. To reduce danger, evolution may have equipped us with fear and anxiety, which are considered two general strategies in the present work. It is assumed that while fear has evolved to get us out of dangerous situations, for example by making us fight, flee or freeze (reactive danger reduction), anxiety helps us to not even get into a dangerous situation in the first place or at least reduce danger of an upcoming situation (proactive danger reduction). To be able to implement these strategies, fear and anxiety have access to a rich array of "tools". For example, they can facilitate shifting our attention to potential signs of threat (Eysenck, 1992; E. M. Mueller et al., 2008), increase our awareness for errors (Hajcak, McDonald, & Simons, 2003a; Pailing & Segalowitz, 2004), potentiate our reflexes (M. Davis, 2001; E. M. Mueller, Hofmann, & Cherry, 2010), influence our peripheral nervous system (Stemmler, 2004; Wager, van Ast et al., 2009), make us mentalize about potential negative futures (i.e. worry; Borkovec, 2002; Borkovec, Robinson, Pruzinsky, & DePree, 1983) and affect how we make decisions (E. M. Mueller, Nguyen, Ray, & Borkovec, 2010). Most importantly, fear and anxiety are associated with an aversive experience, which biases us to behave in a way that reduces fear and anxiety (Mowrer, 1947).

From the perspective of a genome then, it would make sense to establish a selection of genes that provide their carrier with those toolboxes associated with fear and anxiety. For a genome however, wrapped up and packed into hundred billions of cell nuclei, it must be incredibly difficult to influence the complex physiology, cognition and experience of an organ-

ism about a million times larger. How can that be achieved? In the present work this question is addressed from a multilevel perspective inspired from cognitive neuroscience (Churchland & Sejnowski, 1988), where ”level” stands for levels of organization within the central nervous system. Due to gene expression (Molecules Level), neurotransmitters and receptors are built and transported to the synapse (Synapses Level) and thus affect the neurons they connect (Neurons Level). The interconnection of neurons leads to complex networks (Networks Level), which are in turn organized into increasingly higher levels of organization such as anatomical structures (Structures Level), systems of interconnected structures (Systems Level), and the central and peripheral nervous systems (CNS/PNS Level), which eventually compose a human being with feelings of fear or anxiety, physiological symptoms such as increased heart rate and complex behaviors that serve to reduce present or future threats. This path is not unidirectional – in fact, behavior influences activity at anatomical structures and networks, influences synapse formation, and even indirectly influences the expression of genes (Philibert et al., 2010).

Another important aspect of the herein presented view on fear and anxiety is that the abovementioned toolbox for fear and anxiety are considered dynamic. Which tools are used in a given instance of fear or anxiety (e.g., increased worrying vs. increased error monitoring), depends not only on genetic contributions, but also on factors such as individual learning experiences (e.g., whether worrying has helped before, or whether errors have led to dangerous situations before), situational demands (e.g., if anticipated danger will lead to subsequent problems that must be solved, and whether the individual is performing at the moment), as well as their interactions. Both learning experiences and situational characteristics are also represented across levels of neuroscience, and thereby modulate fear and anxiety in complex ways.

There is good support for attempts to discriminate fear and anxiety at multiple levels of neuroscience. For example, substances that modulate fear (i.e., panicolytics) are different

from those that modulate anxiety (i.e. anxiolytics: R. J. Blanchard, Griebel, Henrie, & Blanchard, 1997; A. M. Perkins et al., 2009). Also, brain circuits that are related to fear processing (e.g. amygdala: LeDoux, 2007) only partially overlap with brain circuits implicated in anxiety (e.g. bed nucleus of the stria terminalis, hippocampus: M. Davis, 2006; Gray & McNaughton, 2000). In addition, trait anxiety is largely uncorrelated (Depue & Lenzenweger, 2005, see also table 1 derived from Study 2 data), or moderately correlated (A. M. Perkins, Kemp, & Corr, 2007), with trait fearfulness.

A predominant view on the difference between fear and anxiety is that fear is related to explicit stimuli whereas anxiety pertains to more diffuse situations with no specific threat stimuli (M. Davis, 2006). In contrast, the present view proposes that fear and anxiety should be distinguished with regard to their future orientation – with fear being considered present-oriented, and anxiety future-oriented. This view is not orthogonal to the former given that processing of the present is much more closely tied to bottom-up processing of explicit stimuli, while future-related thinking is more diffuse and includes less specific details (Schacter & Addis, 2007). Note that the proposed link of anxiety and future-oriented processing provides an interesting explanation for the puzzling findings that (a) the main common target for a variety of anxiolytics appears to be the hippocampus (Gray & McNaughton, 2000), which is usually thought of as a mainly memory-relevant structure, and that (b) most anxiolytics affect memory (Gray & McNaughton, 2000). There is now converging evidence that memory – and thus the hippocampus and related structures – are of particular relevance for future-oriented thinking (Addis, Wong, & Schacter, 2007). For the sake of completeness it should also be noted that the present vs. future distinction can also be applied to positive emotions, thereby enabling a distinction between liking (present-oriented) and wanting (future-oriented), which are also associated with distinct neurobiological correlates (Depue & Lenzenweger, 2005).

	BIS	PSWQ	NEO NA	"Wor/Anx"	"Fear"
SS	-.07	-.04	-.01	-.04	**-.83**
HA	.10	.06	.04	.06	**.73**
"Wor/Anx"	**.82**	**.85**	**.71**		.11
"Fear"	.08	.02	-.02	.11	

Table 1: Correlations (unpublished) between personality measures linked to fearfulness (SS, HA), worrying (PSWQ), and anxiety (BIS, NEO NA), with worry/anxiety ("Wor/Anx") and fearfulness ("Fear") factors derived thereof (taken from n=200 participants described in Study 2). BIS: Behavioral Inhibition Scale (BIS/BAS scales; Carver & White, 1994), PSWQ: Penn State Worry Questionnaire (Meyer, Miller, Metzger, & Borkovec, 1990), NEO NA: Neuroticism Anxiety Scale (NEO Personality Inventory - Revised; Costa & McCrae, 1992), SS: Sensation Seeking Scale (Zuckerman-Kuhlman Personality Questionnaire; Zuckerman, 2002), HA: Harm Avoidance Scale (Multidimensional Personality Questionnaire; Tellegen & Waller, 2008).

Taken together, the concepts of fear and anxiety in the present work can be defined as follows:

> Anxiety and fear are multilevel responses to the organisms' challenge of reducing danger. They include complex and dynamic patterns of intra- and interlevel interactions that are orchestrated to adequately respond to anticipated and present situational demands. While the function of anxiety is to *reduce danger in the future* ("don't get yourself into trouble!"), the function of fear is to *reduce danger in the moment* ("get yourself out of trouble!").

It should be noted that this concept of anxiety is in contrast to some prevailing accounts. For example, Gray and McNaughton (2000) define anxiety as "the common actions of all clinically well-established anxiolytic drugs" (p. 4). Despite some circularity inherent in that approach (i.e., what constitutes an anxiolytic in the first place?), the concept of an anxiolytic is not compatible with a dynamic multilevel definition of anxiety because it implies a

direct and stable association between the molecular (i.e., substance) and the whole-system level, and thereby neglects or even negates any dynamics at intermediate levels. As another difference, Gray and McNaughton assume that anxiety is always the *result* of a conflict between incompatible goals (e.g. approach and avoidance tendencies). In contrast, the present framework suggests that avoidance tendencies are secondary to anxiety and its primary goal of proactively reducing danger. Avoidance tendencies are therefore seen as another tool that may become particularly relevant whenever situational or motivational characteristics increase the likelihood of an approach-related behavior that is associated with some risk. In such cases, avoidance tendencies and behavioral inihibition may decrease the likelihood of risky behavior and thereby reduce future danger. As a result, in cases where approach tendencies (driven by other motivational systems) persist, anxiety could thus contribute to (rather than result from) approach-avoidance conflicts. Similarly, this functional perspective provides an explanation for why anxiety is often associated with negative affect and depression: negative affect is a tool, which reduces future danger by tapering approach-motivated behavior (Izard & Ackerman, 2000).

The present concept of anxiety also diverges from M.W. Eysencks' (1992) account that "the primary function of anxiety is to facilitate the detection of danger or threat in potentially threatening environments" (p.11). In the present view, the primary function of anxiety is not the *detection,* but the *reduction* of danger. Although the detection of danger is an elementary *tool* to prevent future or present danger, this tool by itself is without evolutionary purpose if there are no dynamic modulations at multiple levels that ultimately lead to adaptive behavior, physiology, and cognition with regard to previously detected threats.

Others have stated that the main purpose of anxiety is to reduce uncertainty (Depue & Lenzenweger, 2005). Again, from the present perspective it would be argued that the reduction of uncertainty can be an important tool for anxiety – whenever knowledge about the nature of the threat helps to reduce danger in the future. However, there may be instances when

reductions of uncertainty do not resolve anxiety or may even increase anxiety. Hypervigilance-avoidance patterns of attentional biases, for example, suggest that threat-related information intake often is voluntarily avoided in anxious individuals following initial threat detection (E. M. Mueller et al., 2008).

Taken together, the proposed functional definition of anxiety can integrate the concepts of behavioral inhibition (Gray & McNaughton, 2000), threat detection (Eysenck, 1992), and uncertainty reduction (Depue & Lenzenweger, 2005), and can explain why they are often related to anxiety. It further explains why physiological changes, worrying, error monitoring, depression and other phenomena are related to anxiety – because they may have shown phylo-, anthropo- and ontogenetic relevance for the reduction of danger. Importantly, the dynamic multilevel approach states that tools can be used flexibly in order to serve the function of reducing danger in the future. As a major strength, it can thereby explain the otherwise puzzling finding that situational characteristics appear to influence, for example, whether anxiety leads to behavioral inhibition (Gray & McNaughton, 2000) vs. activation (Sidman, 1953), hypervigilance (MacLeod, Mathews, & Tata, 1986) vs. attentional avoidance (Y. P. Chen, Ehlers, Clark, & Mansell, 2002), and increased vs. unaffected error monitoring (Olvet & Hajcak, 2009).

Fear and Anxiety across humans and species

Humans differ with regard to how often, how strong, and in which situations they experience fear and anxiety. Valid and reliable questionnaires have been developed that measure interindividual differences in trait anxiety and fearfulness (Depue & Lenzenweger, 2005). The construct trait anxiety is closely related to what is also known as behavioral inhibition sensu Gray (Gray & McNaughton, 2000), neuroticism (Matthews & Gilliland, 1999), and negative affect (Brown, Chorpita, & Barlow, 1998; Campbell-Sills, Liverant, & Brown, 2004; D. Watson, Clark, & Tellegen, 1988), while fearfulness is more closely linked to some harm avoid-

ance measures (e.g. the harm avoidance scale of the Multidimensional Personality Questionnaire; Tellegen & Waller, 2008) and low sensation seeking (Depue & Lenzenweger, 2005). It has been demonstrated that measures of trait anxiety have high heritability estimates (Lichtenstein & Annas, 2000; M. B. Stein, Jang, & Livesley, 1999) and some genes have already been identified that may explain a small proportion of variance in questionnaire measures (Lesch et al., 1996; Wacker, Reuter, Hennig, & Stemmler, 2005), but also in measures reflecting lower neuroscience levels such as brain activity in anxiety related structures (e.g. Hariri et al., 2002). Derived from the above multilevel approach it can be assumed that interindividual differences in anxiety should be manifest at different levels of neuroscience. As a consequence, the study of interindividual differences may provide valuable insights with regard to the neurobiology of anxiety. For example, the finding that trait anxiety and fearfulness are not identical (A. M. Perkins et al., 2009) suggests that fear and anxiety may be related to distinct processes at different neuroscience levels. In fact, fear and anxiety have been linked to different anatomical structures and are reduced by different types of drugs (see above).

Despite being essential for survival, fearfulness and anxiety may have dramatic consequences for an individual if they are exaggerated. Almost 30 % of the population suffers at least once in their lifetime from a severe anxiety disorder such as Generalized Anxiety Disorder, Social Anxiety Disorder, Panic Disorder, Post Traumatic Stress Disorder, or Obsessive Compulsive Disorder, all of which dramatically decrease life-quality (Kessler, Berglund et al., 2005). Although these conditions are associated with elevated levels of trait anxiety, additional factors such as maladaptive behavior that aims at avoiding the emergence of anxiety and impairments in emotion regulation may be critical for the development of an anxiety disorder (Brown et al., 1998). Understanding anxiety from a multilevel perspective may be of particular value for the treatment of anxiety disorders. For example, pharmacological treatments (focussing on the neurotransmitter level) and psychotherapy (focussing on the cognitive and behavioral level) alone have moderate efficiency for treating anxiety disorders

(Hofmann & Smits, 2008). However, novel translational approaches (Hofmann, 2007), for example administering D-Cycloserine – a pharmaceutical presumably supporting synaptic learning processes – prior to exposure therapy, have been shown to boost effect sizes dramatically (Hofmann et al., 2006). On the other hand, research on anxiety disorders may also inform general models of anxiety. For example, the finding that selective serotonin reuptake inhibitors (SSRIs) not only improve depression but also anxiety disorders (Zohar & Westenberg, 2000) suggests that serotonergic neurotransmission plays an important role in fear and/or anxiety (Hariri et al., 2002; Lesch et al., 1996; E. M. Mueller, Stemmler, Hennig, & Wacker, submitted abstract).

Given the evolutionary advantage of mechanisms that reduce present and future danger, defensive behavioral systems can be observed across vertebrates (D. C. Blanchard, Griebel, & Blanchard, 2001), although the precise content of the "toolboxes" likely varies: worrying would not be expected in rats, ultrasonic vocalization (Wohr, Borta, & Schwarting, 2005) would not be expected in man. However, there are many fear and anxiety-related phenomena that can be observed across species (D. C. Blanchard et al., 2001), such as fear-potentiated startle, which can be found in rats (M. Davis, 2006), mice (E. M. Mueller, Hofmann et al., 2010), rhesus monkeys (Winslow, Parr, & Davis, 2002) and humans (Grillon, Ameli, Woods, Merikangas, & Davis, 1991). Thus, the study of animals – mostly rodents – has proven invaluable for understanding the neuropsychology of human fear and anxiety (D. C. Blanchard et al., 2001; M. Davis, 2006; Fendt & Fanselow, 1999; Gray & McNaughton, 2000; LeDoux, 2007; Mowrer, 1947).

The present thesis

The present thesis on fear and anxiety is composed of four empirical studies. Participants of Studies 1 and 2 were healthy humans with varying levels of trait anxiety, Study 3 tested individuals with Generalized Anxiety disorder, and Study 4 was conducted with fear-

conditioned mice. All studies investigated fear- and anxiety-related tools, including cortically driven modulation of heart rate (Study 1), error monitoring (Study 2), decision-making (Study 3), and fear-induced potentiation of reflexes (Study 4). The manipulated, or quasi-manipulated, levels of neuroscience included the cell level (Study 4), the synapse level (Study 2), the network level (Study 2), the structure level (Study 1), and the whole-system level (Study 3). Modulations where observed at the peripheral nervous system level (Study 1), structure level (Study 2), and whole-system/behavioral level (Study 3 and 4).

The goal of this work was not to map a complete path from the molecule to the experiential, behavioral, and cognitive manifestation of anxiety, but rather to find relevant connections between different levels of neuroscience. In an attempt to bring together (biological) neuroscience and (psychological) cognitive science into an integrative cognitive neuroscience, Churchland and Sejnowski (1988) have suggested that "the ultimate goal of a unified account does not require that it be a single model that spans all the levels of organization. Instead the integration will probably consist of a chain of models linking adjacent levels" (p. 242).

Obviously, we are still far away from such a unified account with regard to anxiety. The multilevel perspective taken in the present work should therefore not be considered a complex model or complete chain of models that are to be tested. Instead the multilevel perspective should be considered a framework that integrates a rich variety of studies that have been conducted on fear and anxiety by others and myself. The following summary of the four studies presented herein may illustrate this variety.

In short, the first study (E. M. Mueller, Stemmler, & Wacker, 2010a) investigated the association of brain and heart activity using a novel method that was based on intraindividual linear correlations between stimulus-locked single-trial EEG and heart period. It was shown that EEG amplitude 300 ms following a performance-feedback stimulus predicted subsequent changes in heart period. Moreover, the level of trait anxiety moderated this prediction such

that more anxious individuals showed heightened neurovisceral (i.e., brain-heart) connectivity.

Using Independent Component Analysis (ICA) on electroencephalographic data (Makeig, Bell, Jung, & Sejnowski, 1996), the second study (Mueller, Makeig, Stemmler, Hennig, Wacker, submitted) investigated how dopamine-related polymorphisms influence the processing of errors in anterior midcingulate cortex (AMC), and whether a dopamine-antagonist (sulpiride) would further modulate such effects. This was based on previous findings that linked error processing to dopamine (Holroyd & Coles, 2002) and anxiety (Gehring, Himle, & Nisenson, 2000). Although there was no direct relationship between error processing and trait anxiety in that study, we found that the COMT Val158Met polymorphism, associated with prefrontal cortex dopamine availability predicted electrophysiological and behavioral correlates of error processing, and that sulpiride reversed the effect associated with COMT Val158Met.

The third study (E. M. Mueller, Nguyen et al., 2010) looked at future-oriented decision-making in Generalized Anxiety Disorder (GAD). Consistent with the conceptualization of anxiety as a strategy to avoid future danger we expected that GAD-participants would make more future-oriented decisions in the Iowa Gambling Task (IGT), which has previously been used to measure the absence of future-oriented decision-making in patients with brain damage. In line with our expectations we found that GAD participants made more future-oriented decisions than non-anxious control participants across two different versions of the IGT, thereby providing evidence for the proposed link between future-orientation and anxiety.

Study four (E. M. Mueller, Hofmann et al., 2010) examined a part of an intracellular signaling cascade that is involved in the acquisition and extinction of fear and anxiety. Extinction learning involves the second messenger cyclic adenosine monophosphate (cAMP), which by modulating intracellular processes ultimately affects protein expression required for long-term potentiation. Importantly, cAMP is broken down by cAMP specific phosphodiesterase 4

(PDE4). In Study 4 (Mueller, Hofmann, Cherry, 2010) we hypothesized that by delivering rolipram, a selective PDE4-inhibitor (Randt, Judge, Bonnet, & Quartermain, 1982), cAMP levels would be elevated and thus extinction learning could be enhanced. This hypothesis was particularly tempting because rolipram was previously shown to boost memory formation (Barad, Bourtchouladze, Winder, Golan, & Kandel, 1998), and because prior studies have succesfully ameliorated extinction learning with substances involving other signalling pathways (Walker, Ressler, Lu, & Davis, 2002). However, in a series of five experiments conducted on fear-conditioned mice, we showed with the fear-potentiated startle paradigm that rolipram disturbed rather than enhanced the consolidation of extinction memory. In addition, rolipram showed panicolytic properties in fear-conditioned mice.

The first study (E. M. Mueller, Stemmler et al., 2010a) was published in *Neuroscience,* and parts of that study were previously published elsewhere (E. M. Mueller, Ahrens, Stemmler, Zangl, & Wacker, 2009; E. M. Mueller, Stemmler, & Wacker, 2010b). The second study (E. M. Mueller, Makeig, Stemmler, Hennig, & Wacker, submitted) was recently submitted to the *Journal of Neuroscience* and parts of that study were previously published (E. M. Mueller, Makeig, Stemmler, Hennig, & Wacker, 2010). The third study (E. M. Mueller, Nguyen et al., 2010) was published in the *Journal of Behavior Therapy and Experimental Psychiatry*, and parts of the study were previously published (E. M. Mueller, Nguyen, Ray, & Borkovec, 2009). The four experiments described in study four (E. M. Mueller, Hofmann et al., 2010) were published in *Neuropharmacology*. All included studies were written in first-authorship. Two earlier studies published in *Psychological Medicine* (E. M. Mueller, Hofmann et al., 2009) and *Neuropsychologia* (Santesso et al., 2008) also investigated anxiety-related tools (attentional biases to threat) at the behavioral and neural levels, but were not included in this dissertation thesis.

Methodological Considerations

The most frequently used statistical tests for interindividual comparisons require normal distributions, homoscedasticity, and group size equality in order to be robust (Erceg-Hurn & Mirosevich, 2008; Keselman, Algina, Lix, Wilcox, & Deering, 2008; see also Figure 1). This is an issue when it comes to the investigation of decreasingly complex neuroscience levels. The further we move towards specificity, the further we likely move away from normality. As we consider cellular or molecular processes, as opposed to orchestrated patterns of behavior, interindividual variations will be composed of fewer and fewer random variables, resulting in distributions with reduced entropy. A good example is independent component activity, which reflects activation of a single brain source, and whose distribution is less gaussian than continuous EEG activity, which reflects a mixture of multiple brain processes (Makeig et al., 1996). If we move toward the molecular level and look at protein expression as a function of genes, for example, the entropy decreases even further and we will find rather discrete levels of protein expression as a function of genotype. In addition to non-normality issues, the investigation of genotype groups in an unselected population will lead to inequal group sizes that are due to the natural genotype distribution in the investigated ethnic group. An additional problem is that some alleles are so infrequent (i.e., the A1 allele of the DRD2 Taq I A polymorphism) that groups have to be combined (i.e., A1/A1 carriers and A1/A2 carriers will become the A1+ group), while others remain homogenous (i.e., A2/A2 carriers). If the dependent variable is related to the allele, such grouping will necessarily lead to inequal variances of the dependent variable in the two groups (i.e., increased variance in the combined group). It is interesting to note that researchers almost exclusively use standard parametric tests even though a violation of its requirements is inherent in the hypothesis it is supposed to test in such cases (i.e., that there is a relationship between the gene and the dependent variable)! This becomes even more fascinating given that it has long been known that

variance and group size heterogeneity may dramatically increase false positive rates (see Figure 1). In order to circumvent these and other methodological issues a statistical package for Matlab (The Mathworks, Inc.) was constructed (E. M. Mueller, Makeig, Delorme, Stemmler, & Wacker, submitted) that uses winsorizing and bootstrap-based statistics which are more robust against the aforementioned violations (Keselman et al., 2008). The script is built on the freeware EEGLAB (Delorme & Makeig, 2004) and contains a series of functions, including (1) bootstrapping, (2) winsorizing, (3) automated independent component selection, (4) group-wise dipole position averaging, (5) displaying, (6) interindividual circular statistics based on the Van-Mises distribution that can be used for phase comparisons of 2 x 2 groups (inter-trial coherence), (7) corrections for multiple comparisons (based on a priori defined clustersize or on surrogate distributions), (8) anovas, (9) t-tests for event-related potentials, event-related spectral perturbations and intertrial coherence, (10) compass plots for inter-subject phase coherence, and many more that are listed in Appendix IV. All analyses of Study 2 were tested with that toolbox. Because they were conducted earlier, all other studies were conducted with parametric tests implemented in SPSS (Study 3 and 4) or matlab code written by myself (Study 1).

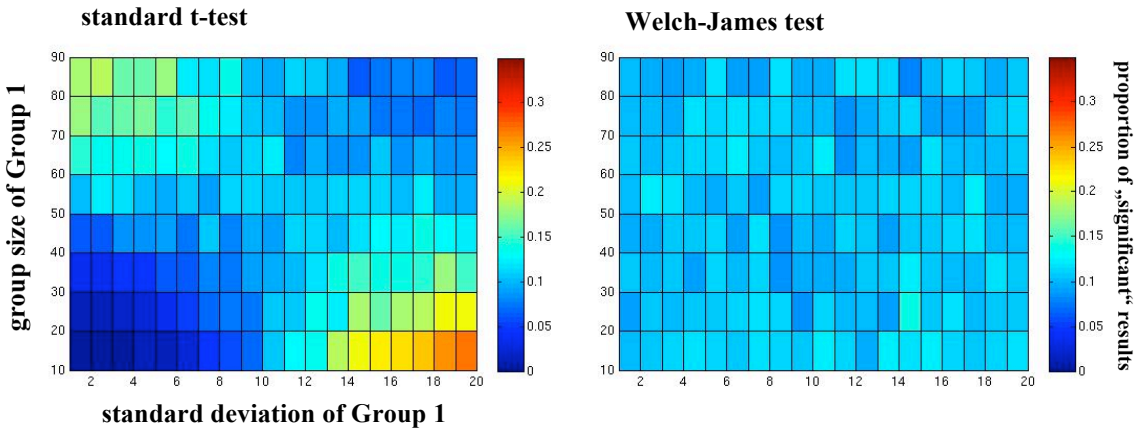


Figure 1: Empirical false positive rates (based on 1000 simulations/cell) for comparing means of two unpaired groups with a nominal alpha of .1 as a function of standard deviation (x-Axis) and group size (y-Axis) of Group 1. Reference Group 2 has a standard deviation of 10 and a group size of 50. Data of both groups is randomly sampled from populations with equal means and normal distribution. Depicted are false positive rates for a standard unpaired t-test (left panel) and a Welch-James test (right panel; Keselman et al., 2008). Light blue indi-

cates that the proportion of false positive rates matches the nominal alpha of .1. Note that the standard t-test but not the Welch-James statistic yields a strong increase in false positives (i.e. lack of robustness) when the relatively smaller group displays high variability and becomes overly conservative when the relatively smaller group displays reduced variability. These and similar simulations were used to test the statistical package developed for Study 2 (see Appendix IV).

Summaries of Empirical Studies

Study 1

"Oh the nerves, the nerves; the mysteries of this machine called man! Oh the little that unhinges it, poor creatures that we are!" ~Charles Dickens

The orchestrated physiological patterns induced by fear (Stemmler, 2004) and anxiety (Epstein & Roupenian, 1970) typically include increases in heart rate. In humans such physiological modulations can be triggered even by highly abstract stimuli, which may require some higher-level (i.e., prefrontal cortex, PFC) processing in order to extract the meaning structure (e.g. Damasio, 1996). One of the primary functions of an emotion like fear is to allocate bodily resources (e.g., increased oxygen supply to extremities) to accomplish the emotions goals (e.g., escape from danger). Because this often must proceed very quickly (as in the case of a present threat) such PFC-heart communications should be able to operate relatively quickly. Although fMRI, lesion and pharmacological studies have indeed identified the medial prefrontal cortex, the insulae, the periaquaeductal grey and other regions as major structural components for the central regulation of autonomic nervous system activity (Critchley, 2005; Damasio, 1996) and heart period in particular (Benarroch, 1997; Critchley et al., 2003; Gianaros, Van Der Veen, & Jennings, 2004; Wager, Waugh et al., 2009) little is known about the timing of neurovisceral communication. While animal studies provide evidence that heart rate may decrease as early as 500 ms after direct stimulation of vagal fibers (Spear, Kronhaus, Moore, & Kline, 1979) and increase at least 1-2 seconds after sympathetic stimulation (Berntson et al., 1997) it is not known how long it takes in humans for cortical activity to trigger changes in heart period. fMRI methods that have been previously used to study neurovisceral connectivity (Wager, Waugh et al., 2009) lack the temporal resolution to identify the precise timing of such quick processes. In addition, the BOLD response itself is a measure of oxygenation and it is thus unclear whether correlations between the BOLD signal and cardio-

vascular activity reflect true neurogenic associations. While EEG does have considerably higher (i.e., real time) temporal resolution and is less confounded with intracerebral blood circulation, the standard approach of averaging EEG over trials does not allow investigating functional (intraindividual) coupling of brain and heart activity because averaging eliminates any intraindividual variance. The primary goal of Study 1 was to develop a method to investigate how quickly cortical activity may trigger modulations of heart rate by using non-averaged EEG. The idea that there may be a signal in the EEG that is indeed linked to modulations of cardiac speed was based on the observation that certain stimuli and/or experimental conditions have similar effects on modulations of event-related potential amplitudes and heart period. For example, when individuals perform a task in which they get performance feedback after each trial (positive vs. negative), both the amplitude of the frontocentral feedback-related negativity (FRN; Miltner, Braun, & Coles, 1997) and the amount of cardiac deceleration (Crone et al., 2003) are increased for negative vs. positive feedback. Other studies found that the same type of stimuli that elicit an increased P300 may also elicit an increased cardiac acceleration from about 2-6 s after stimulus presentation (Lang, Gatchel, & Simons, 1975; Otten, Gaillard, & Wientjes, 1995). Based on these early findings we thus hypothesized that our new method (described below) could reveal relationships between non-averaged EEG (e.g., single-trial FRN or single-trial P300) and evoked heart-period response.

Method

The method, which we have termed Cardio-Electroencephalographic-Covariance-Trace (short: CECT) and which has been chosen for the front cover of the journal *Neuroscience, Volume 166*, can be considered a two-level approach. At the first (intraindividual) level, time-lagged P-correlations (i.e. intraindividual correlations between two variables over time, Cattell, 1952) are computed between single-trial EEG magnitudes and single-trial modulations of heart period. For that, the continuous EEG is first epoched such that each of k epochs

represents one trial. Then, each epoch is divided into a number (e.g., 100) of time-bins and for each time-bin the mean amplitude is calculated. Analogously, the cardiotach (trace of heart-period changes over time) is epoched and each of k epochs is divided into a number (e.g., 10) of time-bins for which the mean heart period values are calculated. A P-correlation (over the k trials) is then computed for each of the 100 x 10 possible EEG x heart-period time-bin combinations. At the second (interindividual) level, correlations are tested for statistical significance. P-correlations are first Fisher transformed (Fisher, 1950) and then for each of the 10 x 100 bins tested against zero over participants with one-sample t-tests. To account for the large number of tests the statistical threshold is adapted accordingly (e.g., using a Bonferonni-correction). In the herein presented study, we analyzed the data of n = 31 college students who performed a gambling task where each trial terminated with a feedback stimulus indicating whether a participant had just won or lost a small amount of money.

Results

Replicating prior studies, conventional data analysis revealed that negative vs. positive feedback led to more negative frontocentral ERP amplitudes from 200 to 260 ms (FRN; Miltner et al., 1997) and to relative cardiac deceleration from 1000 to 3500 ms after the feedback stimulus. Importantly, using the CECT-method we found that frontocentral EEG magnitude in time bins about 200 ms to 400 ms after the feedback stimulus correlated with cardiac acceleration from 2000 to 5000 ms (Figure 2). This correlation (termed N_300_4) was significant across individuals and similarly emerged for both feedback types and even remained highly robust after (a) conservative Bonferroni-correction for 10 x 110 = 1100 comparisons, (b) partialling trial indices (to control for time effects), and (c) partialling baseline heart-rate (to control for baseline-effects on heart-rate modulation and EEG). Moreover, EEG was uncorrelated with HP modulations in the subsequent trial indicating that EEG and cardiac chronotropy were specifically correlated on a trial-by-trial basis.

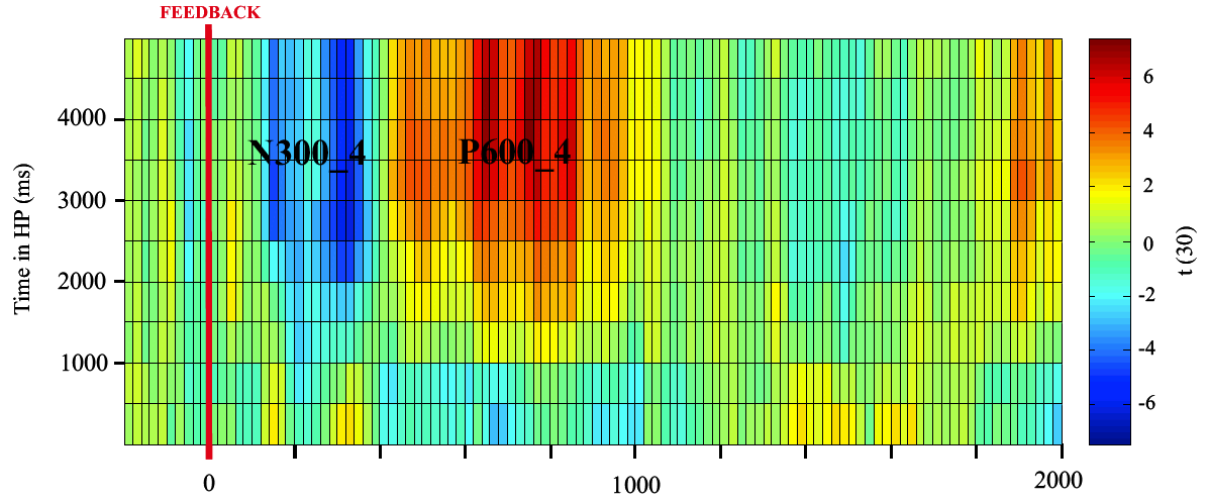


Figure 2: Cardio–Electroencephalographic Covariance Trace (CECT). *t*-values for positive (red) and negative (blue) EEG_heart period P-correlations as a function of time in EEG (horizontal axis) and heart period (vertical axis) at FCz. Clusters are named according to direction of the correlation (positive vs. negative), time in the EEG (in ms) and time in heart period (in s) as N300_4 and P600_4.

Discussion

Although feedback valence modulated both FRN and evoked heart period (replicating earlier studies), the magnitude of single-trial FRN seemed to not specifically predict modulations of heart period, which is consistent with prior observations that different neurotransmitter systems are involved in feedback valence effects on FRN and heart period (van der Veen, Mies, van der Molen, & Evers, 2008). In contrast, the EEG for a much longer time range than the FRN (i.e., from 200 to 400 ms following feedback) was correlated with heart period (such that a more positive amplitude predicted cardiac acceleration). Because both P300 (Donchin, 1981; Rushby, Barry, & Doherty, 2005) – which typically peaks between 200 and 400 ms – and modulations of heart period (Graham & Clifton, 1966) have previously been linked to the orienting response, we interpreted our findings to indicate that "some anterior P300 generators are related to activation of a central autonomic network, for example to prepare the organism for action upon detection of changes in the environment or upon detection of other relevant signals" (p. 496). Interestingly, about one year after our article had been published,

Nieuwenhuis et al formulated a theory that similarly states that P300 and autonomic responses are functionally coupled in the orienting response (Nieuwenhuis, De Geus, & Aston-Jones, 2011).

Because cortico-cardiac connections play a central role in recent models of anxiety (Berntson, Sarter, & Cacioppo, 1998; Friedman, 2007; Thayer & Lane, 2009), we were also interested to probe whether trait anxiety moderated the strength of the correlation between EEG and cardiac acceleration. Interestingly, high trait-anxiety, as measured with the BIS-scale (Carver & White, 1994), tended to be associated with stronger cortico-cardiac coupling following negative ($r = .36$, $p < .055$) but not following positive feedback ($r = .03$). Although this latter finding was excluded from the final article due to a reviewer's concern regarding the p-value, it is interesting to note that the coupling of cortical and subsequent cardiac activity following negative feedback may be elevated in anxious individuals. It should also be noted that there is now strong support for this association as we recently replicated a significant correlation between cortico-cardiac coupling and the BIS scale for negative but not for positive or uncertain feedback in a different task in about 170 participants. For this, and because of predictions derived from recent models of panic, we currently use the CECT method to study cortico-cardiac connectivity in Panic Disorder, Depression and healthy individuals in an ongoing DFG-funded research project. In addition to that project, three further studies are currently being conducted to replicate the coupling of P300 and autonomic activity, and to better understand how lower neuroscience levels are implemented in cortico-cardiac coupling (i.e., involvement of particular neurotransmitters).

The first follow-up study (unpublished) replicated an association of EEG activity from 200 to 400 ms with a different EEG set and a different sample, and further showed that neurovisceral communication can also be triggered if the abstract stimulus is ambiguous (signals neither reward nor punishment). The second ongoing study investigates how dopamine (pharmacological challenge and dopaminergic polymorphisms) may affect neurovisceral re-

sponses to negative, neutral and positive feedback in a different task. The third study investigates – in cooperation with Dr. Van der Veen (Rotterdam, NL) – whether neurovisceral communication is related to serotonergic neurotransmission that was challenged by depleting participants with tryptophan, a precursor of serotonin (van der Veen et al., 2008).

Study 2

"If I had to live my life over again, I'd try to make more mistakes next time." – Nadine Stair

Obviously, the commitment of an error can have quite dangerous consequences across all areas of life. Given that anxiety serves the goal to reduce danger in the future one could expect that anxiety is associated with elevated monitoring of ones actions for possible errors. Consistent with this notion, many researchers have reported a link between potentiated error monitoring and state (Pailing & Segalowitz, 2004), trait (Hajcak et al., 2003a), and pathological (Olvet & Hajcak, 2008) anxiety. Interestingly, error monitoring seems to be closely related to dopamine-based neurotransmission (Frank, D'Lauro, & Curran, 2007; Holroyd & Coles, 2002; Jocham & Ullsperger, 2009) and several studies have reported a link between anxiety and polymorphisms that affect cortical dopamine (Hettema et al., 2008; Hunnerkopf, Strobel, Gutknecht, Brocke, & Lesch, 2007; Joe et al., 2008; Wacker et al., 2005). Accordingly, it could be hypothesized, that such polymorphisms somehow enhance error monitoring as one component of the anxious phenotype.

However, the mechanisms by which interindividual differences in dopamine may affect error monitoring are not well understood. With regard to neuroanatomical structures, there is converging evidence from fMRI and EEG research for a prominent involvement of the anterior midcingulate cortex (AMC; Debener et al., 2005). Dopamine manipulations (i.e., manipulations at the molecular/synapse level) have been shown to affect error-monitoring correlates (Jocham & Ullsperger, 2009), and models have been proposed that link the molecular (i.e., neurotransmitter) to the maps (i.e., neuroanatomical structures) level (Frank, 2005; Holroyd & Coles, 2002; for summaries see Appendix V).

Durstewitz and Seamans (2008) state that the amount of prefrontal cortex dopamine determines qualitatively different network states. According to their dual-state theory of pre-

frontal cortex dopamine function, there exist two discrete dynamical regimes. D_1-dominated states are characterized by higher energy barriers among different network patterns, which supposedly favor the online maintenance and relative stability of representations. In contrast, D_2-dominated states are associated with a lower energy barrier, which may support flexible and fast switching among representational states. Durstewitz and Seamans assume that whether a network is in D_1- or D_2-dominated regimes depends on prefrontal cortex dopamine level. Following an inverted U-shape function, low and high dopamine levels are associated with D_2-dominated states while intermediate dopamine levels are related to D_1-dominated states. Because error-processing likely triggers and/or requires dynamic processes like orienting (Notebaert et al., 2009), updating of representations (Holroyd & Coles, 2002), and adaptation of behavior (Botvinick, Braver, Barch, Carter, & Cohen, 2001), we reasoned that error-processing would be elevated in D_2- rather than D_1-dominated states, and we thus expected potentiated error-processing when prefrontal cortex levels of dopamine are presumably high or low rather than intermediate.

Based on these theoretical accounts we tested whether interindividual differences in dopamine would relate to differences in error processing. For independent variables we (1) assessed a prominent single nucleotide polymorphisms tied to dopamine – Catechol-O-Methyltransferase (COMT) Val158Met, and (2) manipulated dopamine experimentally with sulpiride, a selective dopamine receptor antagonist. For dependent variables we analyzed the error-related negativity (ERN) and post-error slowing (PES) as electrophysiological and behavioral markers of error monitoring, respectively.

COMT. The enzyme COMT is crucial for the elimination of dopamine in humans especially in prefrontal cortex (J. Chen et al., 2004; Lachman et al., 1996). The gene coding for COMT is located on chromosome 22 and a common single nucleotide polymorphism at codon 158 leads to a substitution of the amino acid Methionine for Valine. This substitution is specific to humans (J. Chen et al., 2004) and can be found in 50 to 60% of the alleles in Euro-

pean populations (Palmatier, Kang, & Kidd, 1999). In individuals homozygous for the Met allele, COMT activity and thermostability are strongly decreased relative to Val carriers (J. Chen et al., 2004; Weinshilboum, Otterness, & Szumlanski, 1999), which presumably results in increased levels of prefrontal cortex dopamine in Met vs. Val homozygotes and intermediate dopamine levels for Val/Met carriers (Bilder, Volavka, Lachman, & Grace, 2004). Associations between the Met allele and a plethora of pheno- and endophenotypes including lower risk for schizophrenia (Egan et al., 2001), reduced agentic extraversion (Wacker & Gatt, 2010; Wacker, Mueller, Hennig, & Stemmler, under revision), enhanced fluid intelligence (Wacker et al., under revision), reduced prefrontal cortex activation in cognitive demanding tasks (Mier, Kirsch, & Meyer-Lindenberg, 2009), and enhanced prefrontal theta activity during rest (Wacker & Gatt, 2010) have been demonstrated. Although COMT has also been linked to fearfulness, trait anxiety, and anxiety disorders, the direction of this association was inconsistent across studies and may also be moderated by gender (Hettema et al., 2008; McGrath et al., 2004; M. B. Stein, Fallin, Schork, & Gelernter, 2005; Wray et al., 2008).

Sulpiride. In the present study we pharmacologically challenged dopaminergic neurotransmission by administration of a single, relatively low dose (200 mg) of sulpiride – a substitute benzamide that is often prescribed as an atypical antipsychotic. Sulpiride blocks dopaminergic, but not adrenergic, cholinergic, gamma-aminobutyric (GABA) ergic, histaminergic, or serotonergic, receptors (Caley & Weber, 1995). Sulpiride is slowly absorbed from the gastrointestinal tract, with peak serum levels occurring within one to six hours after oral ingestion, and the average elimination half life in the range of 3 to 10 hours (Mauri, Bravin, Bitetto, Rudelli, & Invernizzi, 1996). There is evidence that sulpiride selectively acts at D_2-like receptors and that lower doses (e.g., 200 mg) have a predominantly pre-synaptic effect resulting in a blockade of inhibitory autoreceptors and presumably increased dopamine release (Frank & O'Reilly, 2006; Serra et al., 1990).

ERN. The ERN is a widely studied electrophysiological index of error monitoring. It is a negative-going event-related potential (ERP) component with a frontocentral scalp distribution that peaks within 100 ms after individuals commit errors in reaction time tasks (Falkenstein, Hoormann, Christ, & Hohnsbein, 2000; Gehring, Coles, Meyer, & Donchin, 1995). Source localization studies (Gehring et al., 2000), theoretical considerations with regard to brain structure (Holroyd & Coles, 2002), and combined EEG/fMRI studies (Debener et al., 2005) have identified the AMC (Vogt, 2005) as the generator of the ERN. Of relevance for the present study, interindividual differences in ERN-amplitude are relatively stable over time (Segalowitz et al.) and highly heritable (Anokhin, Golosheykin, & Heath, 2008), suggesting a strong genetic component.

A variety of studies have investigated whether there is a direct relationship between the ERN amplitude and dopaminergic polymorphisms with inconclusive results (for review see: Ullsperger, 2010). With regard to COMT, Frank and colleagues compared 11 Met/Met to 28 Val carriers and found no significant group differences in ERN amplitude (Frank et al., 2007). Krämer et al. compared 20 Met/Met carriers to 20 Val/Val carriers and found – for one type of error – a marginally significant effect for COMT, indicating that Val homozygotes had relatively larger ERN amplitudes than Met homozygotes (Krämer et al., 2007). However, given the presumably small effect sizes of single polymorphisms on such phenotypes (Ullsperger, 2010), the sample sizes used in those studies were likely insufficient for any effects to reach statistical significance in the first place. Pharmacological tests for the involvement of dopamine in ERN generation also yielded unclear results. Low-dose dopamine-antagonists (de Bruijn, Sabbe, Hulstijn, Ruigt, & Verkes, 2006; Zirnheld et al., 2004) and L-DOPA (Jocham & Ullsperger, 2009) – both increasing phasic dopaminergic transmission – have led to reduced ERN amplitudes. In contrast, amphetamine – likely also increasing extracellular DA levels – has led to increased ERN amplitudes (de Bruijn, Hulstijn, Verkes, Ruigt, & Sabbe, 2004).

The relationship between ERN and anxiety has also been intensively studied. Potentiated ERN amplitudes have been found in individuals with obsessive compulsive disorder (Gehring et al., 2000; Hajcak & Simons, 2002), high worry tendencies (Hajcak et al., 2003a), state anxiety (Pailing & Segalowitz, 2004), and high negative affect/neuroticism (Boksem, Tops, Wester, Meijman, & Lorist, 2006; Hajcak, McDonald, & Simons, 2004; Luu, Collins, & Tucker, 2000; Pailing & Segalowitz, 2004). However, there is now some evidence that this relationship disappears when feedback is given after each trial (Grundler, Cavanagh, Figueroa, Frank, & Allen, 2009; Nieuwenhuis, Nielen, Mol, Hajcak, & Veltman, 2005; Olvet & Hajcak, 2009), possibly because, if available, anxious individuals may rely on external rather than internal information for error monitoring.

ICA. EEG that is recorded at scalp channels reflects a mixture of several brain processes that may be operating independently and in parallel. This is an issue for the interpretation of ERPs because it is unclear whether a certain waveform (such as the ERN), parameters derived thereof (e.g., the amplitude), and any effects of independent variables can be linked to one brain process or a mixture of several processes. Independent Component Analysis (ICA) is a method to find linear combinations of a recorded mixture that are maximally independent from each other (= independent components, ICs) and thus likely reflect separate brain sources. Although ICA is relatively methodologically advanced and is therefore not yet used in many laboratories around the globe, it was used in the present study to investigate the effect of dopamine on error-related brain dynamics. This was facilitated by a generous fellowship Grant from the Society for Psychophysiological Research (SPR) to Erik Mueller to finance a four-month visit at the Swartz Center for Compuational Neuroscience in San Diego, where ICA was first used to analyze EEG data (Makeig et al., 1996). The underlying idea of ICA is summarized in Appendix V.

PES. Following error commission, modulations of behavior can be observed. Among the most studied post-error behavioral phenomena is post-error slowing (PES) – the tendency

of participants to display increased reaction times in trials following an error (Eichele, Juvodden, Ullsperger, & Eichele, 2010; Rabbitt & Phillips, 1967) – possibly reflecting an adaptation (Botvinick et al., 2001) or orienting process (Notebaert et al., 2009). Although some studies have found a correlation between PES and ERN amplitude (Debener et al., 2005), this association has not always been replicated (Hajcak, McDonald, & Simons, 2003b), possibly due to the use of R- rather than P-correlations, tapping into functionally less meaningful sources of variance (E. M. Mueller, Stemmler et al., 2010a). A robust association between neuroticism/anxiety and PES could not been shown in one prior study (Hajcak et al., 2004). With regard to dopamine, two studies using haloperidol as a dopamine-active drug (de Bruijn et al., 2006; Zirnheld et al., 2004) found that it did not affect PES, although olanzapine – a dopamine and serotonin receptor antagonist – did lead to impaired ERN amplitudes and PES (de Bruijn et al., 2006). In addition, there is some evidence that dopaminergic genes modulate PES (Krämer et al., 2007).

The present study. As stated above, the goal of the present study was to investigate the role of dopamine in error processing. Based on the model of Durstewitz and Seamans we expected errors to have a stronger impact on brain activity (i.e., ERN) and behavior (i.e., PES) in Val vs. Met carriers, presumably occupying D_2 states which should facilitate network updating rather than network stability (Durstewitz & Seamans, 2008). Following their inverted u-shaped model (see Figure 3) we further hypothesized that experimentally elevating extracellular dopamine availability by administration of low-dose sulpiride would lead to increased error-processing in Val but decreased error-processing in Met carriers.

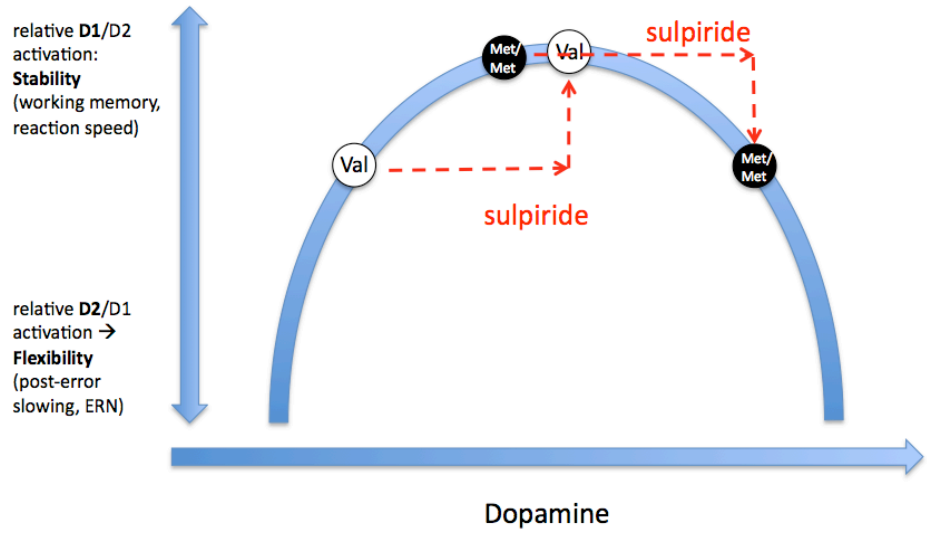


Figure 3: Postulated relationship between prefrontal dopamine level, COMT and relative D1 vs. D2 receptor activation as previously described by D. Durstewitz and J. K. Seamans (2008). Due to enhanced relative D2 receptor activation in Val vs. Met carriers we hypothesized increased error-related negativity and post-error slowing in Val vs. Met carriers. By increasing PFC dopamine activity through presynaptic D2 receptor blockade sulpiride (200 mg) is predicted to shift Val+ carriers into medium and Met/Met carriers into high dopamine levels (dashed arrows) resulting in reduction or enhancement of error-related negativity and post-error slowing.

Method

To test these hypotheses, n = 200 males participated in the present study. Following a clinical interview to rule out the presence of any psychopathologies, they were given sulpiride (200 mg) or placebo (double-blind), which they consumed together with a standardized breakfast. They then filled out questionnaires and performed tasks for which the results will be reported elsewhere (e.g. Wacker et al., under revision). Approximately 4 h after taking the pill, participants performed a standard Eriksen Flanker task in which five-letter strings were presented (S S H S S) and participants were instructed to respond with their index or middle finger if the central letter was an S or an H, respectively. The task was adaptive such that participants received the feedback "too slow" whenever their reaction time exceeded the mean reaction time plus one standard deviation taken from the last preceding trial block. We recorded the EEG during that task and later decomposed the EEG using ICA. For each IC we fitted a

dipole in a standard brain volume and included ICs into a subsequent clustering procedure. ICs of all participants were then clustered based on their estimated dipole position. One cluster included ICs, which were localized in proximity to the anterior midcingulate cortex previously linked to error processing and ERN (Debener et al., 2005). For those midcingulate cortex ICs we computed the ERPs and measured the ERN amplitude (IC-ERN) as is usually done with non-decomposed scalp channel EEG. ERN amplitudes and PES were analyzed using a statistical package implemented in MATLAB by myself that is capable of parametric and bootstrap statistics (see Appendix IV).

Results

As expected, both, PES and IC-ERN amplitudes were modulated by a sulpiride x COMT interaction: Individuals who had received the placebo showed greater IC-ERN amplitudes and more PES if they had a Val allele compared to Met homozygotes, mirroring the (marginally significant) findings from Krämer et al (2007). Importantly, sulpiride enhanced PES and IC-ERN amplitude in Met carriers but reduced IC-ERN and tended to reduce PES in Val carriers (see Figure 4). In contrast to our expectations, we found no association between either the IC-ERN amplitude or dopamine polymorphisms and trait anxiety or neuroticism, neither at the level of individual scales nor at the level of factors derived from factor analysis of several scales from the neuroticism/anxiety spectrum.

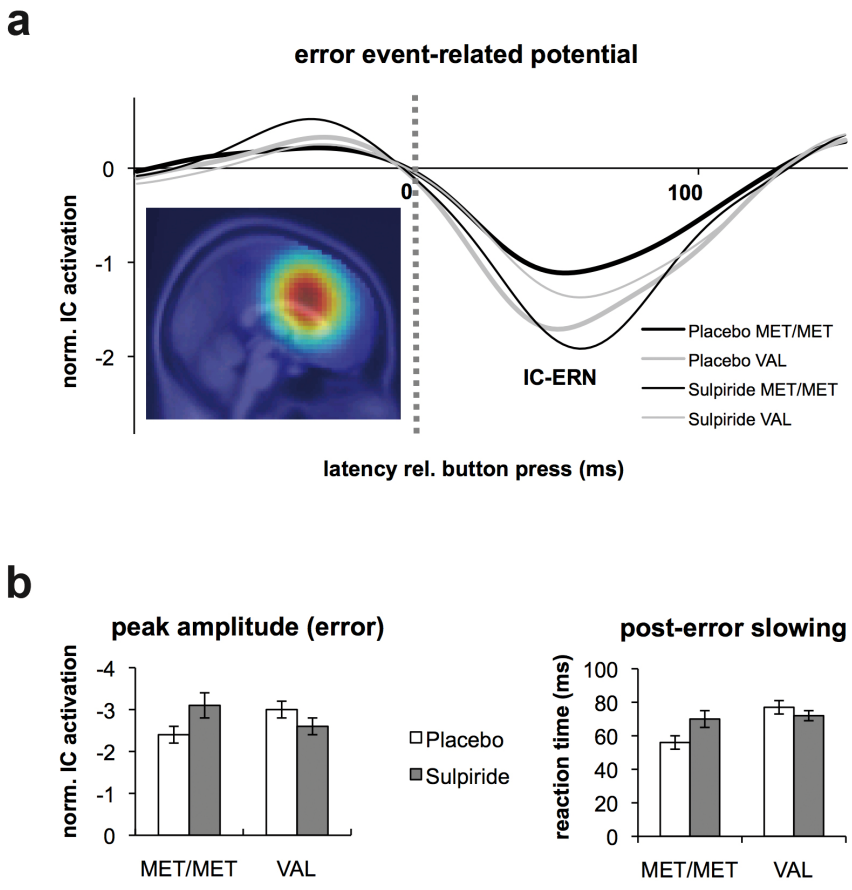


Figure 4: Interactions of sulpiride and COMT on neural and behavioral error-processing correlates. **(a)** Grand average event-related potentials (ERPs) for a medial frontal independent component cluster (IC-cluster) following erroneous button presses (at latency 0) for Val+ (grey) and Met/Met (black) carriers, who received placebo (thick) or sulpiride (thin). Independent component ERPs were normalized by the root mean square over the component scalp map projection to all channels prior to averaging. A standard brain image (Montreal Neurological Institute) indicates the region of maximum concentration (equivalent dipole density) of this IC-cluster. **(b)** Bar plots indicating means (and SEMs) of peak IC-cluster event-related potentials (left) and reaction-time slowing in the subsequent trial (right) following errors in the placebo (white) or sulpiride (grey) groups.

Discussion

The findings of Study 2 clearly support the involvement of dopamine in error-processing and interindividual differences therein. Val carriers, presumably showing lower levels of PFC dopamine than Met homozygotes due to increased COMT activity, had enhanced behavioral (PES) and electrophysiological (IC-ERN) indicators of error processing. Moreover, the administration of low-dose sulpiride, presumably increasing PFC dopamine by

D_2 autoreceptor blockade, reversed this effect. Assuming that reactive error-processing is facilitated in D_2 dominated states of PFC networks, this pattern of findings can be explained with Durstewitz and Seamans' (2008) dual-state theory applied to error-processing. According to their theory, Val carriers – due to low PFC dopamine levels – likely occupy D_2-dominated states, while Met carriers – with medium PFC dopamine levels – more likely occupy D_1-dominated states. Because they assume that the relationship between PFC dopamine level and D_1 vs. D_2 ratio is inverted U-shaped, an elevation of dopamine would be expected to shift PFC network states of Val carriers towards relative D_1, and of Met carriers towards relative D_2, domination.

In addition to demonstrating the involvement of dopamine in error processing, the present finding has an important implication. It suggests that some effects of sulpiride, an antipsychotic that is often prescribed in Germany ("Dogmatil"), may depend on COMT genotype. Thus, sulpiride seems to elevate error processing in Met but reduce it in Val carriers. This should be interpreted with caution because error rates and reaction times were unaffected, and thus it cannot be stated that sulpiride is more beneficial for Met than for Val carriers. In fact, if ERN and PES reflect a distraction from ongoing behavior that is triggered by a self-committed error (Notebaert et al., 2009) rather than an adaptive process, Val carriers may actually benefit more from acute low-dose sulpiride than Met carriers. Accordingly, in order to answer whether Val or Met carriers benefit more from sulpiride with regard to error processing, further research on the functional significance of ERN and PES must be conducted. Such research could be especially informative for the pharmacological treatment of schizophrenia, as error monitoring (Alain, McNeely, He, Christensen, & West, 2002) and the COMT Val158Met polymorphism (Egan et al., 2001) have been linked to schizophrenia.

We found no associations between dopaminergic genes, error processing and anxiety. The missing link between ERN and anxiety in the present study may be explained with the fact that we delivered trial-to-trial feedback in the Flanker task. As explained above, recent

reports suggest that trial-to-trial feedback diminishes the otherwise well-replicated correlation between ERN and neuroticism/anxiety (Olvet & Hajcak, 2009). In contrast to prior studies, we further found Val158Met and Taq I a to be unrelated to any measures of neuroticism or anxiety. While the link between COMT and anxiety may be female-specific (Hettema et al., 2008) and thus would be absent in our male study-participants, the TaqIa correlation was previously found to be male specific (Wacker et al., 2005). A possible explanation for the missing associations with anxiety in the present study is that, due to (a) the exclusion of participants with pathological anxiety and (b) the required intake of a pill that could contain an antipsychotic with potential side-effects (=potential danger in the future), our sample did not include participants with elevated trait anxiety in the first place. In line with this, the standard deviation of several neuroticism/anxiety measures was truncated (e.g. NEO PI-R Neuroticism-Anxiety scale: SD = 4.3) relative to a normative population of young males (SD = 5.4; Ostendorf & Angleitner, 2004). Alternatively, the link between anxiety and error processing may predominantly involve other neurotransmitters such as serotonin, which has been related to anxiety more consistently (Lesch et al., 1996) and may also affect error and feedback processing (Beste et al., 2009; van der Veen et al., 2008). Future studies investigating the interaction of serotonergic polymorphisms (e.g. 5HTTLP-R, Lesch et al., 1996) and serotonin manipulations (e.g. tryptophan depletion, van der Veen et al., 2008) on error processing and how it is correlated with anxiety may shed light on this hypothesis.

Study 3

"Today is the tomorrow we worried about yesterday." ~Author Unknown

Generalized Anxiety Disorder (GAD) is a condition that is characterized by excessive anxiety and worry concerning a number of domains. Individuals with GAD report difficulties to control the worry. The anxiety and worry are so severe that they are often associated with restlessness, being easily fatigued, difficulty concentrating, irritability, muscle tension and sleep disturbance (DSM IV). Within a period of 12 months, approximately 3% of the population suffers from GAD, and its 12-month prevalence is thus comparable to alcohol abuse (2005). GAD shows a high comorbidity with major depression, which in most cases has its onset after the manifestation of GAD (D. J. Stein, 2001), suggesting that GAD may trigger depressive symptoms. According to Gray and McNaughton, GAD is "in essence the only clearly identifiable primary anxiety disorder. That is, it is a case of maladaptive anxiety in which the primary pathology lies in the control of anxiety itself" (p.323). Accordingly, GAD can be considered a clinical condition that is closely linked to the concept of trait anxiety.

Consistent with the proposal that anxiety serves to reduce danger in the future, it has been observed that individuals with GAD are characterized by excessive worrying and being overly concerned with the future (Borkovec et al., 1983). Moreover, worry itself has been defined as ''a future-oriented mood state in which one becomes ready or prepared to attempt to cope with upcoming events'' (Brown, O'Leary, & Barlow, 1993, p. 139) – a state that may have dramatic consequences for life quality, as individuals with GAD often exhibit a failure to enjoy life or to live in the present moment (Borkovec, 2002; Borkovec, Alcaine, & Behar, 2004; Borkovec & Sharpless, 2004). Based on these associations between GAD, anxiety or worry, and a preoccupation with the future, we hypothesized that decision-making under ambiguity may also be biased in GAD, such that individuals with GAD would preferentially make future-oriented decisions.

Method

To test this hypothesis we used the Iowa Gambling Task (IGT). Originally this task was designed to capture the *lack* of future-oriented decisions in individuals suffering from ventromedial prefrontal cortex damage (Bechara, Damasio, Damasio, & Anderson, 1994). These individuals (the case of Phineas Gage being the most prominent example), despite intact intelligence and other cognitive functions, fail to act in a long-term oriented manner in every-day life and also show severe impairments in the IGT (Bechara et al., 1994; Bechara, Tranel, & Damasio, 2000). Similarly, conditions associated with high impulsivity and low future-orientation (e.g., attention deficit hyperactivity disorder, pathological gambling, substance abuse) have been correlated with below-average IGT performance (Bechara, Dolan, & Hindes, 2002; Cavedini, Riboldi, Keller, D'Annucci, & Bellodi, 2002; Garon, Moore, & Waschbusch, 2006). In contrast to these conditions, we hypothesized that if GAD is related to abnormally high future-orientation, individuals with GAD should show above-average performance in the IGT.

The IGT is a relatively simple task. The participant sees four decks of cards and is instructed to draw from each deck as he wishes. Each card leads to a fictional monetary reward that has the same value for all cards in a given deck ($100 for decks A and B, and $50 for decks C and D). Some cards lead to an additional punishment. The reinforcement schedule is designed such that the sum of additional punishments for ten cards taken from decks A or B is larger ($1250) than the sum of rewards for ten cards taken from decks A or B ($1000). In contrast the sum of additional punishments for ten cards taken from decks C or D is smaller ($250) than the sum of rewards ($500). Thus, an advantageous or future-oriented strategy is to take many cards from decks C and D even though the maximum reward value in an individual trial is smaller than in decks A and B. Because punishments are given in an unsystematic fashion, this task is not as easy as it may sound and healthy adults often continue taking cards

from decks A and B even after 100 trials.

An issue with the IGT is that if an individual prefers decks C and D over A and B, it is impossible to state whether he does so because these decisions are more long-term advantageous or because decks C and D are associated with smaller magnitudes of infrequent punishments, which would indicate risk aversion (Smoski et al., 2008) rather than future-orientation. In Study 3 we therefore not only used the standard IGT but also a variant thereof, in which the contingency table was multiplied by -1. That means that in this version each card from decks A and B yielded a punishment of $100, and over 10 trials the sum of infrequent rewards was $1250. Each card from decks C and D yielded a punishment of $50 and over 10 trials the sum of infrequent rewards was $250. Thus, in contrast to the standard IGT decks C and D are long-term disadvantageous although they are still associated with a small magnitude of punishment values.

The study was conducted over the course of two years at the Pennsylvania State University. As a course requirement, $N = 1882$ students of introductory Psychology classes had to complete a battery of questionnaires that included the GADQ-IV (Newman et al., 2002) – a self-report screening instrument for DSM-IV GAD criteria – and the Penn State Worry Questionnaire (Meyer et al., 1990) – a self-report instrument that measures trait worrying. Of these, students who met GAD criteria and non-anxious control participants were invited to participate in the study. The final sample consisted of n = 27 GAD and n = 20 control participants. They performed both versions of the IGT (each 100 trials) in counterbalanced order. The dependent variable was the number of long-term advantageous decisions in blocks of 20 trials.

Results

In the first block of the task, both groups made equally few long-term advantageous decisions, and over the task both groups learned to make more advantageous decisions. As

expected, individuals with GAD learned significantly faster than control participants to make long-term advantageous decisions (see Figure 5). Importantly, this was the case for both the standard and the modified IGT version. Moreover, if participants were classified into learners and non-learners based on their advantageous decisions in the last trial block, learners had a significantly higher mean PSWQ score than non-learners. There were no group differences in the time needed to make a decision or in the level of conscious awareness of which decks were better.

Discussion

Consistent with our expectations, we found an advantage of GAD participants in learning to make long-term advantageous decisions. While the disadvantages of suffering from GAD are indisputable, it is important to be aware that there may be conditions for which a tendency for increased anxiety and heightened worrying is associated with long-term benefits (see also: Adam M. Perkins & Corr, 2005). This finding may have relevant implications for understanding the etiology and maintenance of GAD because under circumstances where heightened worrying leads to long-term success, worrying may be positively reinforced. However, it may be trait rather than state worrying/anxiety that is linked to better IGT performance. In a subsequent follow-up study we found no effects of worry induction vs. relaxation on IGT performance in healthy college students, although we did replicate a positive effect of trait anxiety on IGT performance (Skip and Erkic, 2009, term paper supervised by E.M., Figure 5), and another group reported the same phenomenon (Werner, Duschek, & Schandry, 2009).

While this apparent future-orientation found in individuals with high anxiety is in line with the assumed function of anxiety to reduce danger in the future (see definition), it is unknown *how* anxious individuals perform better in this task without being aware of the good decks. The authors of the IGT hypothesized that neurovisceral communication ("somatic

markers") may be a key requirement for successful performance in the IGT because somatic states conditioned to bad decisions may bias future decisions of an individual towards more adaptive choices (Bechara, Damasio, Tranel, & Damasio, 1997). Our finding of enhanced neurovisceral communication in anxious individuals following negative feedback (cf. Study 1) may explain why individuals with exaggerated anxiety were able to make better long-term oriented decisions despite not being consciously more aware of advantageous decisions. Consistent with that interpretation, Werner et al (2009) reported a correlation between trait anxiety and anticipatory and feedback-evoked peripheral reactions in the IGT and IGT performance. In addition, potentiated PFC reactivity to erroneous responses and/or negative performance feedback has been found in anxious individuals, and may reflect better learning from negative feedback in humans with high anxiety (as discussed in Study 2).

Molecular genetic, electrophysiological or other psychophysiological measures were not collected in that study. Both dopamine and serotonin may relate to IGT performance (Bechara, Damasio, & Damasio, 2001) and GAD symptoms (D. J. Stein, Westenberg, & Liebowitz, 2002), and we have recently found that high PSWQ values in healthy males are linked to the short variant of the serotonin transporter gene (E. M. Mueller et al., submitted abstract), which also predicts better early IGT performance in males (Stoltenberg & Vandever, 2010). Future studies experimentally manipulating dopamine and/or serotonin and assessing the interactions of (1) trait-worrying or GAD, (2) catecholaminergic genes, and (3) neurovisceral communication on long-term oriented decision-making may be helpful to better understand the mechanisms of superior task performance in highly anxious individuals and may inform whether IGT-performance can be considered a valuable endophenotype for neuroticism/anxiety. With regard to understanding neuroscience levels involved in anxiety, such a study would be especially informative if electrophysiological correlates of feedback processing such as ERN/FRN (cf Study 2) and P300 (cf Study 1) would be included into analysis as indicators of relevant processes at medial prefrontal cortices.

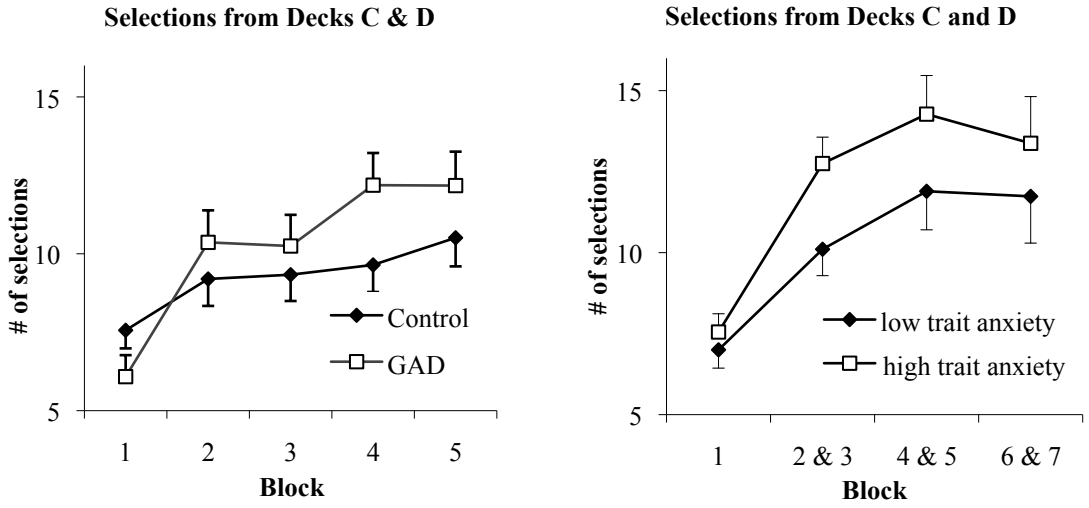


Figure 5: Number of Iowa Gambling Task selections from long-term advantageous decks C and D in Study 3 (left panel) comparing participants with Generalized Anxiety Disorder to non-anxious control participants and an unpublished replication experiment (right panel) with healthy college students with high or low trait anxiety.

Study 4

"We experience moments absolutely free from worry. These brief respites are called panic."
~Cullen Hightower

Despite high heritability estimates, anxiety and specific fears are not stable over the lifetime of an individual. Ever since Little Albert (J. B. Watson & Rayner, 1920), it is known that fears can be acquired or conditioned, and the effectiveness of exposure therapy for anxiety disorders suggests that fears and anxiety can also be extinguished (Foa & Kozak, 1986). Based on the assumption that fear and anxiety are conditioned responses (CR) to stimuli (CS) previously paired with aversive sensations (US), exposure therapy tries to extinguish such associations by repeatedly exposing the individual to the CS while aversive consequences are absent. Although exposure therapy is highly effective for anxiety disorders, there are a large number of non-responders who still suffer from severe anxiety after exposure. In order to improve exposure therapy outcome, there have been several attempts to discover substances that facilitate extinction learning (Hofmann, 2007). The biological mechanisms of extinction are not fully understood, however it is widely accepted that extinction is a particular type of new learning rather than just decay of old associations (Bouton, 2004). Accordingly, it has been speculated that substances that modulate proteins implicated in learning processes (e.g., long-term potentiation) may boost extinction.

The second messenger cyclic adenosine monophosphate (cAMP) is part of an intracellular signaling pathway implicated in learning, memory formation (Alberini, 1999; R. L. Davis, Cherry, Dauwalder, Han, & Skoulakis, 1995) and fear extinction (Myers & Davis, 2007). To give an example for such a pathway, (1) stimulation of dopamine D_1 receptors (see Study 2) may (2) activate G-coupled receptors, which then (3) stimulate adenylil cyclase,

which (4) leads to an accumulation of cAMP, which then (5) affects protein kinase A (PKA) to (6) phosphorylate cAMP responsive element binding protein (CREB) in the cell nucleus, which in conjunction with other proteins may (7) affect the expression of genes, which may ultimately (8) play an important role in memory formation, although the mechanisms for this final step are not yet fully understood (Carlezon, Duman, & Nestler, 2005). Because cAMP is broken down by cAMP-specific type IV phosphodiesterases (PDE4), the administration of rolipram, a selective PDE4 inhibitor has been found to elevate cAMP levels (Barad et al., 1998), increase CREB phosphorylation (Monti, Berteotti, & Contestabile, 2006), and improve learning (Rutten, Basile, Prickaerts, Blokland, & Vivian, 2008).

Based on prior findings that rolipram may enhance learning and memory (Barad et al., 1998; Bourtchouladze et al., 2003; Comery et al., 2005; Rutten et al., 2008; Zhang et al., 2002), we tested in Study 4 whether rolipram may enhance the acquisition and/or extinction of fear. Moreover, based on previously reported anxiolytic properties of rolipram (Li et al., 2009; Silvestre, Fernandez, & Palacios, 1999), we hypothesized that rolipram may affect the expression (or "experience") of fear due to presumably overlapping neural circuits for fear and anxiety (M. Davis, 2006; Gray & McNaughton, 2000). For that purpose we conducted a series of five experiments in which fear was assessed using the fear potentiated startle (FPS) paradigm with mice.

Methods

The FPS paradigm is based on the observation that an acoustic startle reflex can be augmented if the startle stimulus is presented in the presence of a cue that has previously been paired with an aversive stimulus (M. Davis, 2006). In the present study we used a standard FPS paradigm in which shocks were initially conditioned to a neutral CS (i.e., a particular

tone; acquisition phase). To measure the degree of fear associated with the CS, a series of relatively loud (95 – 105 dB) noise bursts were randomly presented either alone or in combination with the CS (test phase). The difference in startle response was taken as a measure of fear.

In the first experiment, we tested the panicolytic (i.e., fear reducing) effect of rolipram by probing whether fear conditioned mice would have reduced FPS if they had received rolipram vs. saline prior to the test phase. In the second experiment, we tested whether rolipram would enhance fear acquisition by delivering rolipram vs. saline prior to the acquisition phase and testing FPS to the CS several days later. In the third experiment, we tested whether rolipram would boost extinction. For that, there was an extinction phase after the first test phase in which the CS was presented alone (no shock) 20 times. Prior to extinction, mice had received rolipram vs. saline. Following that extinction phase, there was a second test phase to compare the reduction of fear after extinction between saline and rolipram groups. In the fourth experiment, there were four extinction sessions, and prior to each session rolipram vs. saline was administered. In contrast to the third experiment, the CS during extinction was not presented alone, but coterminated with a startle burst. This allowed us to measure how startle responses decreased during extinction as an indicator for intra-session reduction of fear (i.e., within-session extinction). Moreover, we could compare whether the extinction effect of one session would be consolidated and still be evident in the next extinction session (i.e., between-session extinction). While within-session extinction is more closely related to acquisition, between-session extinction reflects consolidation, both of which may be differently affected by rolipram. After the four sessions there was a final FPS test without prior rolipram delivery to test whether rolipram or saline groups had benefitted more from the extinction sessions. The fifth experiment was a replication of the fourth experiment.

Results

Experiment 1, in which rolipram was delivered prior to the test phase, revealed that rolipram significantly reduced FPS amplitudes in a dose-dependent manner. Mice that had received 1 mg rolipram per 1 kg body weight 30 minutes prior to the test phase (n = 8) had a lower FPS score than mice that had received 0.2 mg/kg (n = 8), which had a lower FPS score than mice that had received 0 mg/kg (saline, n = 8). This pattern suggests that rolipram reduces the expression (and possibly the experience) of fear in mice in a dose-dependent manner (Figure 5). Experiment 2, in which 1 (n = 9), .2 (n = 9), .03 (n = 9) or 0 mg/kg rolipram were delivered prior to the training phase, FPS scores assessed 24 h later revealed that rolipram did not significantly affect fear acquisition.

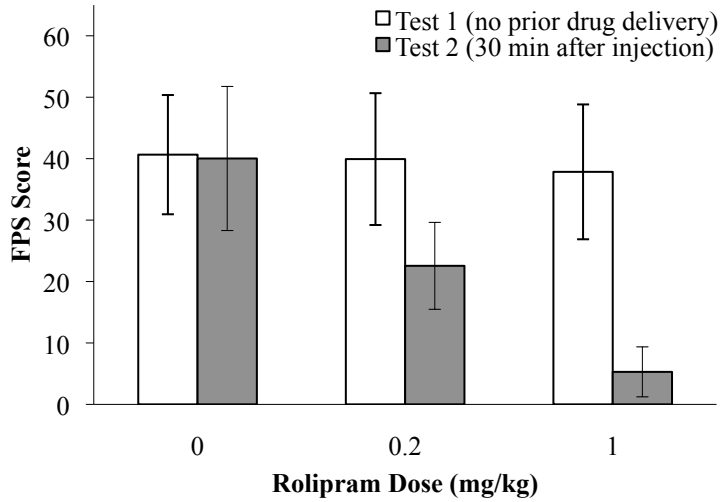


Figure 5: Rolipram reduced fear as measured with the FPS-score. White bars reflect mean FPS-scores (+/-SEM) with no prior injection and grey bars reflect FPS-scores 30 min after injection of saline or rolipram.

In Experiment 3, n = 61 mice were fear conditioned and tested for FPS scores. Based on these scores, mice were then assigned to one of four groups to achieve equivalent mean FPS scores. Groups were randomly determined to receive either 1 (n= 12), 0.2 (n = 16), 0.03 (n = 15) or 0 mg/kg rolipram (n = 18) 30 min prior to an extinction session. FPS scores assessed at a subsequent testing phase revealed that mice that had received saline prior to extinction showed a significant decline in FPS scores from before to after extinction. Importantly, this decline was significantly *reduced* in mice that had received any dose of rolipram. Thus, in sharp contrast to our expectations, rolipram did not ameliorate, but rather impair extinction learning. In fact, very small doses (.03 mg/kg) were sufficient to significantly weaken extinction ($p < .001$).

In Experiment 4, we wanted to better understand why rolipram disturbed extinction as revealed by Experiment 3. N = 18 mice were trained, tested and assigned to two groups to achieve equivalent FPS scores. They then underwent four extinction sessions and were injected saline (n = 9) or 1 mg/kg rolipram (n = 9) prior to each session. In those extinction sessions the CS was presented 21 times and always co-terminated with a noise burst. Instead of FPS scores (as in Experiments 1 - 3) the startle response to that burst averaged over 7 trials (yielding 3 blocks for each session) served to indicate fear (CS-associated startle) during extinction. The results are displayed in Fig 6. As can be seen, for mice receiving saline, CS-associated startle decreased within extinction sessions and between extinction sessions. Mice receiving rolipram were characterized by overall reduced startle amplitudes (replicating panicolytic properties of rolipram found in Experiment 1) and impaired between-session extinction despite intact within session extinction. At a final post-extinction test (i.e., when nothing was injected), the mean CS-associated startle of the rolipram group was significantly larger than that of the control group (replicating impaired extinction found in Experiment 3). These findings suggest that rolipram impairs extinction by affecting consolidation (i.e., between-session extinction) rather than short-term acquisition (i.e., within-session extinction) per se.

The fifth experiment was a replication of Experiment 4 with the only difference being that, due to technical failure, CS-associated startles were not recorded on the fourth extinction session. Again, the between-session extinction was significantly larger in the control compared to the rolipram group, where between-session extinction was not evident. The fifth experiment (Experiment 4B) thus replicated the main finding of Experiment 4 – that rolipram impairs extinction consolidation.

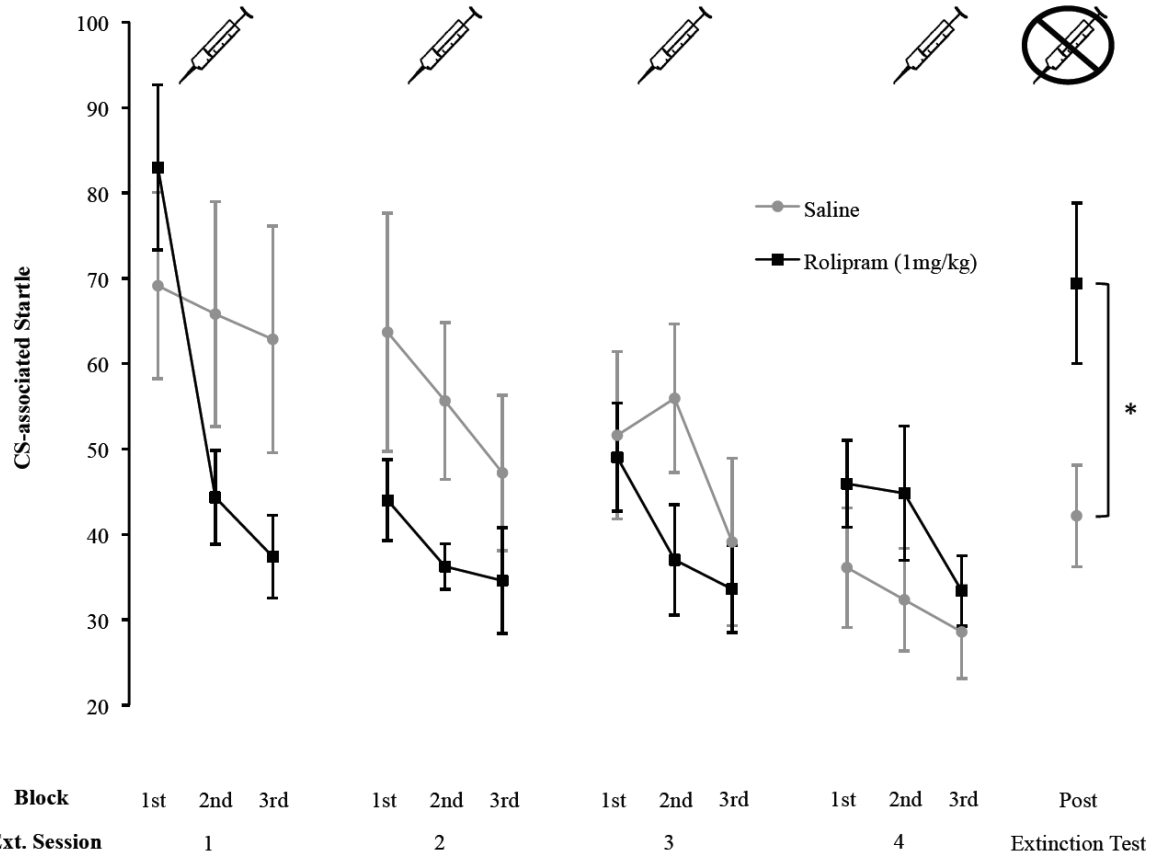


Figure 6: The decline of fear-associated startle responses over four days of extinction training subdivided into three consecutive trial-blocks for each session. While rolipram does not affect within session extinction, increases in startle responses from the last block of one session to the first block of the next session suggest impaired between-session consolidation of fear extinction in the rolipram group (black lines). Consistently, CS-associated startle responses at a final "injection free" Post-Extinction Test indicate that rolipram- but not saline-treated mice still expressed high levels of fear after the extinction treatment.

Discussion

The five experiments of Study 4 revealed that rolipram disturbs expression and extinction consolidation of conditioned fear in mice. Contrary to our expectations, the experimental elevation of cAMP presumably increasing CREB phosphorylation and expression of learning-relevant genes, did not improve acquisition or extinction of fear but – if anything – impaired these processes. This finding contrasts with prior reports that rolipram may serve as a memory enhancer (for review see: Reneerkens, Rutten, Steinbusch, Blokland, & Prickaerts, 2009).

The present study also differs from earlier investigations that found that rolipram boosts the acquisition of fear (Barad et al., 1998; Monti et al., 2006). Of relevance, these studies focussed on contextual fear conditioning, which is crucially dependent on hippocampal processes. Here, however, fear was conditioned to a tone for which the sensory cortices, thalamic nuclei, lateral and basolateral nuclei of the amygdalae compose the core structures (M. Davis, 2006). Similarly, extinction consolidation involves non-hippocampal structures, such as the amygdala and the ventromedial prefrontal cortex (although extinction consolidation of contextual fear likely involves the hippocampus: D. Mueller, Porter, & Quirk, 2008). With regard to hippocampus-independent learning, other studies also indicate that rolipram does not enhance – or may even interfere with – learning (Barad et al., 1998; Gong et al., 2004), suggesting that rolipram may have different effects at different structure-dependent processes. Note that this view supports the herein proposed dynamic multilevel approach to fear and anxiety because it is assumed that the effect of molecular level manipulations (i.e., rolipram) on higher-level phenomena (i.e., extinction, acquisition and expression of fear) may crucially depend on intermediate levels (i.e., involvement of specific structures). In order to identify such intermediate stations on the pathway from molecules to modulation of fear, future extensions of the present study could include the analysis of cAMP and/or pCREB levels in the aforementioned structures.

In contrast to Studies 1 through 3, Study 4 was conducted with mice instead of humans. However, affective modulation of the startle reflex can be found in humans and may be a valid endophenotype for exaggerated fearfulness (Corr et al., 1995; Vaidyanathan, Patrick, & Bernat, 2009). Moreover, rolipram has been considered for pharmacotherapy in humans due to its antidepressant (Li et al., 2009; Wachtel, 1983) and memory enhancing (Reneerkens et al., 2009) properties. Thus, future studies should test whether rolipram has panicolytic and anxiolytic effects in humans as well, and whether it disturbs extinction of fear in humans. While the former may be beneficial in some conditions (e.g., in the treatment of depression with comorbid anxiety disorders), the latter would counter-indicate rolipram treatment during exposure therapy.

On the search for new extinction boosters, the present findings may also be of value – if inhibition of PDE4s impairs the extinction of fear, it could be speculated that cAMP-dependent elevations of PKA hinder extinction. As a result, it could be further speculated that inhibition of PKA ameliorates extinction. Interestingly, Isiegas et al. (Isiegas, Park, Kandel, Abel, & Lattal, 2006) reported that extinction of contextual fear was indeed improved when PKA was inhibited in transgenic mice. With regard to humans, future studies could investigate whether polymorphisms related to cAMP, PKA, and PDE4 are also related to fearfulness and/or exposure therapy outcome success.

Integration and Conclusion

The four studies presented in this thesis independently provided support for a dynamic multilevel account for anxiety-related phenomena (see Table 2). Study 1 showed how medial prefrontal cortex activity (i.e., Structure Level) measured with EEG was related to heart rate (PNS Level) and provided some evidence that this association was dynamically linked to trait anxiety: in conditions of negative but not positive feedback did trait anxiety increase the link between cortical and cardiac activity. This modulation is consistent with the functional definition of anxiety given that negative but not positive feedback is normally associated with increased danger in the future.

Study 2 showed how dopaminergic genes (Molecule Level) and manipulations of dopamine (Synapse Level) presumably affected network states (Network Level), which then influenced brain activity at the AMC (Structure Level) and error-related behavior (Whole System Level). The unexpected finding that trait-anxiety was not related to error monitoring in that study can be explained *post hoc* by task characteristics (Olvet & Hajcak, 2009), again suggesting that some patterns of multilevel interactions are dynamically linked to anxiety.

Study 3 tested individuals with GAD (manifest at the Whole System Level) using a neuropsychological test designed to measure future-orientation in patients with damage of the ventromedial prefrontal cortex (Structure Level) and resulting impairments in neurovisceral connectivity (Bechara et al., 1997) thus affecting the CNS and PNS-Levels. Consistent with (a) the assumed future-orientation of anxiety and (b) increased neurovisceral connectivity in anxiety (Study 1) individuals with GAD performed better in the IGT than non-anxious control participants.

Finally, Study 4 manipulated intracellular signalling cascades (Molecule Level), thereby modulating synaptic learning and extinction learning (Synapse Level), which then affected fear-related reflex potentiation (CNS-Level and Whole Systems Level). In contrast to

prior studies that found improved extinction learning of hippocampus-dependent fear memory (e.g., fear conditioned to a place), Study 4 found that rolipram disturbed extinction learning of presumably hippocampus independent fear-memory (e.g., fear conditioned to a sound). Together with these other studies, Study 4 thus provides further evidence that situational characteristics (place vs. sound as cue for present danger) may influence various levels (including the Molecule Level) with regard to fear processing.

As can be seen in Table 2, some studies covered different levels than others. Of course, the herein proposed subdivision into eight levels of organization should be seen as a flexible framework used for illustrating the multilevel perspective rather than as a rigid model. Future research may uncover that much more levels of organization are needed to explain certain phenomena, and there may also be cases when good predictions can be made based on fewer than eight levels. However, Table 2 also shows that guesses for most empty cells can be made based on existing theories and research findings. A critical exception may be the network level, and it has been noted by others that this level is underrepresented in cognitive neuroscience research. However, the network level may be particularly critical for linking what we know about substances, cells, synapses and neurons (mostly based on in vitro work) to what we know about anxiety relevant structures (based on neuroimaging, EEG and lesion studies). From this perspective, future studies that include the neural network levels when investigating danger-reduction phenomena may be indispensable stations for achieving a wholistic understanding of fear and anxiety.

	Study 1	Study 2	Study 3	Study 4
Danger reduction mechanism	Physiological Adaptation	Error-monitoring	Future-oriented decisions	Reflex potentiation
Whole System Level	Trait anxiety	Post-error slowing	GAD	Startle response
PNS/CNS	Heart Rate	Heart rate change (Hajcak et al., 2003b)	Decision-making Skin conductance change (Bechara et al., 1997)	Potentiation of startle
Systems	Central Autonomous Network (CAN, Benarroch, 1997)	Error-monitoring system (Holroyd & Coles, 2002)	vmPFC-Amygdala connections (Damasio, 1996)	Startle pathway (M. Davis, 2006)
Structure	mPFC	Anterior midcingulate	vmPFC (Bechara et al., 1994)	mPFC/ infralimbic cortex (D. Mueller et al., 2008), Amygdala (M. Davis, 2006)
Network	?	D_2- vs D_1-state (Durstewitz & Seamans, 2008)	?	?
Synapse	Serotonin (van der Veen et al., 2008), Norepinephrine (Nieuwenhuis et al., 2011)	Sulpiride	Serotonin, Dopamine (Bechara et al., 2001)	Norepinephrine (D. Mueller et al., 2008)
Molecule	COMT (unpublished observations)	COMT	5HTTLPR (Stoltenberg & Vandever, 2010)	cAMP, PDE4
Dynamic modulation	Positive vs. negative feedback	Feedback given or not (Olvet & Hajcak, 2009)	Independent of high vs. low risk	Hippocampus-dependent vs. hippocampus-independent

Table 2: The four studies placed in the present multilevel framework. Grey font indicates variables that can be hypothesized to be relevant for the mechanism of interest at this particular neurobiological level although that level was not included into the present analyses. mPFC = medial prefrontal cortex; vmPFC = ventromedial prefrontal cortex; cAMP = cyclic adenosine monophosphate; PDE4 = Phosphodiesterase 4; 5HTTLPR = serotonin transporter linked polymorphic region; GAD = Generalized Anxiety Disorder.

References

Addis, D. R., Wong, A. T., & Schacter, D. L. (2007). Remembering the past and imagining the future: common and distinct neural substrates during event construction and elaboration. *Neuropsychologia, 45*, 1363-1377.

Alain, C., McNeely, H. E., He, Y., Christensen, B. K., & West, R. (2002). Neurophysiological evidence of error-monitoring deficits in patients with schizophrenia. *Cereb Cortex, 12*, 840-846.

Alberini, C. M. (1999). Genes to remember. *J Exp Biol, 202*, 2887-2891.

Anokhin, A. P., Golosheykin, S., & Heath, A. C. (2008). Heritability of frontal brain function related to action monitoring. *Psychophysiology, 45*, 524-534.

Barad, M., Bourtchouladze, R., Winder, D. G., Golan, H., & Kandel, E. (1998). Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci U S A, 95*, 15020-15025.

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition, 50*, 7-15.

Bechara, A., Damasio, H., & Damasio, A. R. (2001). Manipulation of dopamine and serotonin causes different effects on covert and overt decision-making. *Society for Neuroscience Abstracts, 27*, 126.

Bechara, A., Damasio, H., Tranel, D., & Damasio, A. R. (1997). Deciding advantageously before knowing the advantageous strategy. *Science, 275*, 1293-1295.

Bechara, A., Dolan, S., & Hindes, A. (2002). Decision-making and addiction (part II): myopia for the future or hypersensitivity to reward? *Neuropsychologia, 40*, 1690-1705.

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

Benarroch, E. E. (1997). *Central autonomic network: functional organization and clinical correlations*. Armonk, NY: Futura Pub. Co.

Berntson, G. G., Bigger, J. T., Jr., Eckberg, D. L., Grossman, P., Kaufmann, P. G., Malik, M., et al. (1997). Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology, 34*, 623-648.

Berntson, G. G., Sarter, M., & Cacioppo, J. T. (1998). Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behav Brain Res, 94*, 225-248.

Beste, C., Domschke, K., Kolev, V., Yordanova, J., Baffa, A., Falkenstein, M., et al. (2009). Functional 5-HT1a receptor polymorphism selectively modulates error-specific subprocesses of performance monitoring. *Hum Brain Mapp, 31*, 621-630.

Bilder, R. M., Volavka, J., Lachman, H. M., & Grace, A. A. (2004). The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. *Neuropsychopharmacology, 29*, 1943-1961.

Blanchard, D. C., Griebel, G., & Blanchard, R. J. (2001). Mouse defensive behaviors: pharmacological and behavioral assays for anxiety and panic. *Neurosci Biobehav Rev, 25*, 205-218.

Blanchard, R. J., Griebel, G., Henrie, J. A., & Blanchard, D. C. (1997). Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neurosci Biobehav Rev, 21*, 783-789.

Boksem, M. A., Tops, M., Wester, A. E., Meijman, T. F., & Lorist, M. M. (2006). Error-related ERP components and individual differences in punishment and reward sensitivity. *Brain Res, 1101*, 92-101.

Borkovec, T. D. (2002). Life in the future versus life in the present. *Clinical Psychology: Science and Practice, 9*, 76-80.

Borkovec, T. D., Alcaine, O. M., & Behar, E. (Eds.). (2004). *Avoidance Theory of Worry and Generalized Anxiety Disorder*: New York, NY, US: Guilford Press.

Borkovec, T. D., Robinson, E., Pruzinsky, T., & DePree, J. A. (1983). Preliminary exploration of worry: some characteristics and processes. *Behav Res Ther, 21*, 9-16.

Borkovec, T. D., & Sharpless, B. (Eds.). (2004). *Generalized Anxiety Disorder: Bringing Cognitive-Behavioral Therapy into the Valued Present*: New York, NY, US: Guilford Press.

Botvinick, M. M., Braver, T. S., Barch, D. M., Carter, C. S., & Cohen, J. D. (2001). Conflict monitoring and cognitive control. *Psychol Rev, 108*, 624-652.

Bourtchouladze, R., Lidge, R., Catapano, R., Stanley, J., Gossweiler, S., Romashko, D., et al. (2003). A mouse model of Rubinstein-Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. *Proc Natl Acad Sci U S A, 100*, 10518-10522.

Bouton, M. E. (2004). Context and behavioral processes in extinction. *Learn Mem, 11*, 485-494.

Brown, T. A., Chorpita, B. F., & Barlow, D. H. (1998). Structural relationships among dimensions of the DSM-IV anxiety and mood disorders and dimensions of negative affect, positive affect, and autonomic arousal. *J Abnorm Psychol, 107*, 179-192.

Brown, T. A., O'Leary, T. A., & Barlow, D. H. (1993). Generalized anxiety disorder. In D. H. Barlow (Ed.), *Clinical handbook of psychological disorders: A step-by-step treatment manual* (pp. 137–188). New York: The Guilford Press.

Caley, C. F., & Weber, S. S. (1995). Sulpiride: an antipsychotic with selective dopaminergic antagonist properties. *Ann Pharmacother, 29*, 152-160.

Campbell-Sills, L., Liverant, G. I., & Brown, T. A. (2004). Psychometric evaluation of the behavioral inhibition/behavioral activation scales in a large sample of outpatients with anxiety and mood disorders. *Psychol Assess, 16*, 244-254.

Carlezon, W. A., Jr., Duman, R. S., & Nestler, E. J. (2005). The many faces of CREB. *Trends Neurosci, 28*, 436-445.

Carver, C. S., & White, T. L. (1994). Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS Scales. *Journal of Personality and Social Psychology, 67*, 319-333.

Cattell, R. B. (1952). The three basic factor-analytic designs - Their interrelations and derivatives. *Psychological Bulletin, 49*, 499 - 520.

Cavedini, P., Riboldi, G., Keller, R., D'Annucci, A., & Bellodi, L. (2002). Frontal lobe dysfunction in pathological gambling patients. *Biol Psychiatry, 51*, 334-341.

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., et al. (2004). Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet, 75*, 807-821.

Chen, Y. P., Ehlers, A., Clark, D. M., & Mansell, W. (2002). Patients with generalized social phobia direct their attention away from faces. *Behav Res Ther, 40*, 677-687.

Churchland, P. S., & Sejnowski, T. J. (1988). Perspectives on cognitive neuroscience. *Science, 242*, 741-745.

Comery, T. A., Martone, R. L., Aschmies, S., Atchison, K. P., Diamantidis, G., Gong, X., et al. (2005). Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci, 25*, 8898-8902.

Corr, P. J., Wilson, G. D., Fotiadou, M., Kumari, V., Gray, N. S., Checkley, S., et al. (1995). Personality and affective modulation of the startle reflex. *Personality and Individual Differences, 19*, 543-553.

Costa, P. T., & McCrae, R. R. (1992). *The Revised NEO Personality Inventory*. Odessa, FL: Psychological Assesment Resources.

Critchley, H. D. (2005). Neural mechanisms of autonomic, affective, and cognitive integration. *J Comp Neurol, 493*, 154-166.

Critchley, H. D., Mathias, C. J., Josephs, O., O'Doherty, J., Zanini, S., Dewar, B. K., et al. (2003). Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. *Brain, 126*, 2139-2152.

Crone, E. A., van der Veen, F. M., van der Molen, M. W., Somsen, R. J., van Beek, B., & Jennings, J. R. (2003). Cardiac concomitants of feedback processing. *Biol Psychol, 64*, 143-156.

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the pre-frontal cortex. *Philos Trans R Soc Lond B Biol Sci, 351*, 1413-1420.

Davis, M. (2001). Fear-potentiated startle in rats. *Curr Protoc Neurosci, Chapter 8*, Unit 8 11A.

Davis, M. (2006). Neural systems involved in fear and anxiety measured with fear-potentiated startle. *Am Psychol, 61*, 741-756.

Davis, R. L., Cherry, J., Dauwalder, B., Han, P. L., & Skoulakis, E. (1995). The cyclic AMP system and Drosophila learning. *Mol Cell Biochem, 149-150*, 271-278.

de Bruijn, E. R., Hulstijn, W., Verkes, R. J., Ruigt, G. S., & Sabbe, B. G. (2004). Drug-induced stimulation and suppression of action monitoring in healthy volunteers. *Psychopharmacology (Berl), 177*, 151-160.

de Bruijn, E. R., Sabbe, B. G., Hulstijn, W., Ruigt, G. S., & Verkes, R. J. (2006). Effects of antipsychotic and antidepressant drugs on action monitoring in healthy volunteers. *Brain Res, 1105*, 122-129.

Debener, S., Ullsperger, M., Siegel, M., Fiehler, K., von Cramon, D. Y., & Engel, A. K. (2005). Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. *J Neurosci, 25*, 11730-11737.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods, 134*, 9-21.

Depue, R. A., & Lenzenweger, M. F. (2005). A neurobehavioral dimensional model of personality disturbance. In M. L. a. J. Clarkin (Ed.), *Theories of Personality Disorders* (2 ed.). New York: Guilford Press.

Donchin, E. (1981). Presidential address, 1980. Surprise!...Surprise? *Psychophysiology, 18*, 493-513.

Durstewitz, D., & Seamans, J. K. (2008). The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. *Biol Psychiatry, 64*, 739-749.

Egan, M. F., Goldberg, T. E., Kolachana, B. S., Callicott, J. H., Mazzanti, C. M., Straub, R. E., et al. (2001). Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci U S A, 98*, 6917-6922.

Eichele, H., Juvodden, H. T., Ullsperger, M., & Eichele, T. (2010). Mal-adaptation of event-related EEG responses preceding performance errors. *Front Hum Neurosci, 4*, 65.

Epstein, S., & Roupenian, A. (1970). Heart rate and skin conductance during experimentally induced anxiety: the effect of uncertainty about receiving a noxious stimulus. *J Pers Soc Psychol, 16*, 20-28.

Erceg-Hurn, D. M., & Mirosevich, V. M. (2008). Modern robust statistical methods: an easy way to maximize the accuracy and power of your research. *Am Psychol, 63*, 591-601.

Eysenck, M. W. (1992). *Anxiety: The Cognitive Perspective*. Hove: Psychology Press.

Falkenstein, M., Hoormann, J., Christ, S., & Hohnsbein, J. (2000). ERP components on reaction errors and their functional significance: a tutorial. *Biol Psychol, 51*, 87-107.

Fendt, M., & Fanselow, M. S. (1999). The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev, 23*, 743-760.

Fisher, R. A. (1950). *Statistical Methods for Research Workers* (11th ed.). Edinburgh, Great Britain: Oliver and Boyd.

Foa, E. B., & Kozak, M. J. (1986). Emotional processing of fear: exposure to corrective information. *Psychol Bull, 99*, 20-35.

Frank, M. J. (2005). Dynamic dopamine modulation in the basal ganglia: a neurocomputational account of cognitive deficits in medicated and nonmedicated Parkinsonism. *J Cogn Neurosci, 17*, 51-72.

Frank, M. J., D'Lauro, C., & Curran, T. (2007). Cross-task individual differences in error processing: neural, electrophysiological, and genetic components. *Cogn Affect Behav Neurosci, 7*, 297-308.

Frank, M. J., & O'Reilly, R. C. (2006). A mechanistic account of striatal dopamine function in human cognition: psychopharmacological studies with cabergoline and haloperidol. *Behav Neurosci, 120*, 497-517.

Friedman, B. H. (2007). An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biol Psychol, 74*, 185-199.

Garon, N., Moore, C., & Waschbusch, D. A. (2006). Decision making in children with ADHD only, ADHD-anxious/depressed, and control children using a child version of the Iowa Gambling Task. *J Atten Disord, 9*, 607-619.

Gehring, W. J., Coles, M. G., Meyer, D. E., & Donchin, E. (1995). A brain potential manifestation of error-related processing. *Electroencephalogr Clin Neurophysiol Suppl, 44*, 261-272.

Gehring, W. J., Himle, J., & Nisenson, L. G. (2000). Action-monitoring dysfunction in obsessive-compulsive disorder. *Psychol Sci, 11*, 1-6.

Gianaros, P. J., Van Der Veen, F. M., & Jennings, J. R. (2004). Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during work-

ing-memory tasks: Implications for the cortical and subcortical regulation of cardiac autonomic activity. *Psychophysiology, 41*, 521-530.

Gong, B., Vitolo, O. V., Trinchese, F., Liu, S., Shelanski, M., & Arancio, O. (2004). Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J Clin Invest, 114*, 1624-1634.

Graham, F. K., & Clifton, R. K. (1966). Heart-rate change as a component of the orienting response. *Psychol Bull, 65*, 305-320.

Gray, J. A., & McNaughton, N. (2000). *The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septohippocampal System (2nd ed.)* (2 ed.). Oxford, UK: Oxford University Press.

Grillon, C., Ameli, R., Woods, S. W., Merikangas, K., & Davis, M. (1991). Fear-potentiated startle in humans: effects of anticipatory anxiety on the acoustic blink reflex. *Psychophysiology, 28*, 588-595.

Grundler, T. O., Cavanagh, J. F., Figueroa, C. M., Frank, M. J., & Allen, J. J. (2009). Task-related dissociation in ERN amplitude as a function of obsessive-compulsive symptoms. *Neuropsychologia, 47*, 1978-1987.

Hajcak, G., McDonald, N., & Simons, R. F. (2003a). Anxiety and error-related brain activity. *Biol Psychol, 64*, 77-90.

Hajcak, G., McDonald, N., & Simons, R. F. (2003b). To err is autonomic: error-related brain potentials, ANS activity, and post-error compensatory behavior. *Psychophysiology, 40*, 895-903.

Hajcak, G., McDonald, N., & Simons, R. F. (2004). Error-related psychophysiology and negative affect. *Brain Cogn, 56*, 189-197.

Hajcak, G., & Simons, R. F. (2002). Error-related brain activity in obsessive-compulsive undergraduates. *Psychiatry Res, 110*, 63-72.

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science, 297*, 400-403.

Hettema, J. M., An, S. S., Bukszar, J., van den Oord, E. J., Neale, M. C., Kendler, K. S., et al. (2008). Catechol-O-methyltransferase contributes to genetic susceptibility shared among anxiety spectrum phenotypes. *Biol Psychiatry, 64*, 302-310.

Hofmann, S. G. (2007). Enhancing exposure-based therapy from a translational research perspective. *Behav Res Ther, 45*, 1987-2001.

Hofmann, S. G., Meuret, A. E., Smits, J. A., Simon, N. M., Pollack, M. H., Eisenmenger, K., et al. (2006). Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. *Arch Gen Psychiatry, 63*, 298-304.

Hofmann, S. G., & Smits, J. A. (2008). Cognitive-behavioral therapy for adult anxiety disorders: a meta-analysis of randomized placebo-controlled trials. *J Clin Psychiatry, 69*, 621-632.

Holroyd, C. B., & Coles, M. G. (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psychol Rev, 109*, 679-709.

Hunnerkopf, R., Strobel, A., Gutknecht, L., Brocke, B., & Lesch, K. P. (2007). Interaction between BDNF Val66Met and dopamine transporter gene variation influences anxiety-related traits. *Neuropsychopharmacology, 32*, 2552-2560.

Isiegas, C., Park, A., Kandel, E. R., Abel, T., & Lattal, K. M. (2006). Transgenic inhibition of neuronal protein kinase A activity facilitates fear extinction. *J Neurosci, 26*, 12700-12707.

Izard, C. E., & Ackerman, B. P. (2000). Motivational, organizational, and regulatory functions of discrete emotions. In M. Lewis & J. M. Haviland-Jones (Eds.), *Handbook of emotions* (2 ed., pp. 253 - 264). New York: Guilford Press.

Jocham, G., & Ullsperger, M. (2009). Neuropharmacology of performance monitoring. *Neurosci Biobehav Rev, 33*, 48-60.

Joe, K. H., Kim, D. J., Park, B. L., Yoon, S., Lee, H. K., Kim, T. S., et al. (2008). Genetic association of DRD2 polymorphisms with anxiety scores among alcohol-dependent patients. *Biochem Biophys Res Commun, 371*, 591-595.

Keselman, H. J., Algina, J., Lix, L. M., Wilcox, R. R., & Deering, K. N. (2008). A generally robust approach for testing hypotheses and setting confidence intervals for effect sizes. *Psychol Methods, 13*, 110-129.

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Merikangas, K. R., & Walters, E. E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry, 62*, 593-602.

Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry, 62*, 617-627.

Krämer, U. M., Cunillera, T., Camara, E., Marco-Pallares, J., Cucurell, D., Nager, W., et al. (2007). The impact of catechol-O-methyltransferase and dopamine D4 receptor genotypes on neurophysiological markers of performance monitoring. *J Neurosci, 27*, 14190-14198.

Lachman, H. M., Papolos, D. F., Saito, T., Yu, Y. M., Szumlanski, C. L., & Weinshilboum, R. M. (1996). Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. *Pharmacogenetics, 6*, 243-250.

Lang, P. J., Gatchel, R. J., & Simons, R. F. (1975). Electro-cortical and cardiac rate correlates of psychophysical judgment. *Psychophysiology, 12*, 649-655.

LeDoux, J. (2007). The amygdala. *Curr Biol, 17*, R868-874.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science, 274*, 1527-1531.

Li, Y. F., Huang, Y., Amsdell, S. L., Xiao, L., O'Donnell, J. M., & Zhang, H. T. (2009). Antidepressant- and anxiolytic-like effects of the phosphodiesterase-4 inhibitor Rolipram on behavior depend on cyclic AMP response element binding protein-mediated neurogenesis in the hippocampus. *Neuropsychopharmacology, 34*, 2404 - 2419.

Lichtenstein, P., & Annas, P. (2000). Heritability and prevalence of specific fears and phobias in childhood. *J Child Psychol Psychiatry, 41*, 927-937.

Luu, P., Collins, P., & Tucker, D. M. (2000). Mood, personality, and self-monitoring: negative affect and emotionality in relation to frontal lobe mechanisms of error monitoring. *J Exp Psychol Gen, 129*, 43-60.

MacLeod, C., Mathews, A., & Tata, P. (1986). Attentional bias in emotional disorders. *J Abnorm Psychol, 95*, 15-20.

Makeig, S., Bell, A. J., Jung, T. P., & Sejnowski, T. J. (1996). Independent Component Analysis of electroencephalographic data. *Advances in Neural Information Processing Systems, 8*, 145-151.

Matthews, G., & Gilliland, K. (1999). The personality theories of H. J. Eysenck and J. A. Gray: A comparative review. Apr 1999. *Personality and Individual Differences, 26*, 583-626.

Mauri, M. C., Bravin, S., Bitetto, A., Rudelli, R., & Invernizzi, G. (1996). A risk-benefit assessment of sulpiride in the treatment of schizophrenia. *Drug Saf, 14*, 288-298.

McGrath, M., Kawachi, I., Ascherio, A., Colditz, G. A., Hunter, D. J., & De Vivo, I. (2004). Association between catechol-O-methyltransferase and phobic anxiety. *Am J Psychiatry, 161*, 1703-1705.

Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and validation of the Penn State Worry Questionnaire. *Behaviour Research and Therapy, 28*, 487-495.

Mier, D., Kirsch, P., & Meyer-Lindenberg, A. (2009). Neural substrates of pleiotropic action of genetic variation in COMT: a meta-analysis. *Mol Psychiatry, 15*, 918-927.

Miltner, W. H. R., Braun, C. H., & Coles, M. G. H. (1997). Event-related brain potentials following incorrect feedback in a time-estimation task: Evidence for a "generic" neural system for error detection. *Journal of Cognitive Neuroscience, 9*, 788-798.

Monti, B., Berteotti, C., & Contestabile, A. (2006). Subchronic rolipram delivery activates hippocampal CREB and arc, enhances retention and slows down extinction of conditioned fear. *Neuropsychopharmacology, 31*, 278-286.

Mowrer, O. H. (1947). On the dual nature of learning—a re-interpretation of "conditioning" and "problem-solving." *Harvard Educational Review, 17*, 102-148.

Mueller, D., Porter, J. T., & Quirk, G. J. (2008). Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. *J Neurosci, 28*, 369-375.

Mueller, E. M., Ahrens, B., Stemmler, G., Zangl, M., & Wacker, J. (2009). Single trial EEG amplitude predicts subsequent changes in heart rate within individuals – the cardio electroencephalographic covariance trace. *Psychophysiology, 46*, S85.

Mueller, E. M., Hofmann, S. G., & Cherry, J. A. (2010). The type IV phosphodiesterase inhibitor rolipram disturbs expression and extinction of conditioned fear in mice. *Neuropharmacology, 59*, 1-8.

Mueller, E. M., Hofmann, S. G., Santesso, D. L., Meuret, A. E., Bitran, S., & Pizzagalli, D. A. (2008). Electrophysiological evidence of attentional biases in social anxiety disorder. *Psychol Med*, 1-12.

Mueller, E. M., Hofmann, S. G., Santesso, D. L., Meuret, A. E., Bitran, S., & Pizzagalli, D. A. (2009). Electrophysiological evidence of attentional biases in social anxiety disorder. *Psychol Med, 39*, 1141-1152.

Mueller, E. M., Makeig, S., Delorme, A., Stemmler, G., & Wacker, J. (submitted). *Interindividual differences in independent brain components - methodological considerations*. Paper presented at the Psychologie und Gehirn, 2011.

Mueller, E. M., Makeig, S., Stemmler, G., Hennig, J., & Wacker, J. (2010). Both trait anxiety associations and dopamine antagonist-induced reductions of the error-related negativity are moderated by the D2 receptor gene. *Psychophysiology, 47*, S47 - S48.

Mueller, E. M., Nguyen, J., Ray, W., & Borkovec, T. D. (2009). Entscheidungsverhalten bei Generalisierter Angststoerung: Erhoehte Sensitivitaet fuer zukuenftige Verluste. *Zeitschrift für Klinische Psychologie und Psychotherapie, 38*, 50.

Mueller, E. M., Nguyen, J., Ray, W. J., & Borkovec, T. D. (2010). Future-oriented decision-making in Generalized Anxiety Disorder is evident across different versions of the Iowa Gambling Task. *J Behav Ther Exp Psychiatry, 41*, 165-171.

Mueller, E. M., Stemmler, G., Hennig, J., & Wacker, J. (submitted abstract). 5HTTLPR and general condition influence experimentally induced negative affect and trait worry in healthy individuals – evidence for a diathesis-stress interaction (abstract). *Zeitschrift für Klinische Psychologie und Psychotherapie*.

Mueller, E. M., Stemmler, G., & Wacker, J. (2010a). Single-trial electroencephalogram predicts cardiac acceleration: a time-lagged P-correlation approach for studying neurovisceral connectivity. *Neuroscience, 166*, 491-500.

Mueller, E. M., Stemmler, G., & Wacker, J. (2010b). *Studying the brain with the heart cardio-electroencephalographic covariance traces as an alternative to the event-related potential technique?* . Paper presented at the 52. Tagung experimentell arbeitender Psychologen (TeaP).

Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Mol Psychiatry, 12*, 120-150.

Newman, M. G., Zuellig, A. R., Kachin, K. E., Constantino, M. J., Przeworski, A., Erickson, T., et al. (2002). Preliminary reliability and validity of the Generalized Anxiety Disorder Questionnaire-IV: A revised self-report diagnostic measure of generalized anxiety disorder. *Behavior Therapy, 33*, 215 - 233.

Nieuwenhuis, S., De Geus, E. J., & Aston-Jones, G. (2011). The anatomical and functional relationship between the P3 and autonomic components of the orienting response. *Psychophysiology, 48*, 162-175.

Nieuwenhuis, S., Nielen, M. M., Mol, N., Hajcak, G., & Veltman, D. J. (2005). Performance monitoring in obsessive-compulsive disorder. *Psychiatry Res, 134*, 111-122.

Notebaert, W., Houtman, F., Opstal, F. V., Gevers, W., Fias, W., & Verguts, T. (2009). Post-error slowing: an orienting account. *Cognition, 111*, 275-279.

Olvet, D. M., & Hajcak, G. (2008). The error-related negativity (ERN) and psychopathology: toward an endophenotype. *Clin Psychol Rev, 28*, 1343-1354.

Olvet, D. M., & Hajcak, G. (2009). The effect of trial-to-trial feedback on the error-related negativity and its relationship with anxiety. *Cogn Affect Behav Neurosci, 9*, 427-433.

Ostendorf, F., & Angleitner, A. (2004). *NEO-Persönlichkeitsinventar nach Costa und McCrae, revidierte Form (NEO-PI-R)*. Göttingen, Germany: Hogrefe.

Otten, L. J., Gaillard, A. W., & Wientjes, C. J. (1995). The relation between event-related brain potential, heart rate, and blood pressure responses in an S1-S2 paradigm. *Biol Psychol, 39*, 81-102.

Pailing, P. E., & Segalowitz, S. J. (2004). The error-related negativity as a state and trait measure: motivation, personality, and ERPs in response to errors. *Psychophysiology, 41*, 84-95.

Palmatier, M. A., Kang, A. M., & Kidd, K. K. (1999). Global variation in the frequencies of functionally different catechol-O-methyltransferase alleles. *Biol Psychiatry, 46*, 557-567.

Perkins, A. M., & Corr, P. J. (2005). Can worriers be winners? The association between worrying and job performance. *Personality and Individual Differences, 38*, 25-31.

Perkins, A. M., Ettinger, U., Davis, R., Foster, R., Williams, S. C., & Corr, P. J. (2009). Effects of Lorazepam and citalopram on human defensive reactions: ethopharmacological differentiation of fear and anxiety. *J Neurosci, 29*, 12617-12624.

Perkins, A. M., Kemp, S. E., & Corr, P. J. (2007). Fear and anxiety as separable emotions: an investigation of the revised reinforcement sensitivity theory of personality. *Emotion, 7*, 252-261.

Philibert, R. A., Beach, S. R., Gunter, T. D., Brody, G. H., Madan, A., & Gerrard, M. (2010). The effect of smoking on MAOA promoter methylation in DNA prepared from lymphoblasts and whole blood. *Am J Med Genet B Neuropsychiatr Genet, 153B*, 619-628.

Rabbitt, P. M., & Phillips, S. (1967). Error-detection and correction latencies as a function of S-R compatibility. *Q J Exp Psychol, 19*, 37-42.

Randt, C. T., Judge, M. E., Bonnet, K. A., & Quartermain, D. (1982). Brain cyclic AMP and memory in mice. *Pharmacol Biochem Behav, 17*, 677-680.

Reneerkens, O. A., Rutten, K., Steinbusch, H. W., Blokland, A., & Prickaerts, J. (2009). Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. *Psychopharmacology (Berl), 202*, 419-443.

Rushby, J. A., Barry, R. J., & Doherty, R. J. (2005). Separation of the components of the late positive complex in an ERP dishabituation paradigm. *Clin Neurophysiol, 116*, 2363-2380.

Rutten, K., Basile, J. L., Prickaerts, J., Blokland, A., & Vivian, J. A. (2008). Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology (Berl), 196*, 643-648.

Santesso, D. L., Meuret, A. E., Hofmann, S. G., Mueller, E. M., Ratner, K. G., Roesch, E. B., et al. (2008). Electrophysiological correlates of spatial orienting towards angry faces: a source localization study. *Neuropsychologia, 46*, 1338-1348.

Schacter, D. L., & Addis, D. R. (2007). The cognitive neuroscience of constructive memory: remembering the past and imagining the future. *Philos Trans R Soc Lond B Biol Sci, 362*, 773-786.

Segalowitz, S. J., Santesso, D. L., Murphy, T. I., Homan, D., Chantziantoniou, D. K., & Khan, S. Retest reliability of medial frontal negativities during performance monitoring. *Psychophysiology, 47*, 260-270.

Serra, G., Forgione, A., D'Aquila, P. S., Collu, M., Fratta, W., & Gessa, G. L. (1990). Possible mechanism of antidepressant effect of L-sulpiride. *Clin Neuropharmacol, 13 Suppl 1*, S76-83.

Sidman, M. (1953). Avoidance conditioning with brief shock and no exteroceptive warning signal. *Science, 118*, 157-158.

Silvestre, J. S., Fernandez, A. G., & Palacios, J. M. (1999). Effects of rolipram on the elevated plus-maze test in rats: a preliminary study. *J Psychopharmacol, 13*, 274-277.

Smoski, M. J., Lynch, T. R., Rosenthal, M. Z., Cheavens, J. S., Chapman, A. L., & Krishnan, R. R. (2008). Decision-making and risk aversion among depressive adults. *J Behav Ther Exp Psychiatry, 39*, 567-576.

Spear, J. F., Kronhaus, K. D., Moore, E. N., & Kline, R. P. (1979). The effect of brief vagal stimulation on the isolated rabbit sinus node. *Circ Res, 44*, 75-88.

Stein, D. J. (2001). Comorbidity in generalized anxiety disorder: impact and implications. *J Clin Psychiatry, 62 Suppl 11*, 29-34.

Stein, D. J., Westenberg, H. G., & Liebowitz, M. R. (2002). Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. *J Clin Psychiatry, 63 Suppl 6*, 12-19.

Stein, M. B., Fallin, M. D., Schork, N. J., & Gelernter, J. (2005). COMT polymorphisms and anxiety-related personality traits. *Neuropsychopharmacology, 30*, 2092-2102.

Stein, M. B., Jang, K. L., & Livesley, W. J. (1999). Heritability of anxiety sensitivity: a twin study. *Am J Psychiatry, 156*, 246-251.

Stemmler, G. (2004). Physiological Process During Emotion. In P. Philippot & R. S. Feldman (Eds.), *The Regulation of Emotion*. Mahwah, New Jersey: Lawrence Erlbaum Associates.

Stoltenberg, S. F., & Vandever, J. M. (2010). Gender moderates the association between 5-HTTLPR and decision-making under ambiguity but not under risk. *Neuropharmacology, 58*, 423-428.

Tellegen, A., & Waller, N. G. (2008). Exploring personality through test construction: Development of the multidimensional personality questionnaire. In G. J. Boyle, G. Matthews & D. H. Saklofske (Eds.), *The Sage handbook of personality and assessment* (Vol. 2, pp. 161-292). London: Sage.

Thayer, J. F., & Lane, R. D. (2009). Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci Biobehav Rev, 33*, 81-88.

Ullsperger, M. (2010). Genetic association studies of performance monitoring and learning from feedback: the role of dopamine and serotonin. *Neurosci Biobehav Rev, 34*, 649-659.

Vaidyanathan, U., Patrick, C. J., & Bernat, E. M. (2009). Startle reflex potentiation during aversive picture viewing as an indicator of trait fear. *Psychophysiology, 46*, 75-85.

van der Veen, F. M., Mies, G. W., van der Molen, M. W., & Evers, E. A. (2008). Acute tryptophan depletion in healthy males attenuates phasic cardiac slowing but does not affect electro-cortical response to negative feedback. *Psychopharmacology (Berl), 199*, 255-263.

Vogt, B. A. (2005). Pain and emotion interactions in subregions of the cingulate gyrus. *Nat Rev Neurosci, 6*, 533-544.

Wachtel, H. (1983). Potential antidepressant activity of rolipram and other selective cyclic adenosine 3',5'-monophosphate phosphodiesterase inhibitors. *Neuropharmacology, 22*, 267-272.

Wacker, J., & Gatt, J. M. (2010). Resting posterior versus frontal delta/theta EEG activity is associated with extraversion and the COMT VAL(158)MET polymorphism. *Neurosci Lett, 478*, 88-92.

Wacker, J., Mueller, E. M., Hennig, J., & Stemmler, G. (under revision). How to consistently link extraversion and intelligence to the Catechol-O-Methyltransferase (COMT) Gene: On defining and measuring psychological phenotypes in neurogenetic research. *Journal of Personality and Social Psychology*.

Wacker, J., Reuter, M., Hennig, J., & Stemmler, G. (2005). Sexually dimorphic link between dopamine D2 receptor gene and neuroticism-anxiety. *Neuroreport, 16*, 611-614.

Wager, T. D., van Ast, V. A., Hughes, B. L., Davidson, M. L., Lindquist, M. A., & Ochsner, K. N. (2009). Brain mediators of cardiovascular responses to social threat, Part II: Prefrontalsubcortical pathways and relationship with anxiety. *Neuroimage, 47*, 836-851.

Wager, T. D., Waugh, C. E., Lindquist, M., Noll, D. C., Fredrickson, B. L., & Taylor, S. F. (2009). Brain mediators of cardiovascular responses to social threat Part I: Reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. *Neuroimage, 47*, 821-835.

Walker, D. L., Ressler, K. J., Lu, K. T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci, 22*, 2343-2351.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol, 54*, 1063-1070.

Watson, J. B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology, 3*, 1-14.

Weinshilboum, R. M., Otterness, D. M., & Szumlanski, C. L. (1999). Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu Rev Pharmacol Toxicol, 39*, 19-52.

Werner, N. S., Duschek, S., & Schandry, R. (2009). Relationships between affective states and decision-making. *Int J Psychophysiol, 74*, 259-265.

Winslow, J. T., Parr, L. A., & Davis, M. (2002). Acoustic startle, prepulse inhibition, and fear-potentiated startle measured in rhesus monkeys. *Biol Psychiatry, 51*, 859-866.

Wohr, M., Borta, A., & Schwarting, R. K. (2005). Overt behavior and ultrasonic vocalization in a fear conditioning paradigm: a dose-response study in the rat. *Neurobiol Learn Mem, 84*, 228-240.

Wray, N. R., James, M. R., Dumenil, T., Handoko, H. Y., Lind, P. A., Montgomery, G. W., et al. (2008). Association study of candidate variants of COMT with neuroticism, anxiety and depression. *Am J Med Genet B Neuropsychiatr Genet, 147B*, 1314-1318.

Zhang, H. T., Huang, Y., Jin, S. L., Frith, S. A., Suvarna, N., Conti, M., et al. (2002). Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. *Neuropsychopharmacology, 27*, 587-595.

Zirnheld, P. J., Carroll, C. A., Kieffaber, P. D., O'Donnell, B. F., Shekhar, A., & Hetrick, W. P. (2004). Haloperidol impairs learning and error-related negativity in humans. *J Cogn Neurosci, 16*, 1098-1112.

Zohar, J., & Westenberg, H. G. (2000). Anxiety disorders: a review of tricyclic antidepressants and selective serotonin reuptake inhibitors. *Acta Psychiatr Scand Suppl, 403*, 39-49.

Zuckerman, M. (2002). Zuckerman-Kuhlman personality questionnaire (ZKPQ): An alternative five-factorial model. In R. Bd & P. M (Eds.), *Big Five Assessment*. Göttingen, Germany: Hogrefe & Huber.

Empirical Studies

Study 1

Mueller, E. M., Stemmler, G., & Wacker, J. (2010). Single-trial EEG predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity. *Neuroscience, 166,* 491-500.

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

Neuroscience xx (2009) xxx

SINGLE-TRIAL ELECTROENCEPHALOGRAM PREDICTS CARDIAC ACCELERATION: A TIME-LAGGED P-CORRELATION APPROACH FOR STUDYING NEUROVISCERAL CONNECTIVITY

E. M. MUELLER,* G. STEMMLER AND J. WACKER

Philipps-Universitaet Marburg, Faculty of Psychology, Gutenbergstr. 18, 35032 Marburg, Germany

Abstract—Cortical efferences to the heart are important for cardiovascular health, psychopathology, emotion regulation and other dimensions of human functioning. Although researchers have already begun to outline the underlying neuroanatomy, the timing of neurovisceral communication in humans is difficult to study non-invasively. A possible coupling between the brain and the heart can be observed following feedback stimuli, which have been shown to evoke both, early (i.e. <500 ms) signatures in the electroencephalogram (EEG) and changes in the chronotropy of subsequent heart beats. Because standard approaches may be insufficient to study how these responses are related, we suggest a method termed "Cardio–Electroencephalographic Covariance Tracing" (CECT), which is based on time-lagged P-correlations (i.e., correlations within individuals) between single-trial EEG magnitudes and heart period changes. When CECT was applied to data from *n*=31 individuals who performed a gambling task, central midline EEG magnitudes from 280 to 340 ms after feedback reliably P-correlated with cardiac acceleration 2 to 5 s thereafter. In addition positive vs. negative feedback lead to enhanced event related potential amplitudes from 200 to 280 ms and to relative cardiac acceleration from 1 to 3.5 s after feedback presentation. The results imply that neurogenic cardiac modulations begin to be affected 200 to 400 ms after stimulus presentation and demonstrate the utility of CECTs for future investigations. **© 2009 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: EEG, feedback related negativity, P300, P2a, heart period, P-correlation.

The relationships between the activity of the brain and the cardiovascular system are highly complex (Thayer and Lane, 2009) and include multiple descending (e.g., Wager et al., 2009) and ascending (Rau and Elbert, 2001) pathways. Cortico–cardiac and/or cardio–cortical connections play important roles in recent psychophysiological models of anxiety (Berntson et al., 1998; Friedman, 2007), emotion regulation (Thayer and Lane, 2000, 2009), social (Porges et al., 1996) and psychopathological (Beauchaine, 2001) development and may play a role in cardiovascular disease (Thayer and Lane, 2007). Numerous functional magnetic resonance imaging (fMRI), positron emission tomography, neurological, and animal studies have established a dominant role of the medial frontal cortex in neurogenic autonomic regulation in general (Critchley et al., 2005; Damasio, 1996) and cardiovascular modulation in particular (Critchley et al., 2003; Gianaros et al., 2004; Wager et al., 2009). In addition, an extended network of more posterior and subcortical structures including insula, amygdala, hypothalamus, periaqueductal gray, and other regions is implicated in autonomic control (Benarroch, 1997; Thayer and Lane, 2009; Wager et al., 2009).

A widely used indicator for cardiovascular autonomic activity is heart period (HP), which is cortically modulated via the nervus vagus and the sympathetic branch of the autonomic nervous system, both projecting to the sinoatrial node. While sympathetic activations yield decreases in HP with a delay of several seconds, vagal bursts trigger quick increases in HP levels (Berntson et al., 1997). Moreover, high frequency oscillatory HP modulations covary with respiratory phase (respiratory sinus arrhythmia), a phenomenon, which reflects phasic vagal cardiac control (Berntson et al., 2007).

Whether cortico–cardiac or cardio–cortical relationships can be found in the human electroencephalogram (EEG), has been an active area of research (e.g., Dirlich et al., 1998; Elbert et al., 1992; Groen et al., 2007; Kubota et al., 2001; Lacey and Lacey, 1970; Lang et al., 1975; Schandry and Montoya, 1996; van der Veen et al., 2008) that may ultimately yield important information about the timing of neurogenic autonomic changes and may supplement fMRI approaches (Critchley, 2005; Wager et al., 2009), which have lower temporal resolution.

Phenomenological similarities between early event related potential (ERP) components and evoked cardiac responses have recently been reported with regard to feedback processing (Crone et al., 2003; Groen et al., 2007). For example, feedback evokes (a) mediofrontal EEG components about 200 to 300 ms post stimulus such as the feedback related negativity (FRN; Miltner et al., 1997; Sato et al., 2005) and the P2a (Potts et al., 2006), (b) modulations of the P300 (Hajcak et al., 2005; Sato et al., 2005), and (c) changes in HP (Crone et al., 2003; Hajcak et al., 2003; Somsen et al., 2000). More specifically, negative vs. positive feedback evokes a more negative FRN (Gehring and Willoughby, 2002; Miltner et al., 1997; Potts et al., 2006) and cardiac deceleration within the next cardiac cycles (Crone et al., 2003; Hajcak et al., 2003; Somsen et

*Corresponding author. Tel: +49-6421-282-3555; fax: +49-6421-282-6559.
E-mail address: erik.mueller@staff.uni-marburg.de (E. M. Mueller).

Abbreviations: CECT, cardio–electroencephalographic covariance trace; ECG, electrocardiogram; EEG, electroencephalography; EOG, electrooculogram; ERP, event related potential; fMRI, functional magnetic resonance imaging; FRN, feedback related negativity; HP, heart period.

0306-4522/09 $ - see front matter © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

al., 2000; van der Veen et al., 2004). In addition, increasing magnitudes of reward or punishment may have accelerator effects on HP (Tranel et al., 1982) and potentiate the P300 (Sato et al., 2005). Given that both, regions implicated in HP control (Critchley et al., 2000; Wager et al., 2009) and the sources of P2a (Potts et al., 2006) and FRN (Holroyd and Coles, 2008; Miltner et al., 1997), have been mapped to medial frontal cortices including the anterior cingulate cortex, there may even be a structural overlap of the generators of feedback-related ERP components and of neurogenic HP changes. Because feedback stimuli in real-life often require quick behavioral and autonomic responses, a close connection between systems that evaluate external feedback and systems that control the viscera may be evolutionarily adaptive.

Previous investigations using R-correlations (i.e., correlation of variables across individuals, see Cattell, 1952) between EEG components and HP changes have yielded mixed or null findings (Hajcak et al., 2003; Otten et al., 1995; Palomba et al., 1997; van der Veen et al., 2000; Weisz and Czigler, 2006). However, due to differences, for example, in HP reactivity (Turner, 1989) and cortical folding (Zilles et al., 1988), individuals vary greatly in phasic HP modulations and ERP amplitudes. Moreover the slope of the regression between HP modulations and ERP amplitudes may differ between individuals. These and other sources of between-subjects variance would necessarily obscure correlations between HP and EEG when the R-technique is applied. Moreover, relationships between aggregated measures (such as standard ERPs and evoked HP responses averaged across trials) at one level (i.e. the between subjects level) cannot be attributed to the lower level (i.e. the within subjects-level) because this would introduce an aggregation bias (e.g. Tabachnik and Fidell, 2006). In contrast, P-correlations (i.e., correlation of variables across situations) tapping within-subjects variance and covariance only (Stemmler, 1992) may be more efficient for the investigation of intraindividual cortico–cardiac mechanisms. Two issues emerge, however, with the P-technique in the present context: First, correlating across situations would require measuring ERP amplitudes of single events. Single trial EEG however has a very poor signal to noise ratio and some components can hardly be detected and measured without averaging. Second, a P-correlation identified within one individual does not permit to draw conclusions about EEG-HP relationships in the population.

In order to address these issues while exploiting the advantages of the P-technique, we suggest a technique that we term “cardio–electroencephalographic covariance tracing” (CECT). CECT can be used to systematically explore whether there are time windows in event-locked single-trial EEG magnitudes that are linearly related to evoked HP changes within individuals. This is achieved by applying a two-level approach, in which for each individual during a narrow time window EEG magnitude is P-correlated (i.e., across trials) with HP changes (first-level) and then tested for significance across individuals (second level). Importantly, different time windows (i.e., lags) in the EEG recording are P-correlated with different lags in the HP recording yielding covariance traces with two independent time dimensions[1]. We hypothesized that there are particular temporo–temporal positions in which the magnitude of a CECT (reflecting a particular P-correlation between an EEG time window and an HP time window) was different from zero.

EXPERIMENTAL PROCEDURES

Participants

A total of $n=39$ right-handed psychology students (19–31 years) participated in this study in partial fulfillment of course requirements. They provided informed written consent and guaranteed that (a) no alcohol was consumed within the last 24 hours and (b) neither nicotine nor caffeine was consumed within the last 5 hours. The study protocol was approved by the ethics committee of the German Society for Psychology (Ethik-Kommission der Deutschen Gesellschaft für Psychologie). Due to bad EEG ($n=4$) or electrocardiogram (ECG) ($n=4$) recordings a total of $n=8$ individuals had to be excluded from analyses, yielding a total of $n=31$ participants (14 female; average age, 22.6 years; SD=3.2 years).

Procedure

Participants reported to the laboratory and provided informed consent. They then filled out personality measures while EEG and ECG electrodes were applied. Afterwards, participants were brought to the experimental room, where they were instructed to sit and relax for 10 min. During this baseline phase EEG was recorded (data reported elsewhere, see Wacker et al., unpublished observation). After the resting phase the gambling task began. Then participants performed another task unrelated to this study, and completed another resting phase before they were debriefed.

Task and stimuli

Participants performed a gambling task adapted from Sato et al. (2005). A trial began with a white fixation cross presented for 500 ms in front of a black background and followed by a number signaling the amount of money that could be gained or lost on that trial (i.e. “0,” “10,” or “50” cent) for 500 ms. Participants then saw a cue card with a face value of either 4 or 5. They were instructed to press the left button if they believed that the next card would have a higher value, and the right button, if they believed the next card would have a smaller value. As soon as the participants made a response, the cue card disappeared, and 3 s later, a feedback stimulus (500 ms) signaled that the previous decision was either correct (“O”) or incorrect (“X”). Accordingly, the participant either won or lost the amount of money previously shown. Participants started with an amount of €5 and were told to try to win as much as possible. The task had a fixed outcome with equal numbers of losses and gains, which was unknown to the participants. Trials were separated by 5 s intervals. The task consisted of six blocks of 60 trials each, 10

[1] Note that it was not the goal to identify components in single trial EEG (which would allow to measure an amplitude), but instead to measure the average voltages within small-time windows even if no components were visible (thus measuring a magnitude). The underlying logic is that even though each magnitude reflects a mixture of signal and noise, only signal should systematically covary with another variable (e.g., heart period). Accordingly, time windows, where the magnitude contains relatively higher signal to noise ratios, should show higher correlations (see also discussion).

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

of which were dummy trials with a face value of 2, 3, 6, or 7, which were not used for analysis.

EEG recording

EEG was measured with 13 InVivo-Metrics (Healdsburg, CA, USA) Ag–AgCl electrodes (F3, F4, Fz, Fzc, C3, C4, PCz, P3, P4, Pz, left and right mastoid; all referenced to Cz) using Easy Cap electrode caps (Falk Minow Services, Herrsching–Breitbrunn, Germany). Impedance at all channels was kept below 5 kΩ for the EEG electrodes and below 1 kΩ for an additional ground electrode placed on the forehead by cleaning the skin with alcohol and treating it with a mild abrasive. To record eye blinks and vertical eye movements electrooculogram (EOG) was recorded with electrodes midline above and below the right eye (vertical EOG) and on the outer canthi of both eyes (horizontal EOG). In the experimental room the signal was fed into a head box where it was preamplified at a gain of 30 and sent to the recording room. There, a 32-channel SynAmps 5083 amplifier (NeuroScan, Sterling, VA, USA) amplified the EEG with a gain of 500 (including preamplification) and filtered the signal with a 1–50 Hz analog bandpass filter. A Macintosh Power Mac G4/450 (Apple, Cupertino, CA, USA) with a PCI 6503 SCSI card (National Instruments, Austin, TX, USA) performed recording and storage of the digitized EEG data under Labview 5.0 (National Instruments, Austin, TX, USA) at a sampling rate of 2000 Hz.

ECG recording

ECG was recorded with Ag/AgCl surface electrodes from VivoMed (Servoprax, Wesel, Germany) applied in a lead II configuration (right forearm, left leg) and connected to a Biopac MP100 system with an ECG100c amplifier module (Goleta, CA, USA). Analog high- and low-pass filters were set to 0.5 and 35 Hz, respectively, amplification was set to a gain of 1000, and the signal was recorded at a sampling rate of 1000 Hz using Labview-based software.

Data reduction and analysis

EEG. The EEG signal was downsampled to 250 Hz, re-referenced to linked mastoids and visually screened for artifacts using an adaptation of EEGLAB (Delorme and Makeig, 2004), which allowed simultaneous screening of EEG and HP. Whenever there was a non-blink artifact in either EEG or HP the entire trial was excluded from further analyses. Eye movement artifacts were removed using independent component analysis implemented in EEGLAB. The EEG was then segmented into epochs from −200 to 2000 ms relative to feedback markers. For ERP and CECT analyses each segment was further subdivided into 110, 20 ms long bins (i.e., lags). For each bin, the average signal amplitude was calculated. In order to compute ERPs, segments were averaged separately for trials with negative and positive feedback (each condition >150 trials) and the magnitudes of the five bins from 200 to 300 ms at channel Cz were used for statistical analyses of the FRN.

HP. R-waves in the ECG were automatically detected using the algorithm implemented in Brain Vision Analyzer 1 (Brain Products, Germany). The resulting cardiotach (plotting heart periods against time) was manually screened for artifacts. It was then segmented into epochs from 0 to 5000 ms relative to feedback markers and for baseline correction the HP at feedback onset was subtracted. For CECT and statistical analyses each 5000 ms segment was subdivided into 10 bins reflecting the average HPs of 500 ms epochs.

CECTs and statistical analyses. Lagged P-correlations were computed for each participant. For that purpose each of the 110 EEG-bins was correlated with each of the 10 IBI-bins across trials according to Equation 1

$$r_a(d,c)=1/k\sum_{i=1}^{k}\frac{(EEG_{aic}-\overline{EEG}_{ac})(IBI_{aid}-\overline{IBI}_{ad})}{S_{ac}S_{ad}} \quad (1)$$

where EEG_{aic} denotes the mean EEG amplitude for one 20 ms bin in lag c (running from 1 to 110) and trial i (running from 1 to k) of participant a and IBI_{aid} denotes the HP change for a 500 ms bin in lag d (running from 1 to 10) and trial i of the same participant. Thus, for each individual and each EEG channel resulted a matrix with 10×110 P-correlations. Correlations were either computed across all trials (k>300), or separately for positive and negative feedback trials (k>150).

Fisher transformed (Fisher, 1950) P-correlations were then tested against zero over participants with one-sample t-tests. To compare P-correlations of ERPs and HP changes with regard to positive vs. negative feedback, paired t-tests were used. Because for the CECTs there were 10×110 P-correlations for each channel a conservative Bonferroni-corrected alpha of .01/1100=.000009 was chosen as a statistical threshold for all CECTs. For all other measures an alpha of .05 (two-tailed) was applied. CECT-analyses were conducted using routines coded in MATLAB Version 7.5.0 (Mathworks, Natick, MA, USA). All other statistical analyses were conducted using Stata Version 10.0 (StataCorp, College Station, TX, USA).

RESULTS

ERP-components

As shown in Fig. 1, positive and negative feedback evoked a positive complex from 140 to 380 ms. Around 200 ms this complex was maximal at Cz and FCz and propagated to posterior electrodes with a maximum at Pz around 300 ms (Fig. 3c). Within that extended waveform there was a time window from 200 to 260 ms in which positive compared to negative feedback elicited a more positive magnitude (Cz: 200–220 ms: $t(30)=6.96$, $P<.0001$; 220–240 ms: $t(30)=5.18$, $P<.0001$; 240–260 ms: $t(30)=3.06$, $P<.005$), but one bin later this pattern was reversed (280–300 ms: $t(30)=-2.9$, $P<.007$). In addition to the positive waveform,

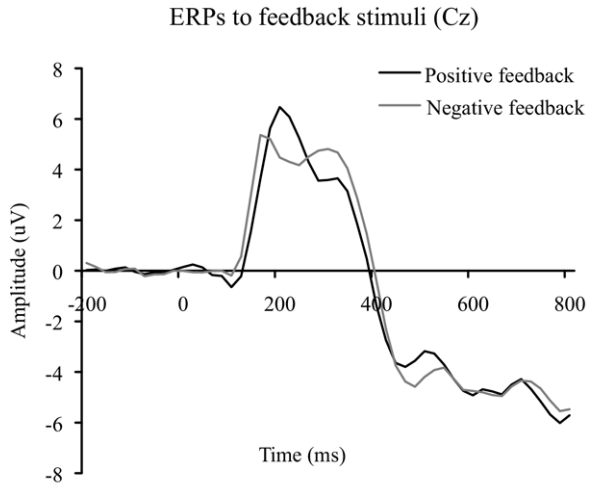


Fig. 1. Grand average event related potentials (ERPs) to positive (black) and negative (grey) feedback measured at Cz.

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

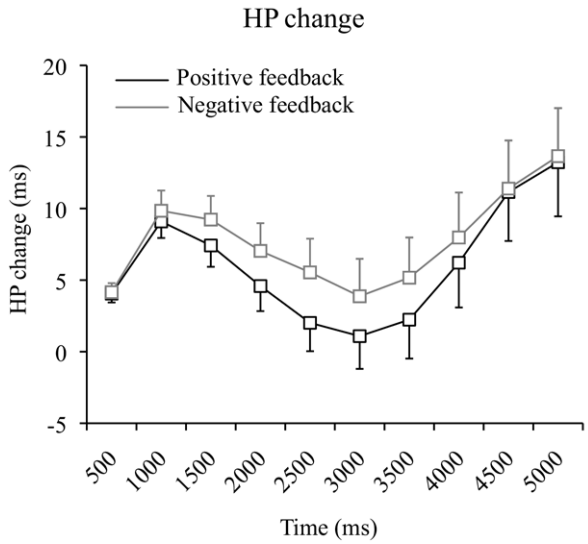


Fig. 2. Changes in heart period locked to positive (black) and negative (grey) feedback stimuli averaged across individuals. Error bars represent the standard error of the mean.

a subsequent negative complex was present from about 400 to 1100 ms. This component also showed a frontal maximum during early, and a posterior maximum at later stages.

HP

Feedback triggered a sinusoidal change in HP (Fig. 2). Negative compared to positive feedback led to relative cardiac deceleration in all five 500 ms bins between 1000 and 3500 ms after stimulus onset (all *P*s$<$.05).

CECT

CECTs for channel FCz are displayed in Fig. 3. The color represents the magnitude of the *z*-transformed P-correlation averaged across individuals, while the horizontal and vertical axes reflect lags in the EEG (in steps of 20 ms) and in the HP recording (in steps of 500 ms), respectively. Fig. 3a shows *t*-values for intraindividual correlations across all 300 trials (not separated with regard to feedback type). Two clusters can be visually identified. The blue upper left cluster indicates that EEG magnitude from about 200 to 400 ms after feedback negatively correlated with HP change from 2000 to 5000 ms after feedback. When CECTs where thresholded with a Bonferroni-corrected *P*-value of $P<.01$, significant *t*-values within that cluster emerged for electrodes FCz, C4, Fz, F4 and Cz (in descending order of maximum *t*-value) with maximum absolute *t*-value at electrode FCz ($t(30)=-6.22$). Temporally, the cluster extended from EEG between 280 and 340 ms and HP changes between 3000 and 5000 ms. Because low HP values reflect a high heart rate, this negative correlation indicates that the larger the ERP magnitude from 280 to 340 ms the larger was the cardiac acceleration 3 to 5 s later. We label this correlation cluster as N300_4 (i.e., negative correlations around lags 300 ms and 4 s for EEG and HP, respectively). In addition to N300_4, a later window of the EEG (460 to 840 ms) showed significant positive correlations with HP from 2500 to 5000 ms (red upper right cluster, P600_4, maximum *t*-value at electrode F4: $t(30)=7.72$, $P<.01$, Bonferroni-corrected)[2].

Fig. 3b displays a CECT for a fixed HP window of 3500 to 4000 ms. As can be seen, later portions of the CECT-waveforms have a similar morphology as the ERP-waveforms. Like ERPs, CECTs have components with amplitudes (i.e., points of maximum EEG-HP correlations), which can be measured for each individual. The spatial distribution of N300_4 is displayed in Fig. 3c. P-correlations, sorted with regard to their *t*-value, are maximal at frontocentral sites with a tendency for larger values in the right hemisphere. In contrast, it should be noted that ERP-magnitudes at the same time range are maximal at parietal electrodes.

Fig. 3d shows correlations that were computed separately for positive and negative feedback. The temporal distribution and the magnitudes of correlations were similar for positive and negative feedback types. P-correlations between EEG and HP did not differ significantly after positive vs. negative feedback (*P*s$>$.05, Bonferroni-corrected).

Supplementary control analyses

To rule out potential alternative explanations for the observed associations we conducted several control analyses. First, we wanted to know whether EEG magnitude around 200 to 400 ms specifically predicted the HP change of that particular trial. We therefore correlated the EEG magnitudes with the HP change of the subsequent trial instead of the same trial. However, when EEG was correlated with HP in the next trial, N300_4 and P600_4 associations completely disappeared suggesting that the reported correlations were trial specific and not driven by carry-over effects from preceding trials or by slow fluctuations of both ERPs and HP across several trials in a row.

Second, due to habituation, EEG responses and HP changes may be smaller at later vs. earlier trials (Rushby et al., 2005). In this case, low (high) EEG magnitudes would be automatically paired with low (high) HP changes and a positive correlation would emerge only due to concurrent habituation in both systems. To rule out this alternative explanation we first linearly detrended the HP signal to remove effects of time on baseline HP. To remove effects of time on the evoked changes in HP we then partialled out the centered trial indices (running from -150 to 150 for the $n=300$ trials) from the HP changes (i.e. the differences between HP at onset and HP at a particular lag after feedback) using a second-degree polynomial (i.e. for each lag and each individual we fitted a cubic regression where the trial index served as predictor). When we repeated the CECT analyses using the residualized HP changes the findings did not change substantially (Ps for

[2] The averages of the Fisher's *z* transformed P-correlations across participants for N300_4 and P600_4 (measured at the spatiotemporo-temporal positions of the maximum *t*-values) were FZ(r)$=-.079$ and FZ (r)$=.061$, respectively.

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

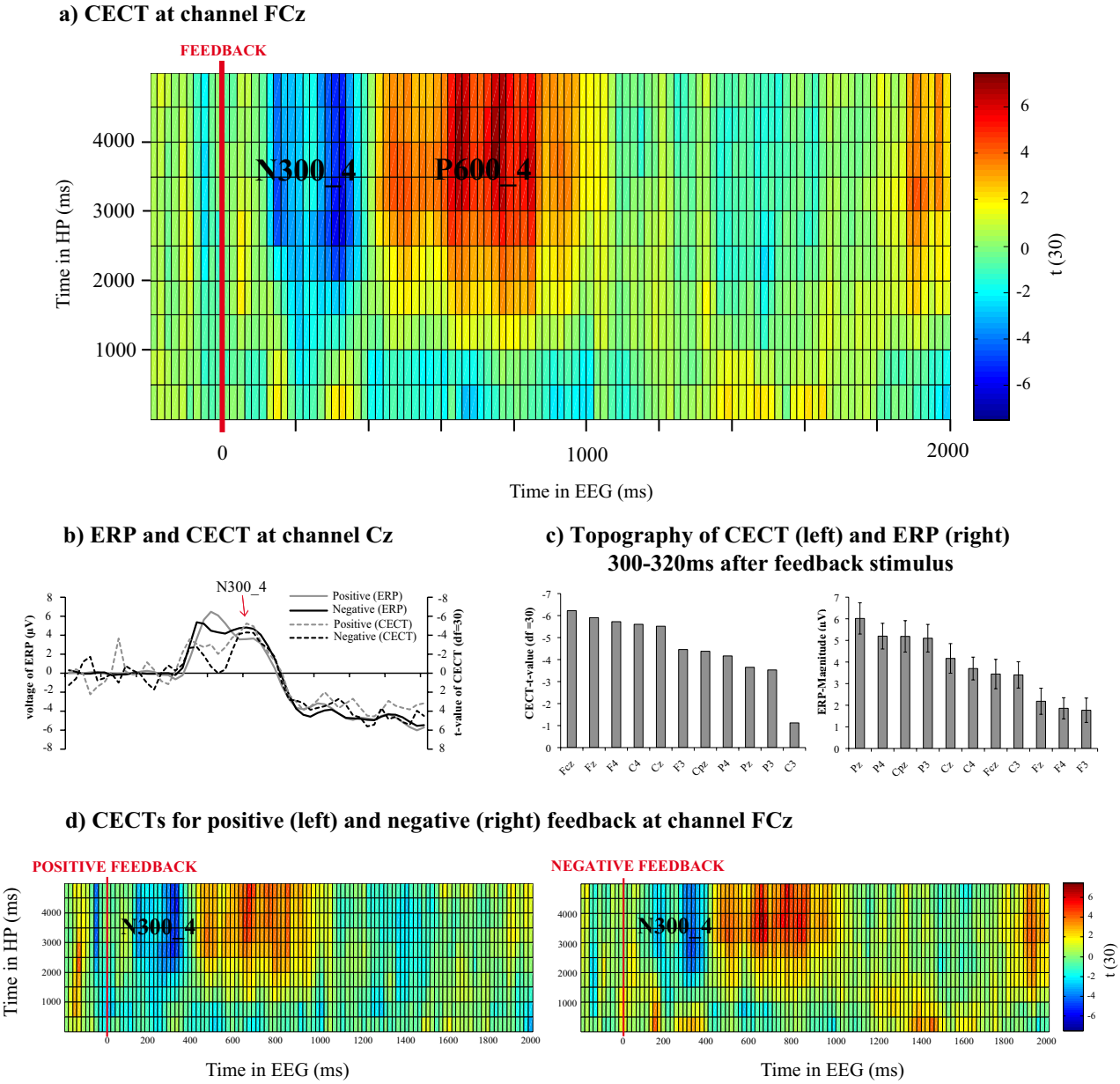


Fig. 3. Cardio–Electroencephalographic Covariance Traces (CECTs). (a) *t*-values for positive (red) and negative (blue) EEG×heart period P-correlations as a function of time in EEG (horizontal axis) and heart period (vertical axis) at FCz. Clusters are named according to direction of the correlation (positive vs. negative), time in the EEG (in ms) and time in heart period (in s) as follows: N300_4, N600_4, P300_0 and N600_0. (b) CECTs for a fixed HP lag of 3500–4000 ms showing how the EEG×heart period P-correlations following positive (black) and negative (grey) feedback change as a function of EEG time (Cz). ERPs time-locked to positive (black) and negative (grey) feedback are also plotted (see primary and secondary *x*-axis for voltage values and *t*-values, respectively) in order to allow direct comparison between EEG and CECTs. (c) *t*-values for the interindividual comparison of P-correlations between EEG magnitude from 300 to 320 ms and HP from 3500 to 4000 ms (N300_4) for all electrodes (left panel) and mean EEG magnitudes from 300 to 320 ms (right panel). Error bars represent the standard error of the mean. (d) Same as (a), but separately for positive and negative feedback.

the maximum *t*-values of N300_4 and P600_4 were below .05 after Bonferroni-correction).

Finally, it is possible that HP at the beginning of a trial modulates both, the EEG (Lacey and Lacey, 1978) and subsequent HP changes (e.g., due to baroreceptor reflexes). To control for that possibility we regressed all HP changes on the HP at feedback onset. Thus, the residuals reflected only changes in HP that were independent of HP at feedback onset. Hence, if the correlations reported above were driven by HP at feedback onset, the correlations with the residuals should disappear. However, maximum *t*-values for the N300_4 and P600_4 clusters were essentially unaffected by this procedure (*P*s<.0005).

Taken together these control analyses show that the observed intraindividual correlation patterns (i.e. N300_4 and P600_4) are (a) trial-specific, (b) cannot be explained

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

by habituation (or other time-related effects) (c) and are not mediated by baseline HP at feedback onset.

DISCUSSION

Cortico–cardiac connectivity

The present study investigated relationships between feedback-evoked modulations of EEG and HP. Thirty-one participants performed a gambling task in which positive and negative feedback was given. In line with previous studies, negative vs. positive feedback led to a more negative frontocentral amplitude from 200 to 260 ms in the EEG (Gehring and Willoughby, 2002; Miltner et al., 1997; Potts et al., 2006) and to a relative deceleration of subsequent heart beats (Crone et al., 2003; Hajcak et al., 2003; Somsen et al., 2000). However, the main question of the present study was, whether there are signals in the EEG that intraindividually relate to changes in HP. This was addressed by first P-correlating EEG magnitude in different lags after feedback with HP changes in other lags and then testing P-correlations across individuals. The resulting CECTs revealed that across participants, EEG magnitude from 200 to 400 ms and from 500 to 1100 ms after feedback correlated with later changes in HP from about 2000 to 5000 ms post-feedback. These findings suggest, that CECTs can be used to assess cortico–cardiac phenomena, and that autonomic reactions to feedback may be triggered in the cortex as early as 200 to 400 ms following the feedback stimulus.

At frontocentral sites, the EEG magnitude from 200 to 400 ms was negatively related to HP change from 2 to 5 s (N300_4). Because the standard ERP waveform showed a positive complex in the same window, and because a negative HP change value reflects a speeding of heart rate, the correlations suggest that an enlarged positive complex predicts subsequent cardiac acceleration. Note, that an acceleration of HP can also be observed in the averaged HP change (Fig. 2), which is preceded by an initial deceleration, which may reflect orienting and/or stimulus intake (Lacey and Lacey, 1978). In addition, there is another deceleration after the acceleration component. Similar triphasic modulations of HP have been reported in prior studies and the deceleration/acceleration components have been linked to different processes (Gatchel and Lang, 1973; Otten et al., 1995). The present findings suggest that the magnitude of the positive complex in the EEG is related to the acceleration component of evoked HP changes following feedback. This interpretation converges with the results from Otten et al. (1995) who demonstrated that specific experimental conditions leading to an increased parietal ERP amplitude around 350 ms (i.e. P300) also increase cardiac acceleration in the event related heart rate (see Lang et al., 1975 for similar findings).

In contrast to the ERP, which was maximal at parietal electrodes from 300 to 320 ms, the N300_4 was maximal at frontocentral midline and right hemisphere sites. The topography of N300_4 is therefore consistent with findings that relate mediofrontal brain regions to autonomic control (Benarroch, 1997; Critchley et al., 2000; Wager et al., 2009) including heart period (Critchley et al., 2000; Wager et al., 2009) and feedback/error processing (Holroyd and Coles, 2002; Potts et al., 2006) and their adaptive integration (Damasio, 1996). Moreover the right vs. left hemisphere has been implicated in chronotropic cardiac control (Ahern et al., 2001; Lane et al., 1992; Thayer and Lane, 2009). Because we recorded EEG with only 11 electrodes, any statements with regard to cortical generators are speculative. However, the fact that right insular activity has been associated with HP modulations (Critchley et al., 2000; Oppenheimer et al., 1992) points to a possible candidate source for N300_4. Pathways by which medial prefrontal cortices, the cingulate cortex and the anterior insulae may influence heart period have been suggested based on retrograde viral staining studies and pharmacological manipulation studies (for example by disinhibiting activity of the central nucleus of the amygdala leading to (a) disinhibition of sympathoexcitatory and (b) inhibition of parasympathoexcitatory neurons, cf. Thayer and Lane, 2009). Even though the present study cannot identify the underlying pathways, we speculate that N300_4 and P600_4 reflect the cortical modulation of vagal (as opposed to sympathetic) input to the sinoatrial node, because when we bandpass-filtered the original HP signal into a range that is affected by vagal but not sympathetic input (i.e. .15 to .5 Hz), neither P300_4 nor N600_4 were severely affected (data available on demand).

An important question is how the observed positive complex in the ERP relates to more traditional components. Because the complex begins rather early (140 to 160 ms) at frontocentral electrodes, it is somewhat similar to a feedback evoked P2a (Potts et al., 2006). However, given its topography moving to posterior electrodes, we suggest that the positive complex is best described as a P300-like component (Sutton et al., 1965; Polich, 2007) the waveform of which may have been slightly blunted due to the chosen high-pass filter settings (Duncan-Johnson and Donchin, 1979; note however, that such a distortion would not severely affect the P-correlations because trials with relatively larger positive complexes will still have relatively larger positive complexes after filtering). Not only is this interpretation in line with the previously discussed findings of Lang et al. (1975) and Otten et al. (1995), but it is also consistent with the general observation that both, P300 (Donchin, 1981; Rushby et al., 2005) and evoked changes of heart period (Graham and Clifton, 1966) are related to the orienting reflex. The P300 can be differentiated into an anterior and a posterior P300 (Polich, 2007) and it has been associated with different cortical generators including the anterior cingulate cortex (Linden, 2005). An intriguing interpretation of the present data could be that some anterior P300 generators are related to activation of a central autonomic network (Benarroch, 1997), for example, in order to prepare the organism for action (Verleger et al., 2005) upon detection of changes in the environment (context updating hypothesis of the P300, Donchin, 1981) or upon detection of other relevant signals (resource allocation: Isreal et al., 1980). CECTs could be

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

applied to oddball paradigms to further investigate this hypothesis.

In line with previous studies we found that negative vs. positive feedback elicited a more negative ERP from 200 to 260 ms. Similar to several other reports (Gehring and Willoughby, 2002; Miltner et al., 1997), the FRN was superimposed on the extended positive complex/P300. When comparing the CECTs and the ERPs in Fig. 1, it appears as if it is rather the positive complex that is related to HP changes and not the FRN. In fact, the CECT seems to increase with the beginning of the positive complex and then to temporarily drop during the FRN time window. Moreover, heart activity also slowed down for negative vs. positive feedback in a time window, in which HP was uncorrelated with EEG magnitudes (i.e., from 1 to 2 s). Thus, even though both, FRN and HP, seem to be sensitive to feedback valence, they appear to be unrelated, which is consistent with other reports (van der Veen et al., 2008; Van der Veen et al., 2004). Instead of the FRN being associated with HP changes, the present findings suggest that it is rather the feedback evoked P300, possibly reflecting an evaluation and/or motivational process (Sato et al., 2005) that relates to HP. This could be tested in future studies.

In addition to the positive complex, there was also a sustained negative complex in the EEG from 500 to 1100 ms and EEG magnitude in that time range correlated positively with HP change 3 to 5 s afterwards (P600_4). Thus, paralleling the N300_4 findings, an enlarged amplitude of the negative complex predicts relative cardiac acceleration. Even though negativities from 600 to 900 ms have previously been found to R-correlate with chronotropic cardiac modulations (Palomba et al., 1997), the present findings imply that components with longer latencies deserve more attention in future research on feedback evoked EEG and cardiovascular relationships.

Two limitations of the present study should be discussed. First, each trial “only” lasted 10 s. Moreover, the time window that could be analyzed in the present study was 5 s and P-correlations appeared to extend beyond that window. Thus, very slow changes in HP driven by feedback related sympathetic activation may not have been fully captured in the present design and/or may have even distorted correlations by carry-over effects into the subsequent trial. However, EEG from 200 to 400 ms was not correlated with HP from 3 to 5 s of the subsequent trial indicating that carry-over effects cannot explain the present findings.

Second, it is possible that the correlations may reflect cardio–cortical instead of cortico–cardiac communication. In fact, a dampening effect of baroreceptor stimulation on late EEG waves has been reported (Elbert and Rau, 1995). However, these effects usually have latencies in the range of seconds, while in the present study EEG as early as 300 ms after feedback correlated with HP. Moreover, N300_4 remained robust even when HP at feedback onset was statistically removed. Thus it is relatively unlikely that the correlations reflect a cardio–cortical direction. However, future studies using CECTs in combination with pharmacological or mechanical challenges to experimentally manipulate cardiovascular afferents may yield further clarification.

The CECT approach

The present method features some unique strengths and constraints worth mentioning. First, in contrast to previous investigations using R-correlations between ERP components and HP modulations, CECTs are based on P-correlations. Therefore they are independent of between-subjects variance and covariance of EEG and HP and are more powerful to detect existing neurovisceral relationships within individuals. Moreover, the current approach avoids the problem of an aggregation bias, which arises when relationships between aggregated measures (such as the standard ERP and evoked cardiac response) found at one level (such as the interindividual level) are used to make inferences about a lower level (i.e. the intraindividual level).

A second characteristic of CECTs is the use of time-lagged correlations. By systematically correlating each EEG bin with each HP bin in defined time windows relative to the stimulus, the temporal dynamics of cortico–cardiac communication can be adequately mapped and explored. To avoid inflation of type I error due to the resulting large number of correlations a conservative Bonferroni correction was used to adjust the alpha level for the statistical threshold.

Third, in order to calculate P-correlations, the CECT approach uses information in the trial-to-trial variability of the EEG signal, which is neglected in standard ERP approaches. By plotting P-correlations against time a waveform emerges that has some similarities with the standard ERP (plotting averaged voltages against time) but is also distinct with regard to topography and specific components. For example, it has been shown that the P300/positive complex is produced by activity in different generators with different functions and latencies (Linden, 2005). As can be seen in Fig. 3b CECT waveforms showed a morphology similar to the later, but not earlier part of the positive complex and had a different topography than the ERP (Fig. 3c). This dissociation suggests that CECTs contain additional information and could be helpful in attempts to decompose a complex ERP waveform (i.e. into components that are associated with autonomic regulation and other components).

A fourth feature of the CECT method is the use of magnitudes vs. amplitudes. Single trial EEG consists of both, noise and cortical activity (including non stimulus-locked activity, oscillatory activity and phasic stimulus-locked activity). This mixture makes it difficult to reliably identify stimulus-evoked components in the single-trial EEG (and then measure their amplitudes). However, by measuring the mean voltages (i.e. magnitudes) during a particular time window (reflecting the sum of noise and cortical activity) and by correlating these magnitudes with subsequent HP changes over trials this issue is circumvented. The reason is that noise is by definition random and can thus not be correlated with changes in HP (or any

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

other variable). Accordingly, any observed correlation can only be driven by that portion of the EEG variance that is signal variance and not by any noise-variance. Thus, even though CECTs reflect relationships between HP and evoked activity in the single-trial EEG, they do not require the identification of any particular components and their amplitudes in the raw data.

Fifth, normalizing P-correlations by Fisher's *z*-transform and then testing these values across individuals allows to determine whether the identified correlations generalize across subjects. Note, that this approach is analogues to the statistical mapping procedure in fMRI research, with the differences that (a) fMRI studies usually test intraindividual correlations between a canonical hemodynamic response function and the measured signal change, whereas CECTs are based on intraindividual correlations between EEG and HP, (b) the goal of fMRI is to detect voxels in the three-dimensional space, whereas CECTs detect significant "voxels" in two dimensions of time (i.e. time in EEG and time in HP) and one dimension of space (i.e. the electrodes), and (c) intraindividual correlations in fMRI are normalized by taking the beta-weights whereas the CECT technique uses a Fisher's *z*-transformation. Note, that in both cases, the grand-averaged values are rather small due to low signal to noise ratios in the single trial data (i.e. averaged standardized beta weights in fMRI studies are often below .1, the averaged *z*-transformed P-correlations for N300_4 was −.08). However, the large *t*-values imply that the phenomenon itself is rather strong and generalizes across individuals.

One constraint of the CECT approach should also be noted. First, CECTs plot the change of a correlation as a function of time(s) and space. This correlation may also be affected by changes in signal to noise ratio and/or changes in signal variance in the EEG or HP recording as a function of time and space. Thus changes in CECT magnitude do not necessarily have to reflect changes in the true underlying relationship between EEG and HP but may also be modulated by changes in signal to noise ratio and signal variance. However, even under circumstances of optimal signal to noise ratio and signal variance, significant correlations would only emerge, if there was a non-zero relationship between EEG and HP. Thus, despite possible influences of signal variance, the present findings do provide evidence that there is a significant relationship between the EEG from 320 to 340 ms and the HP change from 2 to 5 s.

CONCLUSION

The present study demonstrates the existence of intraindividual correlations between feedback-evoked EEG and HP changes and that there are spatiotemporal positions, where these correlations are robust across individuals and independent subsamples. Specifically, the frontocentral EEG around 300 ms after feedback was linearly related to modulations of HP from 2 to 5 s. It was further shown that cardiovascular covariance traces may be used to extract an HP-related signal from the event-related EEG which resembles averaged ERP waveforms for some, but not all, components. We conclude that CECTs can be used to investigate spatiotemporal aspects of neurovisceral connectivity.

Acknowledgments—The authors would like to thank Maria Zangl and Barbara Ahrens who collected the present data as part of their diploma theses. The preparation of this article was facilitated by a grant from the Deutsche Forschungsgemeinschaft (WA 2593/2-1).

REFERENCES

Ahern GL, Sollers JJ, Lane RD, Labiner DM, Herring AM, Weinand ME, Hutzler R, Thayer JF (2001) Heart rate and heart rate variability changes in the intracarotid sodium amobarbital test. Epilepsia 42:912–921.

Beauchaine T (2001) Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. Dev Psychopathol 13: 183–214.

Benarroch EE (1997) Central autonomic network: functional organization and clinical correlations. Armonk, NY: Futura Publishing Co.

Berntson GG, Bigger JT Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, van der Molen MW (1997) Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 34:623–648.

Berntson GG, Cacioppo JT, Grossman P (2007) Whither vagal tone. Biol Psychol 74:295–300.

Berntson GG, Sarter M, Cacioppo JT (1998) Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. Behav Brain Res 94:225–248.

Cattell RB (1952) The three basic factor-analytic designs—their interrelations and derivatives. Psychol Bull 49:499–520.

Critchley HD (2005) Neural mechanisms of autonomic, affective, and cognitive integration. J Comp Neurol 493:154–166.

Critchley HD, Corfield DR, Chandler MP, Mathias CJ, Dolan RJ (2000) Cerebral correlates of autonomic cardiovascular arousal: a functional neuroimaging investigation in humans. J Physiol 523(Pt 1):259–270.

Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, Cipolotti L, Shallice T, Dolan RJ (2003) Human cingulate cortex and autonomic control: converging neuroimaging and clinical evidence. Brain 126:2139–2152.

Critchley HD, Tang J, Glaser D, Butterworth B, Dolan RJ (2005) Anterior cingulate activity during error and autonomic response. Neuroimage 27:885–895.

Crone EA, van der Veen FM, van der Molen MW, Somsen RJ, van Beek B, Jennings JR (2003) Cardiac concomitants of feedback processing. Biol Psychol 64:143–156.

Damasio AR (1996) The somatic marker hypothesis and the possible functions of the prefrontal cortex. Philos Trans R Soc Lond B Biol Sci 351:1413–1420.

Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134:9–21.

Dirlich G, Dietl T, Vogl L, Strian F (1998) Topography and morphology of heart action-related EEG potentials. Electroencephalogr Clin Neurophysiol 108:299–305.

Donchin E (1981) Presidential address, 1980. Surprise! . . . Surprise? Psychophysiology 18:493–513.

Duncan-Johnson CC, Donchin E (1979) The time constant in P300 recording. Psychophysiology 16:53–55.

Elbert T, Rau H (1995) What goes up (from heart to brain) must calm down (from brain to heart)! Studies on the interaction between baroreceptor activity and cortical excitability. In: From the heart to

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

the brain: the psychophysiology of circulation—brain interaction (Vaitl D, Schandry R, eds), pp 133–149. New York, NY: Peter Lang Publishing.

Elbert T, Roberts LE, Lutzenberger W, Birbaumer N (1992) Modulation of slow cortical potentials by instrumentally learned blood pressure responses. Psychophysiology 29:154–164.

Fisher RA (1950) Statistical methods for research workers. Edinburgh, Great Britain: Oliver and Boyd.

Friedman BH (2007) An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. Biol Psychol 74:185–199.

Gatchel RJ, Lang PJ (1973) Accuracy of psychophysical judgments and physiological response amplitude. J Exp Psychol 98:175–183.

Gehring WJ, Willoughby AR (2002) The medial frontal cortex and the rapid processing of monetary gains and losses. Science 295:2279–2282.

Gianaros PJ, Van Der Veen FM, Jennings JR (2004) Regional cerebral blood flow correlates with heart period and high-frequency heart period variability during working-memory tasks: implications for the cortical and subcortical regulation of cardiac autonomic activity. Psychophysiology 41:521–530.

Graham FK, Clifton RK (1966) Heart-rate change as a component of the orienting response. Psychol Bull 65:305–320.

Groen Y, Wijers AA, Mulder LJ, Minderaa RB, Althaus M (2007) Physiological correlates of learning by performance feedback in children: a study of EEG event-related potentials and evoked heart rate. Biol Psychol 76:174–187.

Hajcak G, Holroyd CB, Moser JS, Simons RF (2005) Brain potentials associated with expected and unexpected good and bad outcomes. Psychophysiology 42:161–170.

Hajcak G, McDonald N, Simons RF (2003) To err is autonomic: error-related brain potentials, ANS activity, and post-error compensatory behavior. Psychophysiology 40:895–903.

Holroyd CB, Coles MG (2002) The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychol Rev 109:679–709.

Holroyd CB, Coles MG (2008) Dorsal anterior cingulate cortex integrates reinforcement history to guide voluntary behavior. Cortex 44:548–559.

Isreal JB, Chesney GL, Wickens CD, Donchin E (1980) P300 and tracking difficulty: evidence for multiple resources in dual-task performance. Psychophysiology 17:259–273.

Kubota Y, Sato W, Toichi M, Murai T, Okada T, Hayashi A, Sengoku A (2001) Frontal midline theta rhythm is correlated with cardiac autonomic activities during the performance of an attention demanding meditation procedure. Brain Res Cogn Brain Res 11:281–287.

Lacey JI, Lacey BC (1970) Some autonomic-central nervous system interrelationships. In: Physiological correlates of emotion (Black P, ed), pp 205–228. London: Academic Press, Inc.

Lacey BC, Lacey JI (1978) Two-way communication between the heart and the brain. Significance of time within the cardiac cycle. Am Psychol 33:99–113.

Lane RD, Wallace JD, Petrosky PP, Schwartz GE, Gradman AH (1992) Supraventricular tachycardia in patients with right hemisphere strokes. Stroke 23:362–366.

Lang PJ, Gatchel RJ, Simons RF (1975) Electro-cortical and cardiac rate correlates of psychophysical judgment. Psychophysiology 12:649–655.

Linden DE (2005) The p300: where in the brain is it produced and what does it tell us? Neuroscientist 11:563–576.

Miltner WHR, Braun CH, Coles MGH (1997) Event-related brain potentials following incorrect feedback in a time-estimation task: evidence for a "generic" neural system for error detection. J Cogn Neurosci 9:788–798.

Oppenheimer SM, Gelb A, Girvin JP, Hachinski VC (1992) Cardiovascular effects of human insular cortex stimulation. Neurology 42:1727–1732.

Otten LJ, Gaillard AW, Wientjes CJ (1995) The relation between event-related brain potential, heart rate, and blood pressure responses in an S1-S2 paradigm. Biol Psychol 39:81–102.

Palomba D, Angrilli A, Mini A (1997) Visual evoked potentials, heart rate responses and memory to emotional pictorial stimuli. Int J Psychophysiol 27:55–67.

Polich J (2007) Updating P300: an integrative theory of P3a and P3b. Clin Neurophysiol 118:2128–2148.

Porges SW, Doussard-Roosevelt JA, Portales AL, Greenspan SI (1996) Infant regulation of the vagal "brake" predicts child behavior problems: a psychobiological model of social behavior. Dev Psychobiol 29:697–712.

Potts GF, Martin LE, Burton P, Montague PR (2006) When things are better or worse than expected: the medial frontal cortex and the allocation of processing resources. J Cogn Neurosci 18: 1112–1119.

Rau H, Elbert T (2001) Psychophysiology of arterial baroreceptors and the etiology of hypertension. Biol Psychol 57:179–201.

Rushby JA, Barry RJ, Doherty RJ (2005) Separation of the components of the late positive complex in an ERP dishabituation paradigm. Clin Neurophysiol 116:2363–2380.

Sato A, Yasuda A, Ohira H, Miyawaki K, Nishikawa M, Kumano H, Kuboki T (2005) Effects of value and reward magnitude on feedback negativity and P300. Neuroreport 16:407–411.

Schandry R, Montoya P (1996) Event-related brain potentials and the processing of cardiac activity. Biol Psychol 42:75–85.

Somsen RJ, Van der Molen MW, Jennings JR, van Beek B (2000) Wisconsin Card Sorting in adolescents: analysis of performance, response times and heart rate. Acta Psychol (Amst) 104:227–257.

Stemmler G (1992) Differential psychophysiology: persons in situations. New York: Springer.

Sutton S, Braren M, Zubin J, John ER (1965) Evoked-potential correlates of stimulus uncertainty. Science 150:1187–1188.

Tabachnik BG, Fidell LS (2006) Using multivariate statistics. Needham Heights, MA: Allyn & Bacon, Inc.

Thayer JF, Lane RD (2000) A model of neurovisceral integration in emotion regulation and dysregulation. J Affect Disord 61:201–216.

Thayer JF, Lane RD (2007) The role of vagal function in the risk for cardiovascular disease and mortality. Biol Psychol 74:224–242.

Thayer JF, Lane RD (2009) Claude Bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. Neurosci Biobehav Rev 33:81–88.

Tranel DT, Fisher AE, Fowles DC (1982) Magnitude of incentive effects on heart rate. Psychophysiology 19:514–519.

Turner RJ (1989) Individual differences in heart rate response during behavioral challenge. Psychophysiology 26:497–505.

van der Veen FM, Lange JJ, van der Molen MW, Mulder G, Mulder LJ (2000) Event-related brain potential and heart rate manifestations of visual selective attention. Psychophysiology 37:677–682.

van der Veen FM, Mies GW, van der Molen MW, Evers EA (2008) Acute tryptophan depletion in healthy males attenuates phasic cardiac slowing but does not affect electro-cortical response to negative feedback. Psychopharmacology 199:255–263.

Van der Veen FM, Nieuwenhuis S, van der Molen MW, Crone EAM (2004) Cardiac and electro-cortical responses to performance feedback reflect different aspects of feedback processing. In: Errors, conflicts, and the brain: current opinions on performance monitoring (Ullsperger M, Falkenstein M, eds). Leipzig, Germany: MPI of Cognitive Neuroscience.

van der Veen FM, van der Molen MW, Crone EA, Jennings JR (2004) Phasic heart rate responses to performance feedback in a time production task: effects of information versus valence. Biol Psychol 65:147–161.

Verleger R, Jaskowski P, Wascher E (2005) Evidence for an integrative role of P3b in linking reaction to perception. J Psychophysiol 19:165–181.

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

ARTICLE IN PRESS

Wager TD, Waugh CE, Lindquist M, Noll DC, Fredrickson BL, Taylor SF (2009) Brain mediators of cardiovascular responses to social threat, Part I: reciprocal dorsal and ventral sub-regions of the medial prefrontal cortex and heart-rate reactivity. Neuroimage 47:821–835.

Weisz J, Czigler I (2006) Age and novelty: event-related brain potentials and autonomic activity. Psychophysiology 43:261–271.

Zilles K, Armstrong E, Schleicher A, Kretschmann HJ (1988) The human pattern of gyrification in the cerebral cortex. Anat Embryol (Berl) 179:173–179.

(Accepted 21 December 2009)

Please cite this article in press as: Mueller EM, et al., Single-trial electroencephalogram predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity, Neuroscience (2009), doi: 10.1016/j.neuroscience.2009.12.051

Study 2

Mueller, E.M., Makeig, S., Stemmler, G., Hennig, J.,& Wacker, J. Dopamine effects on human error processing depend on COMT VAL158MET genotype. Submitted to the *Journal of Neuroscience*.

Journal Section: Behavioral/Systems/Cognitive

Dopamine effects on human error processing depend on COMT VAL158MET genotype

Abbreviated title: error processing, dopamine and COMT

Erik M Mueller[1,2], Scott Makeig[2], Gerhard Stemmler[1], Jürgen Hennig[3], & Jan Wacker[1]

[1]Philipps-Universitaet Marburg, Marburg, Germany; [2]University of California, San Diego, CA, USA; [3]Justus-Liebig Universitaet, Giessen, Germany

Corresponding author: Erik M. Mueller, Gutenbergstr. 18, 35032 Marburg, Germany, Email: erik.mueller@staff.uni-marburg.de

Figures: *4* **Tables:** *1* **Supplementary Material: none**

Number of Pages: *28*

Number of words: 6246 (Abstract, Introduction, Methods, Results, Discussion, Figure legends, Tables, References)

Abstract: 248 **Introduction: 498** **Discussion: 1287**

Conflicts of Interest: none

Abstract

Brain dopamine (DA) and serotonin have been linked to error processing. A recent theory suggests that high and low (vs. medium) prefrontal cortex DA levels enhance network flexibility. We hypothesized that such flexibility may promote dynamic error processing. N = 169 male participants were genotyped for the COMT Val158Met polymorphism, associated with low (Val allele) and medium (Met allele) PFC DA levels. In addition DRD2TaqIa and 5HTTLPR, polymorphisms associated with striatal D_2 receptor density and serotonin uptake, respectively, were assessed. Participants received placebo or a selective DA-D_2 receptor blocker (sulpiride, 200 mg) and performed a Flanker task. EEG was recorded and later decomposed into independent brain components (ICs) using Independent Component Analysis. After errors participants displayed (a) a negative deflection in ICs source-localized to the proximity of the anterior midcingulate cortex (IC-error-related negativity, IC-ERN) and (b) slowing in the subsequent trial (post-error slowing, PES). Importantly, both, IC-ERN and PES were modulated by COMT x Sulpiride interactions such that the Val allele predicted elevated IC-ERN and PES after placebo while this association was reversed after sulpiride. Furthermore, this COMT x Sulpiride interaction was potentiated in carriers of lower vs. higher expressing 5HTTLPR alleles. Because by blocking presynaptic autoreceptors low doses of sulpiride presumably increase extrasynaptic DA, the COMT x Sulpiride interaction is consistent with the hypothesis that low (Val, placebo) and high (Met, sulpiride) vs. medium DA (Val, sulpiride; Met, placebo) levels elevate error processing. The influence of 5HTTLPR further indicates that serotonin influences dopaminergic mechanisms of error processing.

Introduction

Errors rapidly trigger cascades of behavioral (Rabbitt and Phillips, 1967) and neural (Gehring and Knight, 2000) sequelae in order to monitor and regulate performance. These include dynamic and flexible modulations of medial prefrontal cortex (PFC) neural activation patterns in response to the error (Holroyd and Coles, 2002) and/or associated response conflicts (Botvinick et al., 2001). The degree of flexibility vs. stability of prefrontal cortex networks has been linked to dopamine (DA) availability. According to the dual-state theory of PFC DA function (Durstewitz and Seamans, 2008), flexible switching among different network states is facilitated when DA levels are either very low or very high and thereby induce a relative dominance of D2 over D1 receptor activation (D2 state). In contrast, medium DA levels promote D1 activation states associated with network stability. Based on the assumption that error processing is a dynamic, DA-related process (e.g. Holroyd and Coles, 2002) it could be hypothesized that error processing is elevated in D2 dominated regimes (i.e. when DA levels are low or high).

A predominance of D2-dominated regimes likely occurs in individuals who carry at least one copy of the Val allele (Val+) of the Catechol-O-Methyltransferase (COMT) Val158Met polymorphism (Durstewitz and Seamans, 2008), because the enzyme COMT metabolizes PFC DA, and carriers of the more functional Val allele presumably show relatively low PFC DA levels (Lachman et al., 1996). In contrast, individuals with no Val alleles (Met/Met carriers) display medium DA levels and are characterized by D1-state networks (Durstewitz and Seamans, 2008). Based on the assumption that D2 facilitates flexible network modulation we hypothesized that Val+ carriers show elevated dynamic error processing in comparison to Met/Met carriers. Furthermore, increasing PFC DA availability by administration of a selective D2 receptor blocker acting at presynaptic sites (e.g. sulpiride; Kuroki et al., 1999) would push Val+ carriers towards medium DA levels (associated with D1 states) while Met/Met carriers would be pushed towards high DA levels (associated with D2

states, see Figure 1). Thus we hypothesized that administration of sulpiride should enhance error processing in Met homozygotes but reduce error processing in Val+ carriers.

We tested these hypotheses using neural and behavioral indices for error processing. The error-related negativity (ERN), is an event-related potential component that peaks within 100 ms after error-commission, originates from anterior midcingulate cortex (Debener et al., 2005) and has been related to DA (Holroyd and Coles, 2002; Jocham and Ullsperger, 2009; Ullsperger, 2010). At the behavioral level we analyzed post-error slowing (PES) which reflects the tendency of participants to slow down after errors have been committed in speeded reaction time tasks (Rabbitt and Phillips, 1967).

In addition to COMT, we investigated the effect of DRD2Taq I a, a polymorphism indirectly linked to presynaptic D2 receptor density in striatum (Zhang et al., 2007) and - because PFC dopaminergic neurotransmission is modulated by serotonin (5-HT; Fink and Gothert, 2007) - we tested whether effects would be modulated by the 5-HT transporter polymorphism (5-HTTLPR), associated with 5-HT transporter expression and ERN amplitude (Fallgatter et al., 2004).

Materials and Methods

Participants

N = 195 non-smoking, right-handed males completed the study. Prior to testing, the absence of any illnesses or DSM-IV diagnoses was confirmed using a standardized clinical interview (Margraf, 1994). All participants reported not having used any prescription or illegal drugs during the past three months. Participants were required to refrain from consuming alcohol, nicotine, and caffeine 12 hours before the beginning of the study. All volunteers gave written informed consent before participating and received a monetary

compensation of 70 EUR (90 USD) for approximately 7 hours involvement in the project. The study protocol was approved by the Ethics Committee of the German Society for Psychology (Deutsche Gesellschaft fuer Psychologie). Datasets containing grossly artifact-contaminated EEG (n = 13; 6.7%) or less than three error trials remaining following artifact correction (n = 8; 4.1%) were excluded. Further, datasets for which the clustering algorithm described below assigned no independent component dipoles to an anterior midcingulate cortex cluster (n = 5; 2.5%) were excluded from further processing, yielding a final sample of n = 169 with an average age of 23.8 years (SD = 3.1; see table 1 for further description).

Procedure

When participants came to the laboratory they first gave informed consent and were then administered the standardized clinical interview. If no exclusion criteria applied, participants then received a standardized breakfast (water/juice, 1-2 rolls with cheese, marmalade or sausage) and ingested either a sulpiride (200 mg) or placebo capsule with a glass of water. Both capsules had the same appearance to ensure that the experimenter and the participants were blind to the pharmacological treatment. Thereafter participants performed several tasks (e.g., intelligence tests, personality questionnaires, learning tasks) for which results will be reported elsewhere. Approximately 4 h after administration of the pill participants started the Flanker task.

Flanker Task

The Flanker task, delivered using Presentation 12.0 (Neurobehavioral Systems, Albany, CA), consisted of three blocks of 140 trials each, plus an initial practice block to determine individual response speed, during which no EEG was recorded. A trial began with central screen presentation of a fixation cross for 1000 ms. Thereafter congruent (SSSSS, HHHHH) or incongruent (SSHSS, HHSHH) stimulus arrays were presented for 600 ms with

the central target letter onset coming 100 ms later than the four flanker letters. Participants were instructed to respond as quickly as possible by pressing one of two buttons under their right index and middle fingers in response to S and H targets, respectively. If participants reacted slower than their mean reaction time plus one standard deviation (both determined from the preceding trial block), the feedback "too slow" appeared for 500 ms beginning 900 ms after the onset of the target stimulus. Otherwise ("error" or "correct") performance feedback was given. If participants did not react within a window 100 ms to 900 ms after presentation of central target letter onset, the feedback read "no button press".

Behavioral data

For behavioral analyses, reaction times were measured as the latency between central target letter presentation and the ensuing subject button press. All responses three standard deviations faster than the average reaction time were discarded. Post-error slowing (Rabbitt and Phillips, 1967) was computed as the difference in reaction times between an error trial and the subsequent trial. To compute a comparable measure for correct trials, for each error trial, a correct trial with similar reaction time was automatically identified and again the difference was taken between this (relatively fast) response and the response time in the subsequent trial. Error rates were also computed.

EEG

Recording

EEG was recorded at a sampling rate of 512 Hz using an Active Two (BioSemi, Amsterdam, Netherlands) active electrode system with DRL and CMS as active and passive reference, respectively. EEG data of the first $n = 99$ and remaining $n = 96$ participants were recorded using 32- and 64-channel configurations, respectively. Because normalizing the

ERN-amplitudes within these two subsets did not affect the effects presented, we provide results for the two subject sub-groups pooled together.

ICA decomposition

Data were analyzed by custom Matlab (The Mathworks, Inc.) scripts built on the open source EEGLAB toolbox (Delorme and Makeig, 2004). The manually inspected and artifact-removed continuous EEG data was 1-Hz high-pass filtered and decomposed using an adaptive mixture ICA algorithm (AMICA; Palmer et al., 2006; Palmer et al., 2008). As explained in more detail elsewhere (Gramann et al., 2010), for each independent component (IC) scalp topography an equivalent current dipole was computed using a standard adult boundary element head model (BEM) implemented in the DIPFIT toolbox (Oostenveld and Oostendorp, 2002). Only ICs with dipoles localized within the brain and whose scalp projection (through a spherical forward head model) explained more than 90% IC scalp topography variance were used in further analyses. ICs of all participants were clustered using a K-means clustering algorithm applied to the estimated equivalent dipole positions. A total of 15 IC clusters, plus one outlier cluster, were thus identified (see Figure 2). The outlier cluster was defined *a priori* to consist of all ICs whose estimated dipole positions were more than 3 SD from any IC cluster centroid. As depicted in Figure 3, IC Cluster 9 had maximum dipole density in and near anterior midcingulate cortex (Vogt, 2005), a region of the brain previously implicated in error processing and ERN generation (Debener et al., 2005). N = 169 participants had at least one IC component in this cluster. For participants with more than one IC in the cluster, the IC exhibiting the largest negative error-response time-locked ERP deflection within 150 ms was automatically identified as this subject's cluster-component ERN (ERN-IC). Both activation time courses and scalp maps of cluster ICs with inverted topographies (e.g., a negative correlation between the IC scalp map and the IC cluster mean scalp map) were polarity reversed (multiplied by -1) before between-subject comparisons to

ensure that all IC activation time courses had comparable polarities. ERN amplitudes for the thus derived ERN-IC activations (for measurement see below) showed a substantial variance overlap with ERN amplitudes at frontocentral scalp channels Cz and Fz (or the average of both channels), yet they were not redundant ($.53 < r\text{s} < .58$). To measure and visualize dipole position spread, dipole density was computed using a 3-D gaussian smoothing function with SD=10 mm. The result is plotted in Figure 3a.

Error-related negativity

For each participant, ERN-IC activations were baseline corrected (using the baseline interval from -800 to -600 ms relative to response onset), then averaged across trials, normalized to make the root-mean square component scalp map projection to all channels (across all time points in the data epochs) 1 μV, and 20-Hz low-pass filtered. Peak-to-peak ERN amplitude was measured as the difference between most negative-going ERN-IC activation value from 0 ms to 150 ms following the button press and the most positive-going activation value from -100 ms to 0 ms before it. In the same way we also measured the standard scalp-channel peak-to-peak ERN at electrode Fz re-referenced to linked earlobes.

Genotyping

DNA was extracted from buccal cells, purified and genotyped for COMT Val158Met as previously described (Reuter and Hennig, 2005). Using the methods reported by (Reuter et al., 2006), all participants were also genotyped for DRD2 TaqIA, a polymorphism associated with individual differences in D_2 dopamine receptor density (Pohjalainen et al., 1998), presumably via linkage disequilibrium with another functional polymorphism located directly on the DRD2 gene (Zhang et al., 2007). Finally, we genotyped participants for 5HTTLPR as described before (Osinsky et al., 2008), which occurs in the

three variants S, L_G and L_A (Nakamura et al., 2000) the former two being associated with low, nearly equivalent 5-HT transporter expression (Hu et al., 2006).

The resulting genotype distributions were as follows. COMT Val158Met: *n* = 33, 86, and 50 for Val/Val, Val/Met, and Met/Met. DRD2TaqIa: *n* = 6, 47, and 116 for A1/A1, A1/A2, A2/A2. 5HTTLPR: *n* = 27, 10, 70, 0, 11, and 50 for S/S, S/L_G, S/L_A, L_G/L_G, L_G/L_A and L_A/L_A. For all statistical analyses, homozygotes of the major allele were compared with the remainder. This resulted in the following comparisons: Met/Met (*n* = 50) vs. Val+ (*n* = 119), A2/A2 (*n* = 116) vs. A1+ (*n* = 53) and LA/LA (*n* = 50) vs. L_G+, S+ (*n* = 119). With regard to 5-HTTLPR L_G+ and S+ were grouped based on the almost identical 5-HT transporter expression (Hu et al., 2006). All genotype distributions were in Hardy-Weinberg equilibrium (all X^2s < .3).

Sulpiride

Sulpiride is a substituted benzamide that acts as a selective D2-receptor antagonist. A single acute dose of 200 mg sulpiride results in considerably lower levels of D2 receptor occupancy than considered efficacious in the treatment of schizophrenia and is thought to primarily block presynaptic autoreceptors thereby leading to increased DA activity (Mereu et al., 1983; Kuroki et al., 1999). Sulpiride is generally well tolerated, does not appear to significantly block other types of receptors (e.g. histaminergic, cholinergic, serotonergic, adrenergic, and Gamma aminobutyric acid (GABA-) receptors), it is slowly absorbed from the gastrointestinal tract, with peak serum levels occurring within one to six hours after oral ingestion, and the average elimination half life is in the range of 3 to 10 hours (Mauri et al., 1996).

Statistical analyses

To reduce the impact of potential outliers, we first winsorized the data (both, reaction-times and IC-activations) by replacing the lower and upper 10% of the data with the respective 10^{th} and 90^{th} percentile values, respectively (Erceg-Hurn and Mirosevich, 2008). To test for main effects or interactions we computed standard parametric ANOVAs with the repeated measures factor Response (error vs. correct) and the between-subjects factors COMT (Val+ vs. Met/Met) and Substance (Sulpiride vs. Placebo) and performed *post hoc* comparisons using unpaired *t*-tests with one-tailed significance thresholds for predicted effects. All statistical tests were conducted using SPSS 15.0.

Results

Behavioral Data

As expected, reaction times following errors were significantly larger than reaction times following rt-matched correct responses, indicating significant PES (Main effect Response: $F_{(1,\ 165)} = 259.6$, $p < .0001$, $\eta_p^2 = .61$). Importantly, the Response x COMT x Substance interaction ($F_{(1,\ 165)} = 4.9$, $p = .03$, $\eta_p^2 = .03$) was significant (see Figure 3). To follow up on this interaction we calculated difference scores (slowing after errors minus slowing after rt-matched correct trials) and compared COMT genotypes on this error-specific net effect separately for placebo and sulpiride. As predicted, in the placebo group, Val+ carriers showed significantly more error-specific slowing than Met/Met carriers ($t_{(78)} = 2.75$, $p = .004$, one-tailed). When analyzing the raw scores in a next step this effect could be attributed to slowing following errors ($t_{(78)} = 4.0$, $p < .001$, one-tailed) rather than slowing following correct responses $t_{(78)} = .99$, $p > .15$, table 1). Of particular relevance, the genotype effect on the difference score was completely planished in participants who received sulpiride ($t_{(87)} = .22$, $p > .4$, one-tailed), primarily due to a significant reduction in Val+ carriers after

sulpiride vs. placebo intake ($t_{(117)}$ = 2.57, p = .006, one-tailed) and a non-significant increase in Met/Met carriers ($t_{(48)}$ = 1.02, p = .16, one-tailed). There were no main effects or interactions on the total number of errors or on the mean reaction time following correct trials supporting the specificity of the present findings with regard to post-error processing.

EEG Data.

Independent-Component data. As shown in Figure 3, errors triggered a clear negative deflection in independent EEG sources localized to anterior mid-cingulate cortex (i.e. IC-ERN) that was dramatically reduced following correct responses (main effect Response: $F_{(1, 165)}$ = 225.3, p < .0001, η_p^2 = .57). Mirroring the behavioral data, there was a significant Response x COMT x Substance interaction ($F_{(1, 165)}$ = 6.8, p = .01, η_p^2 = .04, see Figure 3). Analogous to the behavioral data we followed up on this three-way interaction by first calculating difference scores indicating the error-specific net effect (amplitude error minus amplitude correct) and then testing these difference scores separately for both substance conditions. In the placebo group, Val+ carriers showed significantly higher difference scores than Met/Met carriers ($t_{(78)}$ = 1.98, p = .026, one-tailed). Analysis of the raw amplitudes revealed that this effect was driven by IC-amplitudes after error-trials ($t_{(78)}$ = 1.8, p = .04, one-tailed ,Figure 3) rather than correct responses $t_{(78)}$ = .22, p > .8, table 1). Similar to the behavioral data, sulpiride increased the difference score in Met/Met carriers ($t_{(48)}$ = 1.99, p = .03, one-tailed) but decreased the difference score in Val+ carriers ($t_{(117)}$ = 1.84, p = .04, one-tailed).

Scalp-channel data. Following the same pattern as PES and IC activation, the Response x COMT x Sulpiride interaction on the ERN-amplitude at channel Fz was marginally significant ($F_{(1, 165)}$ = 3.2, p = .07, η_p^2 = .02). With regard to difference scores (amplitude error minus amplitude correct) sulpiride induced a decrease in scalp-channel ERN in Val carriers ($t_{(117)}$ = 1.85, p = .03, one-tailed) and a non-significant increase of amplitude

in Met/Met homozygotes ($p > .15$, see also table 1). The reduced effect sizes in scalp-channel vs. IC data are consistent with lower signal-to-noise ratio in the former (Makeig et al., 2004).

Behavioral and EEG data.

To test, whether individual differences in IC-ERN and PES were related, we correlated IC activation peaks and reaction time slowing separately for error and correct trials. IC-activation after errors (i.e. IC-ERN) showed a significant correlation with PES ($r_{(169)} = .16$, $p = .02$, one-tailed) but not with slowing after rt-matched correct responses ($p > .3$). Moreover, IC-activation after correct responses correlated neither with PES nor with slowing after rt-matched correct responses (all ps $> .15$), indicating that there is an association between anterior midcingulate cortex IC-activation and reaction time slowing that is specific to error responses.

Epistasis effects with DRD2Taq I a and 5-HTTLPR

To test for epistasis effects we separately included DRD2TaqIa and 5-HTTLPR into the previously reported ANOVAs (for the distributions of DRD2TaqIa and 5HTTLPR over the COMT Val158Met genotypes see Table 1). There were no significant main-effects or interactions on PES, error-related IC activation or scalp-channel EEG that involved the DRD2TaqIa polymorphism. However, when 5-HTTLPR was included, there was a COMT x 5-HTTLPR interaction on reaction time slowing independent of Response and Substance ($F_{(1,160)} = 4.52$, $p = .04$, $\eta_p^2 = .03$) indicating that among carriers with the less functional 5-HTTLPR alleles (L_G and S) Val carriers slowed more ($F_{(1,114)} = 8.01$, $p < .006$), while this effect was absent in L_A homozygotes ($p > .5$). Importantly, a significant Response x COMT x 5-HTTLPR x Substance interaction on IC activation ($F_{(1,160)} = 3.18$, $p = .05$, $\boldsymbol{\eta}_p^2 = .02$), indicated that the previously reported Response x COMT x Substance interaction on error-

related IC activation was potentiated in carriers of the less functional 5-HTTLPR variant ($F_{(1,114)} = 8.94$, $p < .005$, $\eta_p^2 = .07$) but absent in L_A homozygotes ($p > .8$; see Figure 4).

Discussion

We have shown that a dopaminergic genotype and a dopaminergic drug interact on human error processing, previously linked to DA. Although prior studies have investigated the effect of DA genotypes (for review: Ullsperger, 2010) or pharmacological challenges (for review: Jocham and Ullsperger, 2009) alone, this is the first study, to the best of our knowledge, that combines the two approaches to investigate the involvement of DA in error processing. Based on (1) the dual-state theory of PFC DA functioning, claiming that D2 states associated with low and high DA levels promote network flexibility (Durstewitz and Seamans, 2008), and because (2) it has been suggested that PFC DA plays a substantial role in error processing (Holroyd and Coles, 2002), we hypothesized that the DA-related COMT Val158Met polymorphism and intake of a selective D_2 blocker would interact such that individuals with presumably low or high DA levels show elevated indices of dynamic error-processing in comparison to individuals with medium DA levels. We tested our hypothesis in $N = 169$ healthy male participants, who were genotyped for COMT Val158Met, and who received a DA D_2 receptor blocker or placebo and then performed a Flanker task while EEG was recorded. We found that carriers of the COMT Val158Met Val allele who had taken placebo and Met homozygotes, who had received sulpiride showed relatively high PES and error-related brain activity. In contrast, Val carriers who had received sulpiride and Met/Met carriers who had received placebo, showed relatively lower PES and error-related brain activity. This pattern of results provides strong support for a role of DA and the COMT Val158Met polymorphism on individual differences in ERN amplitude and PES and for the applicability of the flexibility/stability model to error processing.

In individuals who had received placebo, the IC-ERN amplitude was significantly smaller in Met/Met vs. Val+ carriers. This was mirrored by the behavioral data, where –after placebo- Met/Met carriers showed significantly less PES than Val+ carriers. Because Val+ carriers presumably show reduced DA-levels and may thus occupy D2 rather than D1 states (Durstewitz and Seamans, 2008) these findings are consistent with the hypothesis, that D2 states facilitate dynamic error-processing. The finding of reduced PES and ERN in Met vs. Val carriers who received placebo further is in line with the pattern of results reported by Krämer et al. (2007) for a small sample of unmedicated participants, although the effect failed to reach significance in this former study.

Because 200 mg of sulpiride presumably increase dopamine availability by blocking presynaptic D2-receptors (Mereu et al., 1983; Kuroki et al., 1999) we hypothesized that sulpiride would increase error processing in Met/Met carriers by pushing them from medium towards high DA levels and thereby into D2 states, and decrease error processing in Val+ carriers by pushing them from low DA towards medium DA levels and thereby into D1 states. As expected, sulpiride significantly interacted with COMT on both, ERN and PES, in the expected directions. Following sulpiride intake, Val+ carriers showed a significant reduction of PES and a significant decrease of ERN amplitude. In contrast, Met/Met carriers who had received sulpiride vs. placebo, showed a significant increase in ERN-amplitude and a non-significant increase in PES. Together, this pattern of findings suggests a u-shaped association between PFC DA availability and dynamic error processing. Similar non-linear associations have been found with regard to DA availability and other brain processes (Goldman-Rakic et al., 2000; Tunbridge et al., 2006; Durstewitz and Seamans, 2008). With regard to the processing of infrequently occurring response errors, a possible mechanism for these effects involves D_1- versus D_2-dominated states in PFC as outlined above, which may suppress or enhance network flexibility and thereby affect reactivity to erroneous events (see Figure 1).

Alternatively, thermoinstable COMT in Met carriers may modulate the ERN by elevating postsynaptic DA at mid-cingulate cortex pyramidal cell neurons, phasic dips of which are assumed to underlie ERN generation (Holroyd and Coles, 2002), although this model may not be able to explain why presumably low levels of DA (as in Val carriers who received placebo) would enhance the ERN. While the dual state theory of dopamine functioning (Durstewitz and Seamans, 2008) and the Holroyd and Coles (2002) account focus on PFC, phasic DA signaling in the basal ganglia may also be implicated in error processing (Frank et al., 2007) and may likewise be potentiated in Val vs. Met carriers (Bilder et al., 2004). The extent to which subcortical DA evokes individual differences in error processing could be investigated with future studies combining molecular genetics and pharmacological challenges in conjunction with neuroimaging techniques.

A follow-up analysis revealed that the COMT x Substance interaction was potentiated in carriers of the less functional 5-HTTLPR variant. This variant is linked to reduced 5-HT uptake (Lesch et al., 1996) and 5-HT could affect PFC DA-neuron firing, for example by inhibiting GABAergic interneurons that otherwise constrain DA release (Fink and Gothert, 2007). Of relevance, the same alleles of 5-HTTLPR that predicted reactivity to dopamine manipulation depending on COMT genotype in the present study have previously been linked to enhanced vulnerability for depression after stressful life-events (Caspi et al., 2003). Although 5-HTTLPR may have pleiotropic effects on dopamine-related error-processing and depressive symptoms after stressful life events, together with evidence for stressful life events affecting the DA system (Pruessner et al., 2004) the present findings indicate that epistasis effects involving 5-HT and DA could be targeted in future studies of negative affect/depression, error processing, and their interrelation (Hajcak et al., 2004). The present study may also be of relevance for schizophrenia, for which D_2 antagonists are among the most effective known treatments. Furthermore, the COMT Val allele has been associated with slightly increased risk for schizophrenia (Egan et al., 2001), particularly when present in

combination with the less functional 5-HTTLPR allele (Borroni et al., 2006). Although the presence of psychiatric disorders was an explicit exclusion criterion in this study, the present findings of opposing effects triggered by acute low-dose neuroleptic intake as a function of a dopaminergic and a serotoninergic genotype may inform future research on neuropharmacological treatment of depression and schizophrenia, both of which correlate with error-processing (Alain et al., 2002; Holmes and Pizzagalli, 2008; Olvet and Hajcak, 2008).

Finally, the limitations of the present study should be acknowledged. Because dopaminergic polymorphisms have previously been shown to have sexually dimorphic effects (Stein et al., 2005; Wacker et al., 2005) we constrained our sample to male participants. In addition, participants were recruited from a not previously genotyped sample resulting in heterogeneous cell sizes for some analyses (particularly those involving 5-HTTLPR or DRD2 in addition to COMT). Although the 5-HTTLPR x COMT x Substance interaction did nevertheless reach statistical significance, future studies investigating men and women which were preselected based on genotypes would allow further generalization of the present findings. Finally, for pragmatic reasons (i.e. safety, statistical power, conductibility) we only investigated the effect of 200 mg sulpiride in comparison to placebo. Future studies including different doses and/or D2 agonists may help to verify and/or modify the interpretations we derived from our findings.

Despite these limitations the present study clearly shows a link between dopaminergic genes, a dopaminergic challenge and error processing thereby extending prior work using smaller samples and either genetic or pharmacological approaches alone in important ways (for reviews see: Jocham and Ullsperger, 2009; Ullsperger, 2010). The present findings suggest (1) that a significant effect of COMT Val158Met on error processing can be demonstrated using a sufficiently large sample and ICA-decomposed EEG (Makeig et al., 2002), (2) that modulations of mid-cingulate ERN amplitudes after administration of D_2

antagonists (Zirnheld et al., 2004) depend on COMT Val158Met genotype. Moreover, the present study provides initial evidence for a modulating involvement of 5-HT in dopaminergic mechanisms of error processing opening up novel research perspectives.

Table 1

Sample characteristics and descriptive statistics

	Placebo		**Sulpiride**	
	Val	**Met**	**Val**	**Met**
N	54	25	65	25
A1-	39	16	47	14
S+ and L_G+	39	17	49	13
Age	23.8 (.4)	23.4 (.6)	23.8 (.4)	23.5 (.4)
Weight (kg)	77 (2)	79 (3)	78 (2)	81 (3)
Height (cm)	183 (8)	183 (7)	183 (8)	181 (7)
Number of Errors	19.6 (1.3)	18.5 (1.9)	20.0 (1.2)	20.5 (2.3)
Reaction Time (ms)	366 (4)	357 (5)	364 (3)	358 (4)
Post-error slowing (ms)	77 (4)	56 (4)	72 (3)	70 (5)
Post-correct slowing (ms)	34 (3)	29 (4)	40 (3)	36 (2)
IC-ERN (error trials)	-3.0 (.2)	-2.4 (.2)	-2.6 (.2)	-3.1 (.3)
IC-ERN (correct trials)	-1.1 (.1)	-1.2 (.1)	-1.1 (.1)	-1.1 (.1)
IC-ERN (error – correct)	-1.9 (.2)	-1.2 (.3)	-1.4 (.2)	-1.9 (.3)
Channel-ERN (error – correct)	-23.3 (1.7)	-19.5 (2.5)	-19.4 (1.6)	-23.8 (2.5)

Notes. First row: Number of subjects per group. Second and third row: Number of A1- carriers (DRD2Taq I a) and S+ L_G+ carriers (5-HTTLPR) per group. Below: Values given as means (SEM). Channel ERN reflects the difference score (amplitude error minus amplitude correct) measured at scalp channel Fz.

Figure 1 Postulated relationship between prefrontal dopamine level, COMT and relative D1 vs. D2 receptor activation as previously described by D. Durstewitz and J. K. Seamans (2008). Due to enhanced relative D2 receptor activation in Val vs. Met carriers we hypothesized increased error-related negativity and post-error slowing in Val vs. Met carriers. By increasing PFC dopamine activity through presynaptic D2 receptor blockade sulpiride (200 mg) is predicted to shift Val+ carriers into medium and Met/Met carriers into high dopamine levels (red arrows) resulting in reduction or enhancement of error-related negativity/post-error slowing, respectively.

Figure 2 Cluster-mean independent component (IC) scalp topographies for outlier (top left) and other IC clusters. Cluster 9 (highlighted) was used for analyses of error-related brain activity in the present study.

Figure 3 Interactions of sulpiride and COMT on neural and behavioral error-processing correlates. **(a)** Grand average event-related potentials (ERPs) for a medial frontal independent component cluster (IC-cluster) following erroneous button presses (at latency 0) for Val+ (grey) and Met/Met (black) carriers, who received placebo (thick) or sulpiride (thin). Independent component ERPs were normalized by the root mean square over the component scalp map projection to all channels prior to averaging (see Methods). A standard brain image (Montreal Neurological Institute) indicates the region of maximum concentration (equivalent dipole density) of this IC-cluster. **(b)** Bar plots indicating means (and SEMs) of peak IC-cluster event-related potentials (left) and reaction-time slowing in the subsequent trial (right) following errors in the placebo (white) or sulpiride (grey) groups.

Figure 4 Interactions of sulpiride, COMT and 5HTTLPR on peak IC-cluster event-related potentials. Bar plots indicating means (and SEMs) of peak IC-cluster event-related potentials following errors in the placebo (white) or sulpiride (grey) groups.

Figure 1

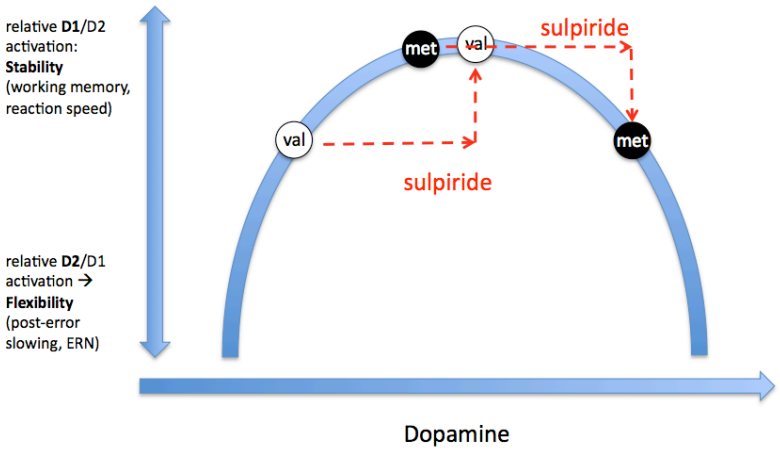

relative **D1**/D2 activation: **Stability** (working memory, reaction speed)
relative **D2**/D1 activation → **Flexibility** (post-error slowing, ERN)
met
val
sulpiride
val
sulpiride
met
Dopamine

Figure 2

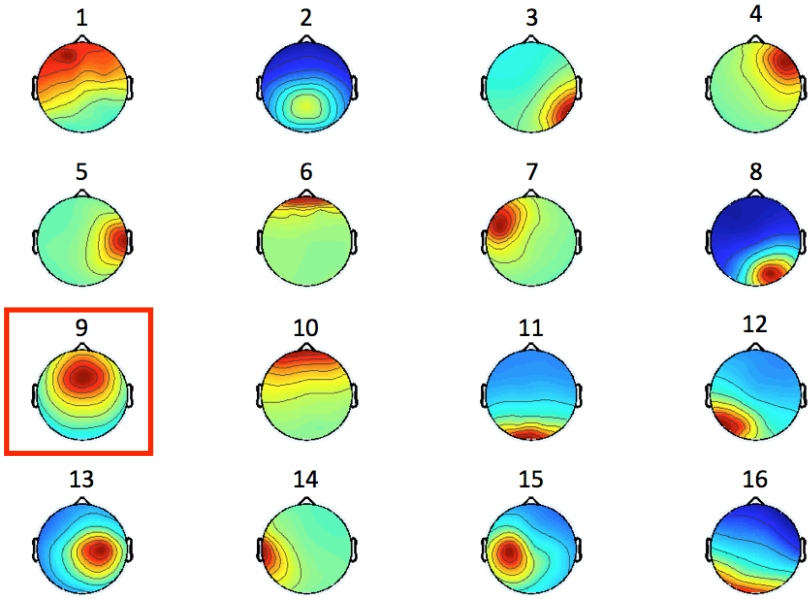
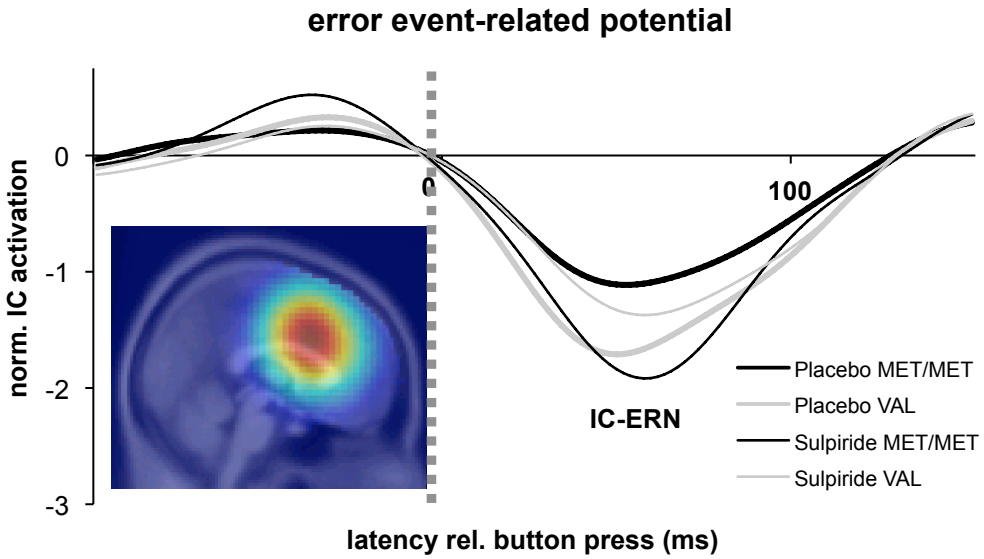


Figure 3

a


error event-related potential
norm. IC activation
0
-1
-2
-3
0
100
IC-ERN
Placebo MET/MET
Placebo VAL
Sulpiride MET/MET
Sulpiride VAL
latency rel. button press (ms)

b

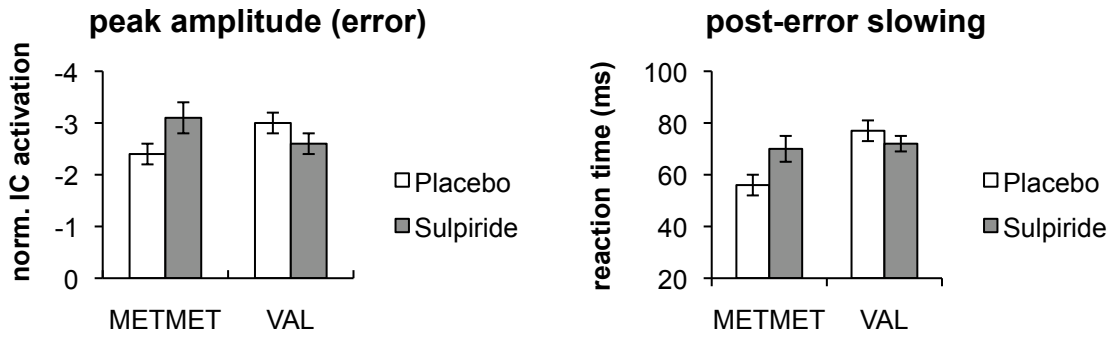
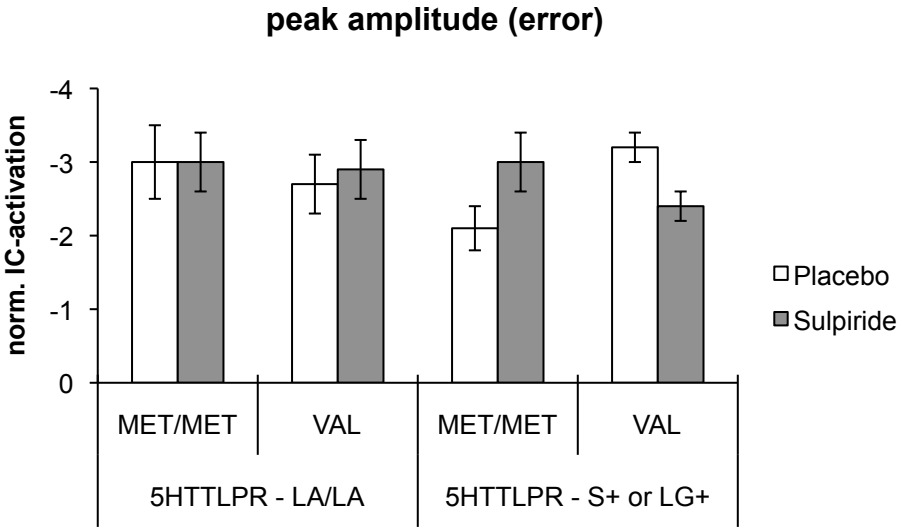

peak amplitude (error)
norm. IC activation
-4
-3
-2
-1
0
METMET
VAL
Placebo
Sulpiride
post-error slowing
reaction time (ms)
100
80
60
40
20
METMET
VAL
Placebo
Sulpiride

Figure 4


peak amplitude (error)
norm. IC-activation
-4
-3
-2
-1
0
MET/MET
VAL
MET/MET
VAL
5HTTLPR - LA/LA
5HTTLPR - S+ or LG+
Placebo
Sulpiride

References

Alain C, McNeely HE, He Y, Christensen BK, West R (2002) Neurophysiological evidence of error-monitoring deficits in patients with schizophrenia. Cereb Cortex 12:840-846.

Bilder RM, Volavka J, Lachman HM, Grace AA (2004) The catechol-O-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology 29:1943-1961.

Borroni B, Grassi M, Agosti C, Archetti S, Costanzi C, Cornali C, Caltagirone C, Caimi L, Di Luca M, Padovani A (2006) Cumulative effect of COMT and 5-HTTLPR polymorphisms and their interaction with disease severity and comorbidities on the risk of psychosis in Alzheimer disease. Am J Geriatr Psychiatry 14:343-351.

Botvinick MM, Braver TS, Barch DM, Carter CS, Cohen JD (2001) Conflict monitoring and cognitive control. Psychol Rev 108:624-652.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301:386-389.

Debener S, Ullsperger M, Siegel M, Fiehler K, von Cramon DY, Engel AK (2005) Trial-by-trial coupling of concurrent electroencephalogram and functional magnetic resonance imaging identifies the dynamics of performance monitoring. J Neurosci 25:11730-11737.

Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134:9-21.

Durstewitz D, Seamans JK (2008) The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. Biol Psychiatry 64:739-749.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR (2001) Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A 98:6917-6922.

Erceg-Hurn DM, Mirosevich VM (2008) Modern robust statistical methods: an easy way to maximize the accuracy and power of your research. Am Psychol 63:591-601.

Fallgatter AJ, Herrmann MJ, Roemmler J, Ehlis AC, Wagener A, Heidrich A, Ortega G, Zeng Y, Lesch KP (2004) Allelic variation of serotonin transporter function modulates the brain electrical response for error processing. Neuropsychopharmacology 29:1506-1511.

Fink KB, Gothert M (2007) 5-HT receptor regulation of neurotransmitter release. Pharmacol Rev 59:360-417.

Frank MJ, D'Lauro C, Curran T (2007) Cross-task individual differences in error processing: neural, electrophysiological, and genetic components. Cogn Affect Behav Neurosci 7:297-308.

Gehring WJ, Knight RT (2000) Prefrontal-cingulate interactions in action monitoring. Nat Neurosci 3:516-520.

Goldman-Rakic PS, Muly EC, 3rd, Williams GV (2000) D(1) receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 31:295-301.

Gramann K, Gwin JT, Bigdely-Shamlo N, Ferris DP, Makeig S (2010) Visual evoked responses during standing and walking. Front Hum Neurosci 4:1 - 12.

Hajcak G, McDonald N, Simons RF (2004) Error-related psychophysiology and negative affect. Brain Cogn 56:189-197.

Holmes AJ, Pizzagalli DA (2008) Spatiotemporal dynamics of error processing dysfunctions in major depressive disorder. Arch Gen Psychiatry 65:179-188.

Holroyd CB, Coles MG (2002) The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. Psychol Rev 109:679-709.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D (2006) Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet 78:815-826.

Jocham G, Ullsperger M (2009) Neuropharmacology of performance monitoring. Neurosci Biobehav Rev 33:48-60.

Krämer UM, Cunillera T, Camara E, Marco-Pallares J, Cucurell D, Nager W, Bauer P, Schule R, Schols L, Rodriguez-Fornells A, Munte TF (2007) The impact of catechol-O-methyltransferase and dopamine D4 receptor genotypes on neurophysiological markers of performance monitoring. J Neurosci 27:14190-14198.

Kuroki T, Meltzer HY, Ichikawa J (1999) Effects of antipsychotic drugs on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens. J Pharmacol Exp Ther 288:774-781.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243-250.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274:1527-1531.

Makeig S, Debener S, Onton J, Delorme A (2004) Mining event-related brain dynamics. Trends Cogn Sci 8:204-210.

Makeig S, Westerfield M, Jung TP, Enghoff S, Townsend J, Courchesne E, Sejnowski TJ (2002) Dynamic brain sources of visual evoked responses. Science 295:690-694.

Margraf J (1994) Mini-DIPS: Diagnostisches Kurz-Interview bei psychischen
Störungen. Berlin: Springer.

Mauri MC, Bravin S, Bitetto A, Rudelli R, Invernizzi G (1996) A risk-benefit assessment of sulpiride in the treatment of schizophrenia. Drug Saf 14:288-298.

Mereu G, Casu M, Gessa GL (1983) (-)-Sulpiride activates the firing rate and tyrosine hydroxylase activity of dopaminergic neurons in unanesthetized rats. Brain Res 264:105-110.

Nakamura M, Ueno S, Sano A, Tanabe H (2000) The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. Mol Psychiatry 5:32-38.

Olvet DM, Hajcak G (2008) The error-related negativity (ERN) and psychopathology: toward an endophenotype. Clin Psychol Rev 28:1343-1354.

Oostenveld R, Oostendorp TF (2002) Validating the boundary element method for forward and inverse EEG computations in the presence of a hole in the skull. Hum Brain Mapp 17:179-192.

Osinsky R, Reuter M, Kupper Y, Schmitz A, Kozyra E, Alexander N, Hennig J (2008) Variation in the serotonin transporter gene modulates selective attention to threat. Emotion 8:584-588.

Palmer JA, Kreutz-Delgado K, Makeig S (2006) Super-Gaussian mixture source model for ICA. In: Lecture Notes in Computer Science (Rosca J, Erdogmus D, Principe JC, Haykin S, eds), pp 854-861. Heidelberg: Springer.

Palmer JA, Makeig S, Delgado KK, Rao BD (2008) Newton method for the ICA mixture model. IEEE International Conference on Acoustics, Speech and Signal Processing, 2008 ICASSP 2008 IEEE, Las Vegas, Nevada:1805–1808.

Pohjalainen T, Rinne JO, Nagren K, Lehikoinen P, Anttila K, Syvalahti EK, Hietala J (1998) The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. Mol Psychiatry 3:256-260.

Pruessner JC, Champagne F, Meaney MJ, Dagher A (2004) Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. J Neurosci 24:2825-2831.

Rabbitt PM, Phillips S (1967) Error-detection and correction latencies as a function of S-R compatibility. Q J Exp Psychol 19:37-42.

Reuter M, Hennig J (2005) Association of the functional catechol-O-methyltransferase VAL158MET polymorphism with the personality trait of extraversion. Neuroreport 16:1135-1138.

Reuter M, Schmitz A, Corr P, Hennig J (2006) Molecular genetics support Gray's personality theory: the interaction of COMT and DRD2 polymorphisms predicts the behavioural approach system. Int J Neuropsychopharmacol 9:155-166.

Stein MB, Fallin MD, Schork NJ, Gelernter J (2005) COMT polymorphisms and anxiety-related personality traits. Neuropsychopharmacology 30:2092-2102.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val158Met and beyond. Biol Psychiatry 60:141-151.

Ullsperger M (2010) Genetic association studies of performance monitoring and learning from feedback: the role of dopamine and serotonin. Neurosci Biobehav Rev 34:649-659.

Vogt BA (2005) Pain and emotion interactions in subregions of the cingulate gyrus. Nat Rev Neurosci 6:533-544.

Wacker J, Reuter M, Hennig J, Stemmler G (2005) Sexually dimorphic link between dopamine D2 receptor gene and neuroticism-anxiety. Neuroreport 16:611-614.

Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, Lee ML, Xiao T, Papp A, Wang D, Sadee W (2007) Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc Natl Acad Sci U S A 104:20552-20557.

Zirnheld PJ, Carroll CA, Kieffaber PD, O'Donnell BF, Shekhar A, Hetrick WP (2004) Haloperidol impairs learning and error-related negativity in humans. J Cogn Neurosci 16:1098-1112.

Study 3

Mueller, E. M., Nguyen, J., Ray, W., & Borkovec, T. D. (2010). Future oriented decision-making in Generalized Anxiety Disorder is evident across different versions of the Iowa Gambling Task. *Journal of Behavior Therapy and Experimental Psychiatry, 41,* 165-171.

J. Behav. Ther. & Exp. Psychiat. 41 (2010) 165–171

Contents lists available at ScienceDirect

Journal of Behavior Therapy and Experimental Psychiatry

journal homepage: www.elsevier.com/locate/jbtep

Future-oriented decision-making in Generalized Anxiety Disorder is evident across different versions of the Iowa Gambling Task

Erik M. Mueller [a,b,*], Jennifer Nguyen [b], William J. Ray [b], Thomas D. Borkovec [b]

[a] Department of Psychology, Universitaet Marburg, Gutenbergstrasse 18, 35032 Marburg, Germany
[b] Department of Psychology, 111 Moore Building, Penn State University, University Park, PA 16802, USA

ARTICLE INFO

Article history:
Received 20 July 2009
Received in revised form
15 December 2009
Accepted 17 December 2009

Keywords:
Decision-making
Iowa Gambling Task
Worrying
Anxiety
Reward

ABSTRACT

Generalized Anxiety Disorder (GAD) and excessive worrying are characterized by a preoccupation with the future. Thus, enhanced identification of potential future punishments or omissions of reward may be related to the disorder. To test this hypothesis, $n = 47$ students meeting GAD criteria according to the GADQ-IV (GAD analogues) or not (control participants) performed the Iowa Gambling Task, which has been related to sensitivity to future consequences. In order to disentangle sensitivity to future loss and sensitivity to high short-term loss magnitudes, which could also lead to enhanced Iowa Gambling Task performance, participants also performed a modified version of the task with reversed contingencies. In both versions, GAD analogues learned to avoid decisions with high probability of long-term loss significantly faster than control participants. Results, therefore, indicate that GAD is characterized by enhanced processing of potential future losses rather than sensitivity to large short-term loss.
© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Worry can be defined as a mental preoccupation with potential negative events that may occur in the future. According to this definition, there are three important elements of worrying. First, worrying is associated with negative events that are, in terms of learning theory, some kind of punishment or omission of reward. Second, these negative events are to some extent unpredictable and thus follow a probabilistic as opposed to deterministic schedule. Third, worrying is mostly future-oriented. Similarly, Brown, O'Leary, and Barlow (1993) describe worry as "a future-oriented mood state in which one becomes ready or prepared to attempt to cope with upcoming events" (p. 139). From these perspectives, it could be hypothesized that people suffering from GAD (characterized by excessive worrying and being overly concerned with the future; Borkovec, Robinson, Pruzinsky, & DePree, 1983) would deploy attentional (e.g. Mathews & MacLeod, 1985; Mogg & Bradley, 1998) and working memory (Hayes, Hirsch, & Mathews, 2008) capacities to constantly search for cues of possible future losses, thus leading to a failure to enjoy life or to live in the present moment (Borkovec, 2002; Borkovec, Alcaine, & Behar, 2004; Borkovec & Sharpless, 2004).

The above specified elements of worrying can be found across different models of Generalized Anxiety Disorder. For example, Wells (1999) has proposed a cognitive model of Generalized Anxiety Disorder according to which there are two types of worry. Type 1 worry refers to worry about external events and non-cognitive internal events (e.g. "my boyfriend will break up with me"). Type 2 worry or metaworry refers to worry about one's own thinking (e.g. "I must stop worrying or I'll lose control."). Metaworry is a critical part of the model because it explains why individuals with GAD avoid worry-inducing situations: they fear that worrying leads to negative future consequences. Thus, a common feature of both types of worry is that they are related to the anticipation of possible negative futures (i.e. loss of a beloved person and losing control).

From a different perspective, Dugas, Gagnon, Ladouceur, and Freeston (1998) emphasize that worrying is characterized by intolerance to uncertainty, including uncertainty about the *future* (i.e. "My mind can't be relaxed if I don't know what will happen tomorrow"; Freeston, Rheaume, Letarte, Dugas, & Ladouceur, 1994). Obviously, not knowing what lies ahead should be especially aversive to those individuals who overly process possible negative futures (Dugas, Freeston, & Ladouceur, 1997). In other words, states of uncertainty may encourage the mental processing of negative probabilistic futures, which according to the above definition reflects worrying.

Finally, the avoidance theory of GAD (Borkovec et al., 2004) assumes that worrying is motivated by its ability to (a) suppress somatic aspects of anxious experience and (b) to remove the perceived threat itself. This perceived threat generally is an

* Corresponding author. Department of Psychology, Universitaet Marburg, Gutenbergstrasse 18, 35032 Marburg, Hessen, Germany. Tel.: +49 6421 895157; fax: +49 6421 28 26559.
E-mail addresses: erik.mueller@staff.uni-marburg.de, muellererik@gmx.de (E.M. Mueller).

0005-7916/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jbtep.2009.12.002

anticipated bad event in the close or distant future, as opposed to a real danger in the present situation (Borkovec et al. 1983). Thus, according to the avoidance theory of worry and GAD, worrying is strongly related to the anticipation and avoidance of future bad events that are probabilistic and rarely happen in reality (Borkovec, Hazlett-Stevens, & Diaz, 1999). Taken together, Borkovec et al.'s (2004), Dugas et al.'s (1998), and Wells' (1999) and models of GAD are consistent with the hypothesis that individuals with GAD show exaggerated processing of uncertain/probabilistic negative events that occur in the future.

In order to experimentally test in the present experiment whether GAD and/or chronic worrying is associated with hypersensitivity for future (probabilistic) outcomes, participants with and without GAD symptoms performed the Iowa Gambling Task (IGT, Bechara, Damasio, Damasio, & Anderson, 1994). In the IGT, participants are instructed to pick cards from four different decks. Each card of a given deck always leads to the same fictional reward, which is $100 for decks A and B, and $50 for decks C and D. In addition, each card can also lead to a loss, which is unpredictable on a trial-by-trial basis. However, in the long run, the losses of decks A and B sum up to be proportionally higher than the losses of decks C and D. Accordingly, participants must learn to avoid decisions that lead to high short-term but low long-term gain (i.e. decks A and B) and to make decisions instead that lead to low short-term but higher long-term gain. In sum, successful IGT performance requires processing of exactly those elements (i.e. probabilistic future losses) that are hypothesized above to relate to GAD and chronic worrying.

The IGT was initially developed in conjunction with the somatic marker hypothesis from Damasio (1994), which relates decision-making to the ventromedial region of the prefrontal cortex (VMPFC) and states that signals from the body may influence decision-making under conditions of ambiguity. It was based on the observation that patients with lesioned VMPFC in real life often (a) do not produce physiological responses to anticipated emotional events and (b) show a failure to act in a future-oriented manner despite intact intelligence (Bechara et al., 1994). Even though some aspects about the hypothesis have been a matter of debate (for review see Dunn, Dalgleish, & Lawrence, 2006), the IGT has been able to experimentally establish the lack of future-oriented behavior in individuals with lesioned VMPFC (Bechara et al., 1994; Bechara, Damasio, Damasio, & Lee, 1999). Moreover, this test has been intensively used to demonstrate *hypo*sensitivity to future outcomes in impulsive, pathological conditions such as pathological gambling (Cavedini, Riboldi, Keller, D'Annucci, & Bellodi, 2002), attention deficit/hyperactivity disorder (Garon, Moore, & Waschbusch, 2006; Toplak, Jain, & Tannock, 2005), delinquency (Schmitt, Brinkley, & Newman, 1999), and substance abuse (e.g. Bechara Dolan et al., 2001; Bechara, Dolan, & Hindes 2002) (for reviews see Buelow & Suhr, 2009 and Dunn et al., 2006).

In contrast to these disorders, which are characterized by a failure to act in a future or long-term oriented manner, it has been hypothesized above that GAD is rather related to enhanced future-oriented processing of cues that may signal punishments and/or reward omissions. Accordingly, one would expect that individuals who frequently worry are better than non-worriers in the IGT. Interestingly, one study (Garon et al., 2006) found that children suffering from ADHD performed worse than healthy children, but this was not the case if they had a comorbid diagnosis of GAD. In addition, some other studies have reported preliminary evidence for *positive* relationships between IGT performance and conditions related to worrying and anxiety (van Honk, Hermans, Putman, Montagne, & Schutter, 2002; Peters & Slovic, 2000; Schmitt et al., 1999; Smoski et al., 2008; but see Miu, Heilman, & Houser, 2008). In sum, even though decision-making deficits with stimuli unrelated to reward and punishment have been reported in GAD (Metzger, Miller, Cohen, Sofka, & Borkovec, 1990), there is theoretical and empirical support for the hypothesis that worrying would be associated with enhanced performance on a decision-making task that measures future orientation with regard to probabilistic punishment.

However, as described above, the upper, long-term disadvantageous decks in the IGT also have larger loss magnitudes in single trials (e.g. $200 or $300 for deck A, $1250 for deck B) than the lower, advantageous decks (e.g. $50 or $25 for deck C, $250 for deck D). Thus, if an individual avoids the upper decks of the IGT, it cannot be judged whether this reflects an enhanced sensitivity for long-term loss or an enhanced sensitivity for short-term loss magnitudes.[1] In order to test whether GAD would rather be associated with the avoidance of short-term loss magnitudes or an enhanced sensitivity for future loss we included an additional IGT version in which future loss could only be avoided by accepting relatively large consistent short-term loss magnitudes (Bechara, Tranel, & Damasio, 2000; Crone, Vendel, & van der Molen, 2003). In this version, the contingency table of the original IGT was inverted (see Table 1). Thus, there were decks with high consistent loss magnitudes and with low consistent loss magnitudes. While the former also brought proportionally higher inconsistent rewards (which made it long-term advantageous) the latter was associated with proportionally smaller inconsistent rewards (which made it long-term disadvantageous).

Taken together, if GAD is characterized by increased sensitivity to short-term loss, lowered IGT performance would be expected in the modified version (in which avoiding decks with larger short-term loss leads to overall long-term loss), whereas enhanced performance would be expected in the standard version (in which sensitivity to both long and short-term loss would lead to advantageous selections). In contrast, if GAD is related to hypersensitivity to inconsistent future long-term losses, these participants should perform better in both versions of the IGT. To test these competing hypotheses, the present study investigated participants with and without GAD symptoms who performed both versions of the IGT in a repeated-measures design.

The pair of advantageous decks (C and D) and the pair of disadvantageous decks (A and B) of the standard IGT each consists of one deck with frequent low punishments (C and A) and one deck with infrequent high punishments (D and B). To test whether GAD is associated with avoidance of infrequent high punishments (regardless of long-term advantage), we also compared groups with regard to the pooled selections from decks D and B of the standard IGT. Because this score is insensitive for long-term consequences, we had no particular hypotheses for these analyses.

2. Method

2.1. Participants

Three cohorts of undergraduate students ($N = 1882$) completed a battery of questionnaires during group screening procedures at the beginning of the semester. Of these, 155 students met GAD criteria according to the GADQ-IV (see below) and agreed to be contacted for participation in later studies. These 155 students and a similar number of students who did not meet GAD criteria were contacted by email and offered class credit for participation in

[1] In the context of the IGT, the avoidance of short-term loss magnitudes could be driven by "risk avoidance", which has previously been linked to anxiety (e.g. Maner & Schmidt, 2006). However, given that the subjective risk for a deck dynamically changes throughout the course of the IGT (as more knowledge about the decks is acquired cf. Brand et al., 2006), and because risk avoidance may not be limited to avoidance of short-term risk but may also include avoidance of long-term oriented risk, we have used the more narrow term "sensitivity to short-term loss" and define it as a tendency to avoid decisions associated with relatively large single-trial loss magnitudes.

Table 1
Contingency table for the first 12 selections of the standard and modified versions of the IGT.

	Standard version				Modified version			
Deck	A	B	C	D	E	F	G	H
Position	Upper left	Upper right	Lower left	Lower right	Upper right	Lower right	Upper left	Lower left
Constant gain/loss	+100	+100	+50	+50	−100	−100	−50	−50
Selection #								
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	−150	0	−50	0	+150	0	+50	0
4	0	0	0	0	0	0	0	0
5	−300	0	−50	0	+300	0	+50	0
6	0	0	0	0	0	0	0	0
7	−200	0	−50	0	+200	0	+50	0
8	0	0	0	0	0	0	0	0
9	−250	−1250	−50	−250	+250	+1250	+50	+250
10	−350	0	−50	0	+350	0	+50	0
11	0	0	0	0	0	0	0	0
12	−350	0	−25	0	+350	0	+25	0
Net gain/loss over 10 trials	−250	−250	+250	+250	+250	+250	−250	−250

For example, every time deck A was chosen in the standard version the participant received a gain of $100. The third time deck A was chosen (regardless of whether other decks were selected in between) the participant also received a loss of $150. Thus, 10 selections from deck A lead to a net loss of $ −250 ((10 × $100) − $150 − $300 − $200 − $250 − $350). In contrast, 10 selections from deck E in the modified version lead to a net gain of $250 ((10 × − $100) + $150 + $300 + $200 + $250 + $350). Note, that the positions of advantageous decks were different in the two versions in order to reduce learning effects. The complete contingency table for the standard version can be found in Bechara et al. (1994) and by its inversion (multiplication by −1) the contingency table for the modified version can be constructed.

a decision-making study. Participants who were selected according to the DSM-IV criteria had an average score in the Penn State Worry Questionnaire (PSWQ, Meyer, Miller, Metzger, & Borkovec, 1990) of 66.5 (SD = 7.6), whereas participants who did not meet DSM-IV criteria had a mean PSWQ score of 45.11 (SD = 12.3). The final sample consisted of $N = 27$ students meeting GAD criteria (23 female, 4 male; age: $M = 19.35$ years; SD = 1.3 years; 4 left-handed; one Asian, one Hispanic) and 20 control participants (12 female, 8 male; age: $M = 18.90$ years; SD = 0.8 years, 3 left-handed, two Asian, one Hispanic) who also met an average of 1.2 GAD criteria (SD = 1.3) in the GADQ-IV. As expected, GAD participants of the final sample had higher scores in the PSWQ ($M = 65.9$, SD = 12.6) than control participants ($M = 43.8$, SD = 8.1), ($t(42) = 0.7.03$, $p < 0.001$) and also had higher scores in the social interaction anxiety scale (SIAS, Mattick & Clarke, 1998) ($M = 37.7$, SD = 10.4, vs. $M = 26.3$, SD = 11.7), ($t(42) = 3.34$; $p < 0.005$). Groups did not differ with regard to handedness ($p > 0.9$), age ($p > 0.15$) or ethnicity ($p > 0.6$). However, there were significant differences in the number of females ($\chi^2(1) = 3.83$; $p = 0.05$). The factor 'Gender' was, therefore, included in all subsequent analyses. In addition, we re-conducted the main analyses with subsamples of the two groups ($n = 14$) that were matched according to gender, age and handedness.

2.2. Materials

2.2.1. Questionnaires

The GADQ-IV is a 9 item self-report measure that assesses DSM-IV GAD criterial symptoms and reliably identifies participants who meet criteria for GAD (Newman et al., 2002). Because the suggested cutoff score of the GADQ-IV has been found to overdiagnose individuals (Behar, Alcaine, Zuellig, & Borkovec 2003), we used the more conservative criterion that *all* of the following DSM-IV criteria had to be satisfied[2]: (a) excessive worry, (b) difficulty in controlling their worrying once it started, (c) having at least two topics of worry, (d) being bothered by excessive and uncontrollable worries more days than not during the past six months, and (e) reporting at least three out of six associated symptoms. In addition to the GADQ-IV, participants filled out the Penn State Worry Questionnaire (Meyer et al., 1990) and the Social Interaction Anxiety Scale (SIAS, Mattick & Clarke, 1998) during the group screening procedure and for 44 participants of the present study this data was also available for analysis. The SIAS is a measure of social anxiety, which shows moderate correlations with depression questionnaires (Mattick & Clarke, 1998) and lower correlations with the GADQ-IV (Newman et al., 2002). The SIAS and the PSWQ were included in the present study to test the specificity of the hypothesized role of worrying.

2.2.2. Iowa Gambling Task

The IGT was adapted from the descriptions in Bechara et al. (1999) study. A trial began with a display of four decks of cards (A, B, C and D) and immediately after the participant selected a card by button press, feedback on the amount won and lost in that trial and on the total amount left was given. For the standard version of the IGT, the selection of the upper (A and B) and lower (C and D) decks always lead to a gain of $100 and $50, respectively. The amount of losses per trial was non-systematic (see Bechara et al., 1994). However, over 10 selections, losses summed up to $1250 for the upper and $250 for the lower decks, leading to a net loss of $250 for the upper ($1250 − 10 × $100) and a net gain of $250 for the lower decks. Therefore, every selection of a lower deck in the standard version was considered 'advantageous', because despite being unpredictable at a molecular trial-by-trial level, every lower deck selection was advantageous on a global molar level.

The modified version of the IGT had the reversed contingencies of the standard version (Bechara et al., 2000). The disadvantageous decks (G and H) had a constant loss of $50 and a total win of $250 over 10 trials, whereas the advantageous decks (E and F) had a constant loss of $100 and a total win of $1,250 over 10 trials. Whereas A and B vs. C and D were arranged horizontally in the standard version G and H vs. E and F were arranged vertically in the modified version in order to reduce effects of learning. Contingencies and positions of the decks for the two versions are further illustrated in Table 1.

[2] To validate the increased specificity of the all-criteria vs. standard composite score, we compared the PSWQ scores of the two GAD groups that would result from either method in the first cohort. As expected, the GAD group according to the all-criterion score had a higher average PSWQ (M= 66.7; SD= 7.43) than the GAD group that would result from applying the composite score (M= 63.4; SD= 9.9).

Each version consisted of a total of one hundred selections, separated into five blocks of 20 trials. After a block, there was a subjective awareness check asking the participants which deck they believed lead to the highest overall gain. Due to a software problem, this variable was not recorded for $n = 9$ (GAD: $n = 4$; Control: $n = 5$) participants.

2.3. Procedure

Participants reported to the laboratory and read and signed an informed consent form. They were then brought to a small sound-shielded room where participants read the instructions for the gambling task and performed one practice trial. Half of the participants in each GAD and non-GAD group began with the standard version of the gambling task, and the other half with the modified version, followed by a 1-min break. After the break, participants engaged in the other version of the task. The order of the conditions was randomly assigned within the GAD- and the control group.

2.4. Statistical analyses

For both versions, the primary dependent variable was the number of advantageous selections within a block of 20 trials (C and D in the standard version and E and F in the modified version). Repeated-measures ANOVAs with the between-subject factors GROUP and GENDER and the within factors BLOCK and VERSION were performed to analyze the number of advantageous decisions (i.e. the main hypothesis) and to analyze the number of selections from decks B + D (i.e. the disadvantageous and advantageous infrequent high loss decks). Preliminary tests were conducted prior to these main analyses. First, independent *t*-tests on the number of advantageous decisions in the first block were performed to assure that there were no group differences at the beginning of the task. To further confirm that participants in both groups had taken similar amounts of time to come to their decisions, and to confirm that groups did not differ in the cognitive penetration of the task we also compared reaction times (measured from the beginning of a trial until the button press and averaged across conditions) and the number of correct answers in awareness checks between groups with independent *t*-tests. Because it has recently been criticized that not enough is known about the reliability of the IGT (Buelow & Suhr 2009), we also assessed internal consistency of the five blocks (across groups). This also allowed investigating whether performance in each block was related to a homogenous construct (cf. Brand, Labudda, & Markowitsch, 2006). All statistical analyses were conducted using SPSS version 11.5.1.

3. Results

3.1. Preliminary analyses and reliability of the measure

Groups did not differ in the number of advantageous decisions in the first block of the standard ($t(45) = 1.05$; $p > 0.3$) or the modified version of the IGT ($t(45) = 0.08$; $p > 0.9$), indicating that there were no *a priori* differences in preference for particular choices. In addition, participants in the two groups did not differ in the time they took to make a decision ($t(45) = 0.12$; $p > 0.9$) and for those participants where subjective awareness data was recorded, there were no GAD vs. control group differences in awareness of advantageous decks ($t(36) = .79$; $p > .4$). A preliminary Order- × Version × Block ANOVA revealed no main effect or interaction involving order (all $ps > 0.2$), suggesting that there were no substantial test re-test effects. Cronbach alphas for the standard ($\alpha = 0.83$) and the modified ($\alpha = 0.86$) versions were high.

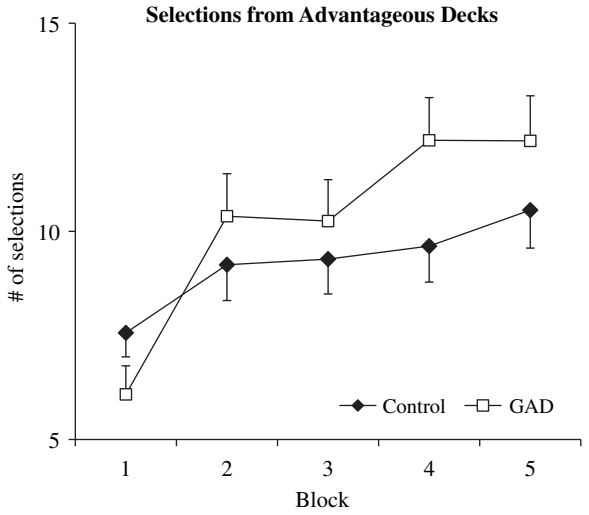


Fig. 1. Estimated marginal means ± SEM of the total number of cards selected from advantageous decks (C and D) in each block of 20 cards for GAD (squares) and control participants (diamonds) reflecting the averaged performance from both versions of the task.

3.2. Advantageous selections

As shown in Fig. 1, GAD participants showed a more pronounced increase of advantageous selections over the five blocks than did the control participants. Statistically this was confirmed with the Group × Gender × Block × Version ANOVA which revealed a significant Group × Block interaction ($F(1, 43) = 3.14$; $p < 0.023$; partial $\epsilon^2 = 0.07$).[3] This interaction was characterized by a group difference in the linear increase across blocks ($F(1, 43) = 5.07$; $p < 0.03$; partial $\epsilon^2 = 0.11$), with a steeper slope in the GAD than non-GAD group. In addition, there were main effects for Block ($F(1, 43) = 17.42$; $p < 0.0001$; partial $\epsilon^2 = 0.28$) and Version ($F(1, 43) = 13.78$; $p < 0.001$; partial $\epsilon^2 = 0.24$), which were further qualified by a Version × Block interaction ($F(1, 43) = 2.79$; $p < 0.033$; partial $\epsilon^2 = 0.06$), indicating that learning (i.e. a linear increase in advantageous decisions over the five consecutive blocks) occurred faster in the modified version of the task. Group × Version and Group - × Version × Block interactions were not significant ($ps > 0.9$).

To further test whether enhanced learning was specifically related to worrying, we divided the sample into learners (i.e. individuals that made less than 50% disadvantageous decisions in the final block of both versions) and non-learners (i.e. individuals that made less than 50% advantageous decisions) and then compared the PSWQ and SIAS scores of these groups. Consistent with the previous analyses, learners ($n = 35$) had higher mean PSWQ scores ($M = 58.6$; SD = 15.3) than non-learners ($n = 9$, $M = 47.7$, SD = 10.5) ($t(42) = 2.01$; $p < 0.05$). In contrast, learners and non-learners did not differ in their SIAS scores ($t(42) = 1.08$, $p > 0.2$).

3.3. High vs. low frequency loss selections in the standard version of the IGT

To test whether aside from long-term advantageous selections, GAD vs. control participants also differed in their avoidance of infrequent large loss magnitudes (as opposed to more frequent but smaller losses) we analyzed the sum of selections from decks A

[3] When analyses were re-conducted with subsamples of the two groups (both: $n = 14$) which were matched according to age, gender and handedness this effect (i.e. group × block interaction) was replicated [$F(1,24) = 3.55$; $p < 0.018$].

(disadvantageous, high frequency of relatively low losses) and C (advantageous, high frequency of relatively low losses) for the five blocks of 20 trials. When we conducted a Group × Gender × Block ANOVA on these scores there was a main effect for Group ($F(1, 43) = 9.37$, $p < 0.01$, partial $\epsilon^2 = 0.18$), indicating that GAD analogues avoided decks with large infrequent loss (Fig. 2). In addition, there also was a Gender × Group interaction ($F(1, 43) = 8.67$, $p < 0.01$, partial $\epsilon^2 = 0.17$), indicating that the group difference was stronger in male vs. female participants.

4. Discussion

The present study used a decision-making paradigm to assess sensitivity to future reward and loss among participants with and without GAD symptoms. GAD analogues learned significantly faster than control participants to avoid selections associated with long-term loss. This effect was observed regardless of whether long-term loss was due to decisions associated with a larger probability of high punishments (standard version) or with a smaller probability of high rewards (modified version). Moreover, groups did not differ in their reaction times and subjective awareness scores, making it unlikely that GAD analogues simply took more time to decide on which choice to make or that GAD analogues had a higher level of cognitive penetration of the task. Finally, GAD participants in the standard IGT also showed significant avoidance of decks with infrequent large vs. frequent small punishments when selections were collapsed across long-term advantageous and disadvantageous decks but separated with regard to loss frequency.

Previous studies have found a relationship between performance in the standard IGT and anxiety (Schmitt et al., 1999; Werner, Duschek, & Schandry, 2009) or depression (Smoski et al., 2008). This study not only replicated these prior results in a different clinical sample but also extended the finding of superior IGT performance in anxious participants to the modified version. Here, even though the long-term advantageous decks were associated with large consistent short-term loss, participants with GAD symptoms selected from them. This pattern of findings suggests, that improved IGT performance in anxious individuals is not due to enhanced sensitivity for short-term loss. Instead, in line with models of GAD (e.g. Borkovec et al. 2004), the findings support the view, that GAD is associated with an enhanced sensitivity for unpredictable long-term loss. However, IGT performance (in both versions) reflects the balancing effects of sensitivity to loss and sensitivity to reward. Thus, the interpretation that GAD analogues reliably learned in both versions to choose decks with high long-term gain (instead of avoiding long-term loss) at the cost of missing opportunities for even higher (relative) short-term gains is also possible. The design of the IGT does not allow disentangling reward and punishment sensitivity regarding the two groups. However, the long-term advantageous selections across inverted contingency matrices of the IGT provide experimental evidence for long-term oriented decision-making in GAD.

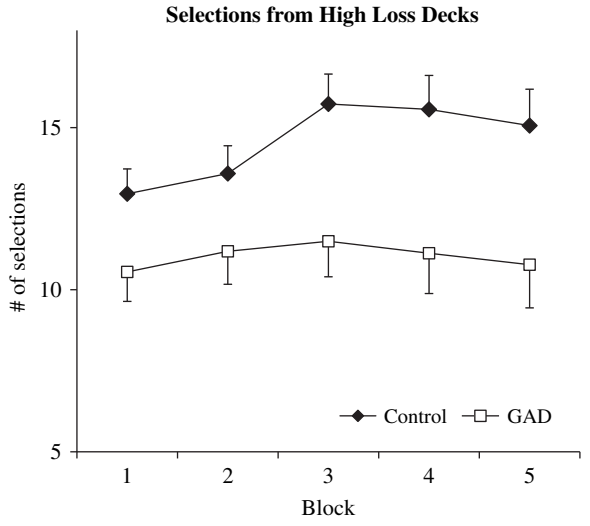


Fig. 2. Estimated marginal means ± SEM of the total number of cards selected from infrequent loss decks (B and D) in each block of 20 cards for GAD (squares) and control participants (diamonds) in the standard version of the IGT.

At least two separate processes are involved in complex real-life decision-making as simulated by the IGT. Individuals must learn about probabilistic contingencies between stimuli and outcomes, and they must make a decision based not only on this learning experience but also on general motivational tendencies (e.g. to avoid punishment or approach reward). The present findings may reflect either one or both of these processes, because both enhanced acquisition of negatively valenced associations (Zinbarg & Mohlman, 1998) and related motivational aspects (Maner & Schmidt, 2006) have been related to anxiety before. However, the group difference in the linear increase of advantageous selections across blocks suggests that enhanced learning to avoid decks associated with long-term losses plays a major role in the present findings and thus may potentially be a characteristic of GAD behaviour in general.

Additional support for the involvement of learning differences comes from recent electrophysiological studies. Error-related negativity (ERN; Gehring, Coles, Meyer, & Donchin, 1995), or specifically the feedback-related negativity (Miltner, Braun, & Coles 1997), is an EEG-component that is enlarged for negative as opposed to positive feedback. It is likely triggered by dopamine signals involved in learning via negative (Holroyd & Coles, 2002) and possibly also positive reinforcement (Santesso et al., 2008) and has been shown to be sensitive to feedback in similar gambling tasks (e.g. Gehring & Willoughby, 2002; Hajcak, Moser, Holroyd, & Simons, 2006, 2007). Importantly, potentiated ERN amplitudes have also been associated with GAD (Ladouceur, Dahl, Birmaher, Axelson, & Ryan, 2006), worrying (Hajcak, McDonald, & Simons, 2003), and related personality traits (e.g. Boksem, Tops, Wester, Meijman, & Lorist, 2006; Hajcak, McDonald, & Simons, 2004). Thus, investigating whether the behavioural results reported here are mediated by the ERN may not only yield important insight on long-term oriented reward and punishment processing in excessive worriers but also information concerning the hypothesized role of dopaminergic transmission in GAD (Stein, Westenberg, & Liebowitz, 2002) and IGT performance (Bechara, Damasio, & Damasio, 2001).

Independent of IGT performance, GAD analogues chose less often decks with infrequent large losses (i.e. decks B and D) than control participants. On average, control participants selected 15 out of 20 cards from large infrequent loss decks, which is consistent with prior reports that healthy participants fail to recognize the infrequent loss deck B as disadvantageous (Lin, Chiu, Lee, & Hsieh, 2007). In contrast to the controls, GAD participants selected only 10 out of 20 cards from large infrequent loss decks. This pattern may reflect that non-anxious participants have an optimistic bias during the task (e.g. "I will not receive another large punishment when choosing deck B/D"), whereas chronic worriers may show a more pessimistic (or in this case realistic) anticipation of loss contingencies. Because this pattern was more pronounced in the male participants, who constitute a smaller part in the GAD population (and similarly, in the present sample) it should be interpreted with some caution. Note, however, that a pessimistic/realistic anticipation of loss contingencies in GAD is consistent with an enhanced sensitivity for future loss as reflected in the performance related analyses.

The present results indicate that chronic worrying does not have to be non-adaptive *per se*. By showing that GAD analogues outperformed non-GAD participants, these findings contribute to reports that trait worrying in combination with high mental ability may be associated with relatively better job performance than trait non-worrying (Perkins & Corr, 2005). These types of findings are crucial to the understanding of GAD and related disorders, as they provide a candidate mechanism for how excessive worrying may be strengthened by positive reinforcement. It has been noted that most individuals believe that worrying may prevent disaster (Freeston et al., 1994) and that positive meta-beliefs about worry play a crucial role in the initiation and maintenance of worry (Borkovec et al., 2004; Wells, 1999). Heightened awareness of possible future punishments and opportunities that could be missed may sometimes indeed lead to more advantageous decisions in real life. However, the cost may be reduced awareness for the present, and thus an appropriate therapeutic strategy is to teach clients to focus more on the present moment (Borkovec, 2002; Borkovec & Sharpless, 2004). This may be achieved through cognitive therapy or applied relaxation techniques (Borkovec et al., 2004), both of which have been found effective for the treatment of GAD (Arntz, 2003).

Two limitations of the present study should be acknowledged. First, although the groups were formed on the basis of a reliable self-report diagnostic measure (Newman et al., 2002), the GAD participants were not treatment seeking. However, the relatively high point-prevalence of students meeting GAD criteria in the present study (8%) compared to GAD patients in the general population (3%, e.g. Kessler, Chiu, Demler, Merikangas, & Walters, 2005) is similar to other studies using student samples (e.g. 7% in Ruscio, 2002). Moreover, GAD patients identified with the Anxiety Disorders Interview Schedule for DSM-IV (ADIS-IV; Di Nardo, Brown, & Barlow, 1994) have been shown to have average PSWQ score of 67 (Behar et al., 2003) and 68 (Fresco, Mennin, Heimberg, & Turk, 2003), which closely resemble the values of the present sample (66). Second, we did not control for depression, which based on very recent reports (Smoski et al., 2008) that could have been another variable of interest. However, the relationship between IGT and depression is inconsistent (Buelow & Suhr, 2009) and we did not find an association between IGT performance and a measure for social anxiety, which is also closely related to depression (Mattick & Clarke, 1998). Moreover, in a recent study (manuscript in preparation) with German college students, we were able to show a significant positive correlation between trait-anxiety, PSWQ score, and IGT performance, thus replicating the present findings on a subclinical sample. Importantly in that study, depression scores and negative mood scores were unrelated to IGT performance. Together these and the present findings support the interpretation that IGT performance is rather related to worrying and/or anxiety than to depression or negative affect per se.

Aside from these limitations, the present study shows that GAD is related to superior performance in gambling tasks in which probabilistic punishment and reinforcement histories must be integrated in order to achieve high long-term gains. The results suggest that GAD is not necessarily characterized by a tendency to avoid consistent short-term losses, but rather by an increased sensitivity for unpredictable future losses.

Acknowledgements

Erik Mueller is now at Marburg University, Marburg, Germany. The stay of Erik Mueller at the Pennsylvania State University was supported by a scholarship from the 'Deutscher Akademischer Austauschdienst' (DAAD) and from the Marburg University – Pennsylvania State University exchange program.

The present findings were been presented in preliminary form at the conferences "6. Workshopkongress für Klinische Psychologie und Psychotherapie" in Zürich, Switzerland, 21–23 May 2009 and "51. Tagung experimentell arbeitender Psychologen" in Jena, Germany, 29 March to 1 April 2009.

We wish to thank Andrea Otto, Kevin J. Fields, Christina A. Ciorrocco, Ashley Tarabay, Brett Brashers, and Lea Moradi for their skilful help with data collection and Greg Hayes and Stefan Westermann for technical support. We also wish to thank Dr. Jan Wacker for comments on the manuscipt.

References

Arntz, A. (2003). Cognitive therapy versus applied relaxation as treatment of generalized anxiety disorder. *Behaviour Research and Therapy, 41*, 633–646.

Bechara, A., Damasio, A. R., Damasio, H., & Anderson, S. W. (1994). Insensitivity to future consequences following damage to human prefrontal cortex. *Cognition, 50*, 7–15.

Bechara, A., Damasio, H., Damasio, A. R., & Lee, G. P. (1999). Different contributions of the human amygdala and ventromedial prefrontal cortex to decision-making. *Journal of Neuroscience, 19*, 5473–5481.

Bechara, A., Damasio, H., & Damasio, A. R. (2001). Manipulation of dopamine and serotonin causes different effects on covert and overt decision-making. [Abstract]. *Society for Neuroscience Abstracts, 27*, 126.

Bechara, A., Dolan, S., Denburg, N., Hindes, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia, 39*, 376–389.

Bechara, A., Dolan, S., & Hindes, A. (2002). Decision-making and addiction (part II): myopia for the future or hypersensitivity to reward? *Neuropsychologia, 40*, 1690–1705.

Bechara, A., Tranel, D., & Damasio, H. (2000). Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. *Brain, 123*, 2189–2202.

Behar, E., Alcaine, O., Zuellig, A. R., & Borkovec, T. D. (2003). Screening for generalized anxiety disorder using the Penn State Worry Questionnaire: a receiver operating characteristic analysis. *Journal of Behavior Therapy and Experimental Psychiatry, 34*, 25–43.

Boksem, M. A., Tops, M., Wester, A. E., Meijman, T. F., & Lorist, M. M. (2006). Error-related ERP components and individual differences in punishment and reward sensitivity. *Brain Research, 1101*, 92–101.

Borkovec, T. D. (2002). Life in the future versus life in the present. *Clinical Psychology: Science and Practice, 9*, 76–80.

Borkovec, T. D., Alcaine, O. M., & Behar, E. (2004). Avoidance theory of worry and generalized anxiety disorder. In R. G. Heimberg, C. L. Turk, & D. S. Mennin (Eds.), *Generalized anxiety disorder: Advances in research and practice* (pp. 77–108). London: Guilford Press.

Borkovec, T. D., Hazlett-Stevens, H., & Diaz, M. L. (1999). The role of positive beliefs about worry in generalized anxiety disorder and its treatment. *Clinical Psychology and Psychotherapy, 6*, 126–138.

Borkovec, T. D., Robinson, E., Pruzinsky, T., & DePree, J. A. (1983). Preliminary exploration of worry: some characteristics and processes. *Behavior Research and Therapy, 21*, 9–16.

Borkovec, T. D., & Sharpless, B. (2004). Generalized anxiety disorder: bringing cognitive behavioral therapy into the valued present. In S. Hayes, V. Follette, & M. Linehan (Eds.), *Mindfulness and acceptance: Expanding the cognitive behavioral tradition* (pp. 209–242). New York: Guilford Press.

Brand, M., Labudda, K., & Markowitsch, H. J. (2006). Neuropsychological correlates of decision-making in ambiguous and risky situations. *Neuralal Networks, 19*, 1266–1276.

Brown, T. A., O'Leary, T. A., & Barlow, D. H. (1993). Generalized anxiety disorder. In D. H. Barlow (Ed.), *Clinical handbook of psychological disorders: A step-by-step treatment manual* (pp. 137–188). New York: The Guilford Press.

Buelow, M. T., & Suhr, J. A. (2009). Construct validity of the Iowa Gambling Task. *Neuropsychological Review, 19*, 102–114.

Cavedini, P., Riboldi, G., Keller, R., D'Annucci, A., & Bellodi, L. (2002). Frontal lobe dysfunction in pathological gambling patients. *Biological Psychiatry, 51*, 334–341.

Crone, E. A., Vendel, I., & van der Molen, M. W. (2003). Decision-making in disinhibited adolescents and adults: insensitivity to future consequences or driven by immediate reward? *Personality and Individual Differences, 35*, 1625–1641.

Damasio, A. R. (1994). *Descartes' error: Emotion. Rationality and the human brain*. New York, NY: Putnam.

Di Nardo, P. A., Brown, T. A., & Barlow, D. H. (1994). *Anxiety disorders interview schedule for DSM-IV (ADIS-IV)*. San Antonio, TX: The Psychological Corporation.

Dugas, M. J., Freeston, M. H., & Ladouceur, R. (1997). Intolerance of uncertainty and problem orientation in worry. *Cognitive Therapy and Research, 21*, 593–606.

Dugas, M. J., Gagnon, F., Ladouceur, R., & Freeston, M. H. (1998). Generalized anxiety disorder: a preliminary test of a conceptual model. *Behaviour Research and Therapy, 36*, 215–226.

Dunn, B. D., Dalgleish, T., & Lawrence, A. D. (2006). The somatic marker hypothesis: a critical evaluation. *Neuroscience and Biobehavioral Reviews, 30*, 239–271.

Freeston, M. H., Rheaume, J., Letarte, H., Dugas, M. J., & Ladouceur, R. (1994). Why do people worry? *Personality and Individual Differences, 17*, 791–802.
Fresco, D. M., Mennin, D. S., Heimberg, R. G., & Turk, C. L. (2003). Using the Penn State Worry Questionnaire to identify individuals with generalized anxiety disorder: a receiver operating characteristic analysis. *Journal of Behavior Therapy and Experimental Psychiatry, 34*, 283–291.
Garon, N., Moore, C., & Waschbusch, D. A. (2006). Decision making in children with ADHD only, ADHD-anxious/depressed, and control children using a child version of the Iowa Gambling Task. *Journal of Attention Disorders, 9*, 607–619.
Gehring, W. J., Coles, M. G., Meyer, D. E., & Donchin, E. (1995). A brain potential manifestation of error-related processing. (EEG Suppl. 44). In G. Karmos, M. Molnar, V. Csepe, I. Czigler, & J. E. Desmedt (Eds.), *Perspectives of event-related potentials research* (pp. 261–272). Amsterdam: North-Holland/Elsevier Science.
Gehring, W. J., & Willoughby, A. R. (2002). The medial frontal cortex and the rapid processing of monetary gains and losses. *Science, 295*, 2279–2282.
Hajcak, G., McDonald, N., & Simons, R. F. (2003). Anxiety and error-related brain activity. *Biological Psychology, 64*, 77–90.
Hajcak, G., McDonald, N., & Simons, R. F. (2004). Error-related psychophysiology and negative affect. *Brain and Cognition, 56*, 189–197.
Hajcak, G., Moser, J. S., Holroyd, C. B., & Simons, R. F. (2006). The feedback-related negativity reflects the binary evaluation of good versus bad outcomes. *Biological Psychology, 71*, 148–154.
Hajcak, G., Moser, J. S., Holroyd, C. B., & Simons, R. F. (2007). It's worse than you thought: the feedback negativity and violations of reward prediction in gambling tasks. *Psychophysiology, 44*, 905–912.
Hayes, S., Hirsch, C., & Mathews, A. (2008). Restriction of working memory capacity during worry. *Journal of Abnormal Psychology, 117*, 712–717.
Holroyd, C. B., & Coles, M. G. (2002). The neural basis of human error processing: reinforcement learning, dopamine, and the error-related negativity. *Psychological Review, 109*, 679–709.
van Honk, J., Hermans, E. J., Putman, P., Montagne, B., & Schutter, D. J. (2002). Defective somatic markers in sub-clinical psychopathy. *Neuroreport, 13*, 1025–1027.
Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry, 62*, 617–627.
Ladouceur, C. D., Dahl, R. E., Birmaher, B., Axelson, D. A., & Ryan, N. D. (2006). Increased error-related negativity (ERN) in childhood anxiety disorders: ERP and source localization. *Journal of Child Psychology and Psychiatry, 47*, 1073–1082.
Lin, C. H., Chiu, Y. C., Lee, P. L., & Hsieh, J. C. (2007). Is deck B a disadvantageous deck in the Iowa Gambling Task? *Behavioral and Brain Functions, 3*, 16.
Maner, J. K., & Schmidt, N. B. (2006). The role of risk avoidance in anxiety. *Behavior Therapy, 37*, 181–189.
Mathews, A., & MacLeod, C. (1985). Selective processing of threat cues in anxiety states. *Behaviour Research and Therapy, 23*, 563–569.
Mattick, R. P., & Clarke, J. C. (1998). Development and validation of measures of social phobia scrutiny fear and social interaction anxiety. *Behaviour Research and Therapy, 36*, 455–470.
Metzger, R. L., Miller, M. L., Cohen, M., Sofka, M., & Borkovec, T. D. (1990). Worry changes decision making: the effect of negative thoughts on cognitive processing. *Journal of Clinical Psychology, 46*, 78–88.
Meyer, T. J., Miller, M. L., Metzger, R. L., & Borkovec, T. D. (1990). Development and validation of the Penn State Worry Questionnaire. *Behaviour Research and Therapy, 28*, 487–495.
Miltner, W. H. R., Braun, C. H., & Coles, M. G. H. (1997). Event-related brain potentials following incorrect feedback in a time-estimation task: evidence for a "generic" neural system for error detection. *Journal of Cognitive Neuroscience, 9*, 788–798.
Miu, A. C., Heilman, R. M., & Houser, D. (2008). Anxiety impairs decision-making: psychophysiological evidence from an Iowa Gambling Task. *Biological Psychology, 77*, 353–358.
Mogg, K., & Bradley, B. P. (1998). A cognitive-motivational analysis of anxiety. *Behaviour Research and Therapy, 36*, 809–848.
Newman, M. G., Zuellig, A. R., Kachin, K. E., Constantino, M. J., Przeworski, A., Erickson, T., et al. (2002). Preliminary reliability and validity of the Generalized Anxiety Disorder Questionnaire-IV: a revised self-report diagnostic measure of generalized anxiety disorder. *Behavior Therapy, 33*, 215–233.
Perkins, A. M., & Corr, P. J. (2005). Can worriers be winners? The association between worrying and job performance. *Personality and Individual Differences, 38*, 25–31.
Peters, E., & Slovic, P. (2000). The springs of action: affective and analytical information processing in choice. *Personality and Social Psychology Bulletin, 26*, 1465–1475.
Ruscio, A. M. (2002). Delimiting the boundaries of generalized anxiety disorder: differentiating high worriers with and without GAD. *Journal of Anxiety Disorders, 16*, 377–400.
Santesso, D. L., Dillon, D. G., Birk, J. L., Holmes, A. J., Goetz, E., Bogdan, R., et al. (2008). Individual differences in reinforcement learning: behavioral, electrophysiological, and neuroimaging correlates. *Neuroimage, 42*, 807–816.
Schmitt, W. A., Brinkley, C. A., & Newman, J. P. (1999). Testing Damasio's somatic marker hypothesis with psychopathic individuals: risk takers or risk averse? *Journal of Abnormal Psychology, 108*, 538–543.
Smoski, M. J., Lynch, T. R., Rosenthal, M. Z., Cheavens, J. S., Chapman, A. L., & Krishnan, R. R. (2008). Decision-making and risk aversion among depressive adults. *Journal of Behavior Therapy and Experimental Psychiatry, 39*, 567–576.
Stein, D. J., Westenberg, H. G., & Liebowitz, M. R. (2002). Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. *Journal of Clinical Psychiatry, 63*(Suppl. 6), 12–19.
Toplak, M. E., Jain, U., & Tannock, R. (2005). Executive and motivational processes in adolescents with Attention-Deficit-Hyperactivity Disorder (ADHD). *Behavioral and Brain Functions, 1*, 8.
Wells, A. (1999). A cognitive model of generalized anxiety disorder. *Behavior Modification, 23*, 526–555.
Werner, N. S., Duschek, S., & Schandry, R. (2009). Relationships between affective states and decision-making. *International Journal of Psychophysiology, 74*, 259–265.
Zinbarg, R. E., & Mohlman, J. (1998). Individual differences in the acquisition of affectively valenced associations. *Journal of Personality and Social Psychology, 74*, 1024–1040.

Study 4

Mueller, E. M., Hofmann, S.G., & Cherry, J. (2010). The type IV phosphodiesterase inhibitor rolipram disturbs expression and extinction of conditioned fear in mice. *Neuropharmacology, 59,* 1 – 8.

Neuropharmacology 59 (2010) 1–8

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

The type IV phosphodiesterase inhibitor rolipram disturbs expression and extinction of conditioned fear in mice

Erik M. Mueller [a], Stefan G. Hofmann [b], James A. Cherry [c,*]

[a] Department of Psychology, Marburg University, Gutenbergstr. 18, 35032 Marburg, Germany
[b] Department of Psychology, Boston University, 648 Beacon Street, Boston, MA 02215, USA
[c] Department of Psychology, Boston University, 64 Cummington Street, Boston, MA 02215, USA

ARTICLE INFO

Article history:
Received 30 November 2009
Received in revised form
1 March 2010
Accepted 6 March 2010

Keywords:
Anxiety
cAMP
Fear-potentiated startle
Memory
Response-inhibition

ABSTRACT

Recent studies suggest that intracellular signaling pathways involving cyclic adenosine monophosphate (cAMP) may be related to fear processing and long-term memory formation. The type IV phosphodiesterase (PDE4) inhibitor rolipram prevents breakdown of cAMP, enhances long-term memory and may reduce anxiety. In the present study we investigated the role of rolipram in the expression (0, 0.2, or 1 mg/kg), acquisition (0, 0.03, 0.2 or 1 mg/kg), and extinction (0, 0.03, 0.2, 1 mg/kg) of fear using a fear-potentiated startle paradigm in mice. It was shown that rolipram reduced the expression (Experiment 1), did not influence acquisition (Experiment 2) and disturbed between-session extinction (Experiments 3 and 4) of fear responses to conditioned tones. Because within-session extinction was not impaired by rolipram and because low (i.e. 0.03 and 0.2 mg/kg) doses strongly affected extinction but not expression of fear, these findings suggest that the effect of rolipram on extinction is not directly dependent on its effect on fear expression. Taken together, these experiments indicate that preventing breakdown of cAMP interferes with the expression and extinction consolidation of conditioned fear.

© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Understanding the neurobehavioral mechanisms underlying the expression and reduction of fear may help improve pharmacologic, psychotherapeutic and combined treatments for anxiety disorders (Hofmann, 2007). The intracellular signaling pathway involving the second messenger cyclic adenosine monophosphate (cAMP) is part of a mechanism implicated in learning and memory formation (Davis et al., 1995; Alberini, 1999) and possibly also in fear extinction (Myers and Davis, 2007). Cyclic AMP triggers protein kinase A (PKA)-mediated phosphorylation of the cAMP response element binding protein (CREB), which in turn activates intracellular signaling cascades that have been implicated in memory processes, anxiety and other phenomena (Carlezon et al., 2005). Importantly, cAMP is broken down by cyclic nucleotide phosphodiesterases, a large family of enzymes that includes the type IV or cAMP-specific phosphodiesterases (PDE4) that are expressed throughout the brain (Cherry and Davis, 1999; Perez-Torres et al., 2000). Administration of the PDE4-specific inhibitor rolipram leads to elevation of cAMP levels (Barad et al., 1998), increased CREB phosphorylation (Monti et al., 2006) and a number of behavioral sequelae, including anti-depressant (Li et al., 2009; Wachtel, 1983; Zeller et al., 1984; Zhang et al., 2002), anti-psychotic (Kanes et al., 2007), spinal neurotransmission (Kehne et al., 1991) and anxiolytic effects (Li et al., 2009; Silvestre et al., 1999a).

Acute (Silvestre et al., 1999a) and chronic (Li et al., 2009) delivery of rolipram has been shown to reduce the expression of anxiety. Consistent with that finding, low levels of CREB in the central nucleus of the amygdala and bed nucleus of the stria terminalis have been associated with relatively high anxiety and fear, respectively (Meloni et al., 2006; Pandey et al., 2005). Fear and anxiety are regulated by distinct, albeit overlapping neural circuits (Davis, 2006; Gray and McNaughton, 2000) with varying degrees of responsiveness to different pharmacologic treatments (Blanchard et al., 1997). Because the effect of rolipram on anxiety but not fear expression has been previously tested, Experiment 1 investigated whether the administration of rolipram would also lead to suppression of fear.

There is also substantial evidence to suggest that rolipram can improve long-term hippocampal-dependent fear memory (Barad et al., 1998) as well as reverse memory deficits induced pharmacologically (Randt et al., 1982; Zhang et al., 2000, 2004; Rutten et al., 2006) or by genetic lesions (Bourtchouladze et al., 2003; Comery et al., 2005). We reasoned that rolipram, which has been shown

* Corresponding author. Tel./fax: +1 617 353 3254.
E-mail address: jcherry@bu.edu (J.A. Cherry).

0028-3908/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2010.03.002

to increase hippocampal CREB (Nibuya et al., 1996; Monti et al., 2006) might also potentiate cue-specific fear conditioning, for example, by elevating CREB levels in the basolateral amygdala (Josselyn et al., 2001). Experiment 2, therefore, tested whether rolipram supplied prior to acquisition would enhance the conditioning of fear.

Further, PDE4 inhibitors may affect fear *extinction*. Mice that exhibit increased cAMP levels due to an overexpression of adenylyl cyclase display impaired extinction (Wang et al., 2004), suggesting that cAMP elevation, through PDE4 inhibition (e.g. Barad et al., 1998) might interfere with extinction of conditioned fear. In fact, mice receiving subchronic rolipram during contextual fear conditioning displayed delayed extinction of fear, perhaps because rolipram produced a stable fear conditioning (Monti et al., 2006). It is not known what effect rolipram may have if it is present during extinction. Therefore, Experiment 3 investigated the hypothesis that delivery of rolipram interferes with the extinction of fear.

Like other types of learning, extinction involves acquisition and consolidation phases (Quirk and Mueller, 2008), which may be differentially affected by drugs. To test whether rolipram affects the acquisition or consolidation phase of fear extinction, Experiment 4 probed the dynamics of fear expression both during extinction sessions (as an index for extinction acquisition) and between extinction sessions (as an index for extinction consolidation).

In the present study rolipram was administered to mice prior to examining the expression (Experiment 1), conditioning (Experiment 2) or extinction (Experiments 3 and 4) of conditioned fear in a fear-potentiated startle (FPS) paradigm, a valid and widely used measure for studying conditioned fear (for review see Davis, 2006).

2. Methods

2.1. Subjects

Six to eight week old male Swiss Webster mice were obtained from Taconic Farms (Germantown, NY) and used within one week of arrival. Mice were housed in groups of four and maintained on a 12/12-h light/dark cycle (lights on at 07:00 h). Food and water were available at all times. All procedures involving animals followed National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Boston University Charles River Campus Institutional Animal Care and Use Committee.

2.2. Apparatus

Testing was conducted in sound-attenuating cubicles (30 × 26 × 17 cm) using an acoustic startle system from Coulbourn Instruments (Allentown, PA). Each cubicle contained a load-cell platform onto which an animal holder was placed. The holder was an opaque plastic tray (16 × 9 × 4.5 cm) with a gridded floor consisting of six, 4.5 mm dia. steel rods spaced 13 mm apart, and a hinged, dome-shaped top constructed of 6.5 mm dia. aluminum bars spaced 6 mm apart. White noise and pure tones were generated by a small speaker located in the chamber. Sound pressure levels were determined with a Radio Shack (Fort Worth, TX) digital sound level meter.

2.3. Testing procedures

Parameters for assessing and analyzing fear-potentiated startle followed those described in Waddell et al. (2004), and are detailed below. An outline of specific procedures used in each experiment is provided in Table 1.

2.4. Acclimation tests

In all tests, a 5-min acclimation period during which no stimuli were presented was given after placing mice in the startle apparatus. Mice were habituated to handling and the behavioral chambers by exposing them on each of three separate days to the startle stimuli (20-ms white noise pulses). Ten stimuli each at intensities of 95, 100, and 105 dB were presented each day with a mean inter-trial interval (ITI) of 30-s within a range of 15–45 s. Performance in these tests was not a part of any subsequent statistical analysis.

2.5. Training

Fear-conditioning sessions consisted of 10 tone plus shock trials in which a 30-s, 4-kHz 70-dB tone coterminated with a 0.25-s, 0.4-mA foot shock (see descriptions of individual experiments for the number of sessions given). The ITI for conditioning was a pseudorandom duration of between 1 and 3 min that averaged 2 min for the 10 trials.

2.6. Rolipram delivery

Rolipram (Sigma, St. Louis, MO) was administered by intraperitoneal injections at 0, 0.03, 0.2, or 1.0 mg/kg in 10% cremophor to facilitate dissolubility.

2.7. Pre-training/FPS tests

A pre-training test was given prior to fear conditioning to determine the unconditioned effect on startle amplitudes of the 4 kHz tone that served as the conditioned stimulus (CS). The pre-training test consisted of nine startle stimulus-alone trials (three each at 95, 100 and 105 dB) followed by an additional 18 trials: nine startle stimulus-alone trials (three each at 95, 100 and 105 dB), and nine CS + startle stimulus trials (three each at 95, 100 and 105 dB) in which the startle stimulus occurred 29.75 s after the onset of the CS. Trials were presented in a pseudorandom order such that at least one startle stimulus of each intensity occurred within any block of 6 trials, and no stimulus occurred more than twice in a row. Parameters for the FPS test were identical to the pre-training test.

2.8. FPS scores

Startle responses occurring following noise pulses were transduced by the load cell and digitized at 1 kHz. The peak response within 200 ms after the onset of the startle stimulus was recorded as the startle response for each trial. FPS scores were computed separately for 95, 100 and 105 dB pulses by subtracting for each animal the mean startle amplitude in response to noise pulses only from the mean startle amplitude in response to noise pulses preceded by the CS.

Table 1
Experimental Design for Experiments 1–4.

Experiment 1	Training	FPS Test	Extinction (4 days)	Training 2	FPS Test
Rolipram/saline delivery					X
Fear assessment		O			O
Experiment 2	Training	FPS Test			
Rolipram/saline delivery	X				
Fear assessment		O			
Experiment 3	Training	FPS Test	Extinction (1 day)	FPS Test	
Rolipram/saline delivery			X		
Fear assessment		O		O	
Experiment 4 (A and B)	Training	FPS Test	Extinction (4 days)*	FPS Test	
Rolipram/saline delivery			X		
Fear assessment		O	O	O	

Stages for each experiment are shown from left to right in temporal order. The letter indicates stages where fear was measured (O) and/or rolipram/saline was administered (X). Mice also went through an acclimation stage and a pre-training test prior to their first training session (not listed in table); mice in Experiment 4 had received additional training and extinction prior to being given the series of tests shown. * denotes extinction tests with a CS (tone) only (no noise pulses). See Methods for additional details of procedure.

2.9. Rolipram and expression of fear (Experiment 1)

Prior to evaluating the effect of rolipram on the expression of conditioned fear, we conducted preliminary tests to verify that the chosen parameters were appropriate for training, testing and extinguishing fear in mice. Following an initial acclimation and pre-training test, $n = 24$ mice were fear conditioned for three days (10 trials of tone plus shock each day) and on the following day given a test for FPS without rolipram injections. As expected, relative to startle responses in the pre-training test (Mean: 3.3; SEM: 1.5) mice showed significant FPS responses (averaged over loudness levels) in the first FPS test ((Mean: 39.5; SEM: 4.4), $t(23) = 9.15$, $p < 0.001$), demonstrating that the fear-conditioning procedure was effective. Thereafter mice received four days of 42 extinction trials (presentation of CS only) to confirm that the procedure would lead to extinction of conditioned fear (data not shown). Mice next received two re-training days during which 10 trials with tone plus shock were delivered. 24 h later mice were injected with 0 ($n = 8$), 0.2 ($n = 8$) or 1 ($n = 8$) mg/kilogram of rolipram. Assignment of mice to substance groups was based on scores from the first FPS test: mice were pseudorandomly placed in groups to achieve equivalent mean FPS scores, and groups were then randomly assigned to receive one of three rolipram doses. Mice were given a second FPS test 30 min after injections to measure the effect of rolipram on the expression of fear.

2.10. Rolipram and acquisition of fear (Experiment 2)

Following the initial acclimation and pre-training tests, $n = 36$ mice were pseudorandomly assigned to groups to achieve equivalent mean pre-training test scores. On the next day mice received an injection of 0 ($n = 9$), 0.03 ($n = 9$), 0.2 ($n = 9$) or 1 ($n = 9$) mg/kg rolipram and fear conditioning began 30 min later. To test the effect of rolipram on fear acquisition, an FPS test was conducted 24 h later.

2.11. Rolipram and extinction of fear (Experiment 3)

Following initial acclimation and pre-training tests, $n = 61$ mice were fear conditioned. Conditioning consisted of one session conducted 24 h (cohorts 2 and 4) or two sessions conducted 24 and 48 h (cohorts 1 and 3) after the pre-training test. One or two days after conditioning, mice were returned to the startle chamber for an FPS test identical to the one described in Section 2.5 except that 6 more acclimation trials were included. The FPS scores were determined and mice were assigned to one of four groups to achieve equivalent mean FPS scores. Twenty-four hours later mice were given 0 ($n = 18$), 0.03 ($n = 15$), 0.2 ($n = 16$) or 1.0 ($n = 12$) mg/kg of rolipram. 30 min later extinction trials ($n = 20$) consisting of the 30-s CS only (no startle stimuli) were delivered in the cubicle, with a mean ITI of 45 s (range = 30–60 s). Forty-eight hours later, FPS tests were administered that consisted of 51 startle trials. The first nine trials were used for acclimation and consisted of the white noise pulse alone. The following 42 consisted of the noise pulse alone ($n = 21$) intermixed with noise plus CS trials ($n = 21$). Because there was strong within-session extinction across groups (i.e. comparison of FPS in the first vs. the second half of the session: $t(60) = 7.27$, $p < 0.001$), only the first 21 trials were analyzed. Because mice were tested in a total of 4 cohorts, the comparability of cohorts was confirmed with a one-factorial (i.e. cohort) ANOVA on the initial FPS scores (prior to extinction), $F(3, 57) = 1.11$, $p > 0.3$.

2.12. Rolipram and expression of fear during extinction training (Experiment 4)

In Part A of this experiment (4A), $n = 18$ mice participated that were previously subjects in another fear-conditioning study where they had received saline ($n = 3$), rolipram (0.03 mg/kg, $n = 1$; 0.2 mg/kg, $n = 3$; or 1 mg/kg, $n = 5$), or D-cycloserine (10 mg/kg, $n = 3$, or 30 mg/kg, $n = 3$) 21 days before the first day of this experiment. All mice had the same level of exposure to previous procedures and were fear extinguished two weeks prior to the present experiment.

Mice were trained with one session of 10 tone plus shock trials. A pre-extinction FPS test was conducted 24 h later in order to assign mice to one of two groups with equivalent FPS. Two, 4, 6 and 8 days later, mice received an extinction session, during which $n = 21$ tones with a duration of 30 s were presented. Each tone coterminated with a noise pulse (95, 100 or 105 dB) in order to assess fear expression during extinction. Thirty min prior to each extinction session mice received either 0 ($n = 10$) or 1 ($n = 10$) mg/kg of rolipram (groups did not differ with regard to substance history in the previous study, $X^2(5) = 5.2$, $p > 0.35$). Four days after the last extinction session, a post-extinction FPS test was conducted in order to assess the effectiveness of the prior extinction sessions.

Because noise pulses were never presented without the CS during extinction trials, no FPS scores could be calculated for the extinction sessions. Therefore, the startle amplitudes measured in response to noise pulses preceded by the CS (CS-associated startle) served as an indicator of fear.

To show that prior use of mice from an earlier experiment would not affect the reproducibility of results, the above experiment was replicated (Experiment 4B) using the same procedure with $n = 14$ fear conditioned mice that were either given saline ($n = 8$) or rolipram ($n = 6$) 30 min prior to each of four extinction sessions. As in Experiment 4A, these mice had previously been exposed to rolipram ($n = 7$) or DCS ($n = 7$) in an unrelated experiment (washout period: 16 days).

2.13. Statistical analyses

For Experiments 1 and 2, the FPS scores were analyzed using an ANOVA with the within-factor *Loudness of noise pulse* (95 vs. 100 vs. 105 dB) and the between factor *Substance Group* (0 vs. 0.2 vs. 1 mg/kg). In Experiment 1, where FPS scores were obtained for animals before they had been exposed to the drugs (i.e. the first FPS test), the within-factor *Session* (first vs. second FPS test) was also included. Sphericity was confirmed using the Mauchley test.

For Experiment 3 the Mauchley test revealed a violation of the sphericity assumption with regard to the *Loudness of noise pulse* × *Session* (pre vs. post-extinction) covariance structure, ($X^2(2) = 12.8$, $p < 0.003$). Therefore a MANOVA with the FPS scores for the three loudness types as dependent variables, *Session* as a repeated measures factor and *Substance Group* as a between subjects factor was computed.

In Experiments 4A and 4B, CS-associated startle amplitudes were analyzed using an ANOVA with the within-factors *Extinction Session* (1–4) and *Block* (1–3), and the between factor *Substance Group* (0 vs. 1 mg/kg). The factor *Block* reflects the first vs. second vs. third set of $n = 7$ trials during extinction and was added in that experiment to investigate effects of rolipram on within-session extinction (startle amplitudes to 95, 100 and 105 dB tones were used to achieve a sufficient number of trials for each block). To test the effect of rolipram on the long-term effectiveness of extinction, CS-associated startle amplitudes at the post-extinction FPS test were compared between groups using an independent *t*-test.

Also in Experiment 4B, to control for the difference in past exposure to drugs, the type of previous drug was evenly distributed over the saline and rolipram groups and the factor *Prior Exposure* (to rolipram vs. DCS) was included in the *Substance Group* × *Extinction Session* × *Block* ANOVA model. Due to apparatus failure only data for the first three extinction sessions were recorded and analyzed (although mice did receive four extinction sessions).

3. Results

3.1. Rolipram and expression of fear (Experiment 1)

As shown in Fig. 1a, the administration of rolipram 30 min prior to the second FPS test reduced FPS amplitudes relative to the first FPS test (i.e. when no rolipram was given) in a dose-dependent manner. In the second FPS test (given after fear was extinguished and subjects were subsequently re-trained) FPS scores were maximally reduced for the 1 mg/kg group (mean FPS: 5.3; SEM: 3.8), marginally reduced for the 0.2 mg/kg group (mean FPS: 22.5; SEM: 3.4) and unchanged relative to the first FPS test in the saline group (mean FPS: 40.0, SEM: 10.8). Statistically this was confirmed with a significant *Substance Group* × *Session* interaction: ($F(1,21) = 3.86$, $p < 0.05$, partial eta squared = 0.27). Post hoc *t*-tests revealed significant differences between the 0 vs. 1 mg/kg group, $t(14) = 3.03$, $p < 0.01$, and between the 0.2 vs. 1 mg/kg group, $t(14) = 3.40$, $p < 0.005$, with regard to scores of the second FPS test, but not of the first FPS test. In addition there was a main effect for *Session* indicating that FPS scores were reduced in the second FPS test, $F(1,21) = 12.91$, $p < 0.005$, partial eta squared = 0.38. Because there was no main effect or interaction involving *loudness of noise pulse* the means presented in Fig. 1 represent the average over the three loudness types. As can be seen in Fig. 1b the *Substance Group* × *Session* interaction cannot be ascribed to general group differences in startle reactivity (e.g. Kehne et al., 1991): rolipram treatment had no effect on startle amplitudes to noise only trials. Note also that FPS scores from rolipram-treated mice were elevated again in a follow-up FPS test conducted four days after the second FPS test (without prior injections, data not shown), suggesting that rolipram did not affect the fear memory per se.

3.2. Rolipram and acquisition of fear (Experiment 2)

Rolipram injected 30 min prior to training did not enhance the acquisition of conditioned fear. In contrast there was a mild decline of FPS scores as a function of rolipram dose (Fig. 2). However, none of the effects reached significance ($p > 0.4$).

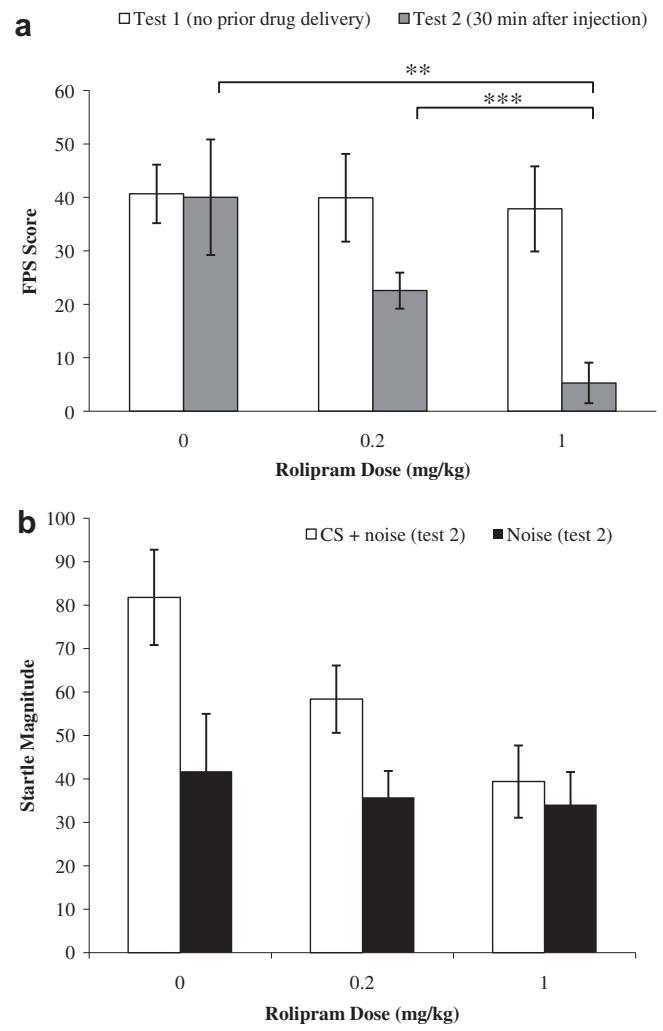


Fig. 1. (a) Mean (±SEM) FPS scores (averaged over the 95, 100 and 105 dB tones) measured at the first (open bars) and second (gray bars) FPS Test. Prior to the second but not first test mice had received 0, 0.2 or 1 mg/kg rolipram. (b) Mean (±SEM) absolute startle amplitudes to noise pulses alone (black) and noise pulses preceded by the CS (open) during the second FPS Test. Note that rolipram did not affect startle amplitudes to unpaired noise pulses. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

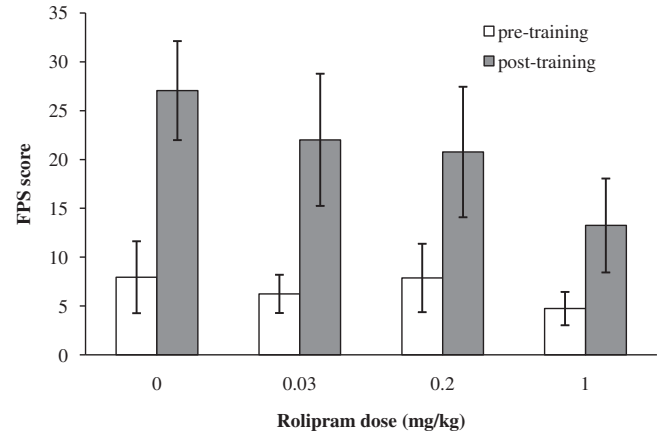


Fig. 2. Mean (±SEM) pre-training (open) and post-training (gray) FPS scores (aggregated over the 95, 100 and 105 dB tones) measured before and after fear conditioning, respectively. Prior to fear-conditioning mice had received 0, 0.03, 0.2 or 1 mg/kg rolipram. Differences between groups are not significant ($p > 0.4$).

3.3. Rolipram and extinction of fear (Experiment 3)

The administration of rolipram strongly disturbed extinction (multivariate interaction *Session* × *Substance Group*: $F(3,57) = 2.66$, $p < 0.008$, partial eta square = 0.125). Univariate analyses revealed that this interaction was significant for the 100 dB tones ($F(3,57) = 6.08$, $p < 0.001$) but not for the 95 dB and 105 dB tones ($p > 0.5$). To analyze this interaction further we computed the decrease of FPS scores after extinction (i.e. post-extinction FPS minus pre-extinction FPS) for 100 dB tones. Post hoc *t*-tests on these difference scores revealed that in comparison to saline, 0.03 mg/kg ($t(31) = 4.69$; $p < 0.001$), 0.2 mg/kg ($t(32) = 2.11$ $p < 0.027$) and 1 mg/kg ($t(28) = 3.53$; $p < 0.001$) of rolipram decreased the effectiveness of extinction (Fig. 3). There were no significant differences between doses of rolipram (all $p > 0.1$).

3.3.1. Rolipram and fear expression during extinction training (Experiment 4A)

As expected, there was a decline of CS-associated startle amplitudes over extinction sessions, $F(1, 16) = 11.80$, $p < 0.001$ (Fig. 4). Importantly, and consistent with Experiment 1, rolipram-treated mice displayed generally low CS-associated startle amplitudes during extinction sessions, which only moderately declined over the four sessions. In contrast, control mice initially displayed large amplitudes, which continuously declined over the four sessions. Statistically this was confirmed by a significant *Substance Group* × *Extinction-Session* interaction, $F(1, 16) = 3.89$, $p < 0.03$, which represents a significant linear decrease of CS-associated startle amplitudes over the four sessions in the control group $F(1, 8) = 17.20$, $p < 0.005$, but not in the rolipram group $F(1, 8) = 4.00$, $p = 0.08$. Moreover, there was a strong decrease of CS-associated startle amplitudes within sessions as reflected by a main effect for *Block*, $F(1, 16) = 23.66$, $p < 0.001$. This effect was stronger in rolipram-treated mice (*Substance Group* × *Block*, $F(1, 16) = 4.04$, $p < 0.032$), especially during the first two sessions (*Substance Group* × *Block* × *Extinction Session*, $F(1, 16) = 2.33$, $p < 0.012$). Notably, when subjects' substance history (Rolipram vs. D-Cycloserine vs. Saline) was included in the above ANOVA model, there was no main effect or interaction involving this factor. However,

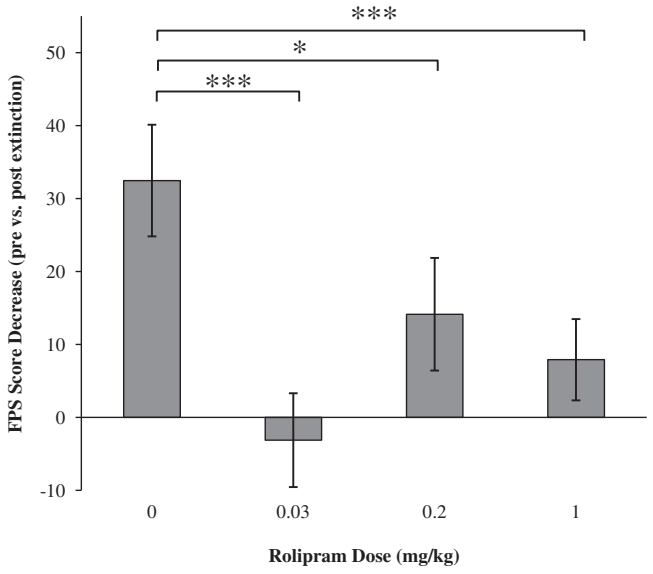


Fig. 3. Mean (±SEM) decrease in FPS scores in the post-extinction FPS test relative to the pre-extinction FPS tests in mice that had received 0, 0.03, 0.2 or 1 mg/kg rolipram 30 min prior to extinction. Only data from the 100 dB noise pulses are shown. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

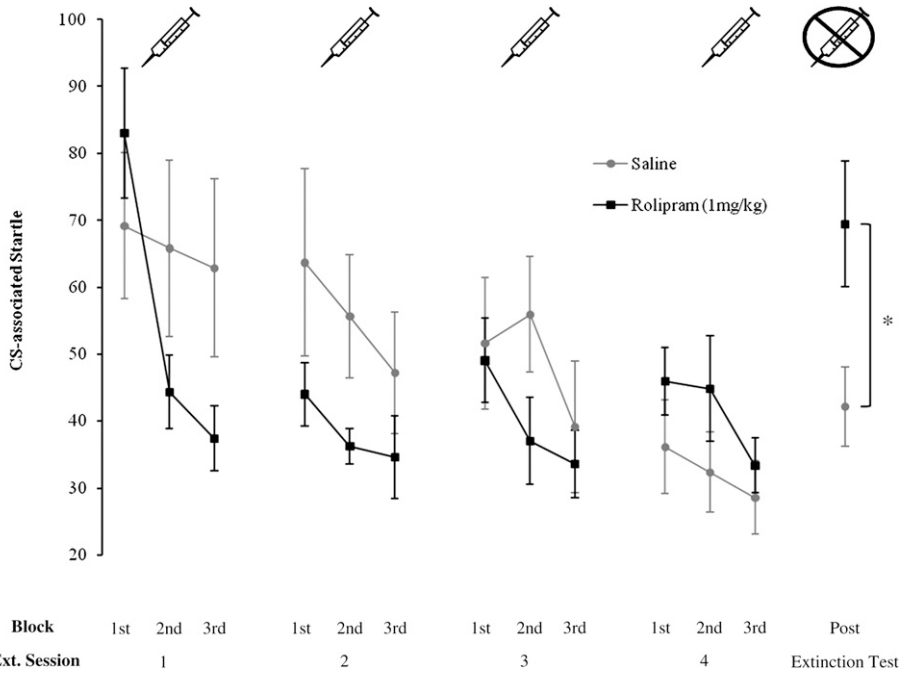


Fig. 4. Mean (±SEM) CS-associated startle amplitudes (i.e. startle amplitudes to noise pulses preceded by the fear conditioned CS) for each of four blocks of $k = 7$ trials during four consecutive extinction sessions and at the final post-extinction test (aggregated over the 95, 100 and 105 dB tones). Mice received 1 mg/kg of rolipram (black) or saline (gray) before extinction sessions but not prior to the post-extinction test. $^{*}p < 0.05$.

the *Substance Group* × *Extinction Session* interaction remained significant ($p < 0.05$). This control analysis shows that pharmacological adaptations or related processes are unlikely to have influenced the reported results (see also replication experiment).

Consistent with Experiment 3, the post-extinction FPS test revealed that mice that had received rolipram during extinction showed significantly larger CS-associated startle responses than control mice, $t(16) = 2.32$, $p < 0.04$.

3.3.2. Rolipram and fear expression during extinction training (Experiment 4B)

Paralleling Experiment 4A, there was a significant *Extinction Session* × *Substance-Group* interaction $F(1, 10) = 4.49$, $p < 0.032$ indicating significant between-session extinction in the control ($F(1,6) = 16.99$ $p < 0.001$) but not rolipram group ($F(1,4) = 1.32$, $p > 0.3$). The factor *Prior Exposure* showed neither main effects nor interactions (ps > 0.25). Again, mice that had received rolipram during extinction displayed a tendency for larger CS-associated startle amplitudes (Mean: 68.3; SEM: 10.1) than control mice (Mean: 52.8; SEM: 8.6) at the post-extinction test ($t(12) = 1.17$, $p < 0.13$, one-sided) and when data for Experiments 4A and 4B were pooled to increase power this effect was statistically confirmed ($t(30) = 2.54$, $p < 0.017$). Moreover, when a *Substance Group* × *Prior Exposure* ANOVA was performed on the post-extinction startle scores there was only a main effect for *Substance Group*, $F(1,27) = 4.67$, $p < 0.04$ but no effects involving *Prior Exposure* ($p > 0.25$). Thus, in neither the original or follow-up experiment was there evidence that prior drug exposure could account for the effect of rolipram on extinction.

4. Discussion

Experiment 1 indicates that rolipram reduces fear expression in a dose-dependent manner. Fear was first conditioned and assessed (first FPS test), then extinguished, re-conditioned and assessed again (second FPS test). Mice received 0, 0.2 or 1 mg/kg rolipram only prior to the second FPS test. Relative to the first FPS test, saline-treated mice showed very similar mean FPS scores. In contrast, mice that received 0.2 or 1 mg/kg rolipram displayed moderate and high reductions of FPS scores, respectively, suggesting that higher doses lead to more suppression of fear. Because rolipram had no effects on startle amplitudes per se, the reduced FPS scores cannot be attributed to effects of rolipram on spinal neurotransmission or other unspecific effects. Instead they imply that rolipram specifically interferes with processing of the conditioned fear stimulus, possibly disturbing retrieval of the fear memory and/or the expression of fear. We acknowledge that because mice were previously trained and fear extinguished the rolipram-related decrease of fear expression could also reflect enhanced retrieval of extinction memory. However, as outlined below, the finding of decreased between-session extinction in rolipram-treated mice (Experiment 4A) indicates that rolipram disturbs rather than enhances retrieval of cued fear extinction memory.

Previous studies have found that rolipram has anxiolytic properties (Li et al., 2009; Silvestre et al., 1999a). The present study extends these findings by showing that rolipram also reduces *fear* expression in mice. Fear and anxiety may be regulated by different neuronal structures: while fear regulation has been primarily attributed to the amygdala (LeDoux, 2007), the bed nucleus of the stria terminalis (BNST) may play a role in anxiety (Davis, 2006). However, others have suggested that the BNST may also be implicated in fear processing (Meloni et al., 2006). Because PDE4 genes are also expressed in these regions (Cherry and Davis, 1999; Perez-Torres et al., 2000), it could be speculated that rolipram prevents fear expression by elevating pCREB levels in one of these structures.

Experiment 2 probed the effects of rolipram on the acquisition of fear. When rolipram vs. saline was given 30 min prior to fear conditioning, mice that had received rolipram did not show enhanced FPS scores on a test that was conducted three days later. This finding is consistent with other reports that rolipram does not enhance (or may even interfere) hippocampus-independent memory formation (Barad et al., 1998; Gong et al., 2004). Given that

studies reporting enhanced fear acquisition after rolipram delivery only examined contextual (i.e. hippocampus-dependent) fear conditioning (Barad et al., 1998; Monti et al., 2006), the present results suggest that fear acquisition per se is not enhanced by PDE4 inhibition. In light of the literature that emphasizes the therapeutic potential of rolipram as a memory enhancer (for review see: Reneerkens et al., 2009), the results of Experiment 2 thus deserve attention, because they imply that rolipram could also retard the consolidation of some types of associations. Although non-significant, the trend of higher rolipram doses given during training to increasingly disrupt FPS acquisition indicates that the potential for non-hippocampal effects of rolipram (and other PDE4 inhibitors) should be carefully considered in future studies.

Experiment 3 found that in contrast to controls, mice that had received any dose of rolipram prior to an extinction session (0.03, 0.2 or 1 mg/kg) showed almost no reduction of previously acquired FPS after extinction training. Follow-up analyses of the significant MANOVA suggested that this effect was most evident for the 100 dB tones, which is consistent with reports from other groups that also reported larger effect sizes for 100 vs. 95 or 105 dB pulses in startle paradigms with mice (Kanes et al., 2007). Fear-potentiated startle reflects the modulation of a polysynaptic startle reflex (Davis, 2006), which requires that (a) the reflex itself is reliably produced and (b) the magnitude of the (unpaired) startle response does not approach the limit of what is physiologically possible (i.e. there is room for potentiation). Possibly, in the present experiment prerequisites (a) and (b) were not met for the 95 and 105 dB pulses, respectively.

Previous work has established that 0.1 μM/kg (=0.028 mg/kg) and larger doses of rolipram given directly after fear conditioning enhances retrieval of fear memory 24 h later (Barad et al., 1998; Randt et al., 1982), suggesting that rolipram may prevent forgetting and/or unlearning of conditioned contextual fear. In addition, 0.1 μM/kg rolipram given prior to training may also improve contextual fear-conditioning in vivo, and long-term potentiation in vitro is enhanced when hippocampal slices are stimulated in solutions of 0.1 μM rolipram (Barad et al., 1998). The present findings extend these prior studies by suggesting that similar doses of rolipram may interfere with the extinction of fear, a process that is clearly distinct from forgetting (Bouton, 2004). Such interference could be driven by elevation of cAMP levels, which could result in heightened protein kinase A activity and subsequent modulation of CREB phosphorylation. This interpretation is consistent with Wang et al. (2004), who reported disturbed extinction when cAMP levels were heightened through overexpression of adenylyl cyclase, as well as with Isiegas et al. (2006), who found improved extinction when protein kinase A activity was inhibited. Because extinction is highly dependent on the prefrontal cortex (Morgan et al., 1993; Quirk and Mueller, 2008) the current result corroborates the more general finding that administration of rolipram/increasing protein kinase A activity impairs prefrontal cortical functioning in aged monkeys and rats (Ramos et al., 2003).

Results from Experiment 4 suggest that rolipram disturbs extinction consolidation but not extinction acquisition. Mice that received rolipram during extinction showed significantly potentiated startle responses at the post-extinction FPS test (i.e. after extinction sessions and rolipram delivery). This finding replicates and extends the results from Experiment 3 by showing that rolipram also disturbs the extinction of fear when (a) several rather than one extinction sessions are performed and (b) when fear is not measured with FPS scores but with CS-associated startle amplitudes.

The decreased slope of the CS-associated startle amplitudes over the four sessions in the rolipram vs. control group provides further evidence for the rolipram-induced disturbance of between-session extinction. Note that this finding also rules out state-dependent learning (e.g. Overton, 1991) as an alternative explanation: even though the same substance was administered prior to each extinction session (i.e. thus inducing the same "state"), rolipram-treated mice showed a blunted decline of fear expression over the extinction sessions compared to control mice.

Moreover, the decreased extinction in the rolipram group cannot be ascribed to impaired within-session extinction (i.e. extinction acquisition). In fact, mice that received rolipram showed even greater within-session extinction than control mice (although this effect was not significant in the replication experiment). Of relevance, the intact within-session extinction in the rolipram group demonstrates that rolipram-induced fear suppression (as demonstrated in Experiment 1) is unlikely to account for the effects of rolipram on extinction: if extinction was impaired due to blockade of the CR (Rescorla, 1997; Krupa and Thompson, 2003), both within- and between-session extinction should have been attenuated in the rolipram group. However, only between-session extinction was impaired in rolipram-treated mice. In addition, rolipram maximally suppressed fear expression at a dose of 1 mg/kg and only moderately suppressed fear at lower doses (Experiment 1). If fear extinction were directly related to fear expression, one would expect that fear extinction would show a similar dose-dependent pattern. However, very low doses of rolipram (i.e. 0.03 mg/kg) were sufficient to produce a maximum disturbance of extinction (Experiment 3). Taken together, the pattern of findings suggests that rolipram has two independent effects on fear processing: one that is rather initiated by larger doses, is immediate and reduces the expression and/or experience of fear, and another for which small doses are sufficient and which is rather related to retarded long-term consolidation of fear extinction. While the former parallels anxiolytic properties of rolipram reported previously (Silvestre et al., 1999a), the latter shows more similarities with deficits in fear extinction due to cAMP elevation (Wang et al., 2004) and is consistent with prior rolipram studies insofar that rolipram affects long- but not short-term memory processes (Barad et al., 1998; Bourtchouladze et al., 2003). However, it should be emphasized that these studies reported *enhanced* long-term memory of hippocampus-dependent fear memory whereas the present findings show that rolipram *hinders* the formation of enduring cued fear extinction memory.

As a potential limitation of Experiment 4, subjects had previously been used in another fear-conditioning study. However, mice received only a single injection of a drug (or saline control) and then were given a 2–3 week washout period before commencing these experiments; moreover, there were no statistically significant relationships between prior drug exposure and performance in Experiments 4A or 4B. A second issue is that these mice had also been fear conditioned and extinguished in the previous experiment in which they participated, so strictly speaking the present experiment investigated re-extinction, which may differ from initial extinction in some regards (Kim and Richardson, 2008). Even though differences in these processes have been linked to NMDA receptor activation (Laurent et al., 2008; Langton and Richardson, 2008), future studies may also systematically probe the role of cAMP-related mechanisms in re-extinction. The present findings, however, suggest, that rolipram similarly disturbs both initial extinction (Experiment 3) and re-extinction (Experiment 4).

In recent years much interest in PDE inhibitors has focused on their ability to act as cognitive enhancers (Reneerkens et al., 2009). In particular, their potential to improve memory function has been shown in paradigms involving contextual fear conditioning (e.g. Barad et al., 1998; Gong et al., 2004), suggesting a potential use of rolipram for the treatment of Alzheimers Disease (Gong et al., 2004) and other conditions of mnestic dysfunctions (Barad, 2003; Bourtchouladze et al., 2003). The present study, however, shows

with multiple experiments that in cued fear conditioning, rolipram retards performance and extinction learning and possibly also interferes with the acquisition of cued fear. This implies that the use of rolipram as a cognitive enhancer should be more carefully evaluated with paradigms that probe hippocampus-independent memory processes before it is considered for pharmacotherapy. Given the high comorbidity of depression and anxiety disorders in humans (e.g. Kessler et al., 2003), the present findings may further indicate that potentially negative effects of PDE4 inhibitors on extinction and exposure-based treatments for anxiety could undermine the practical usefulness of PDE4 inhibitors for antidepressant therapy (Wachtel, 1983; Li et al., 2009).

In sum, the present study has demonstrated that the PDE4 inhibitor rolipram disturbs the expression and between-session extinction of fear. These findings strongly suggest that the potential clinical usability of rolipram should be further evaluated with paradigms that probe negative effects of rolipram on hippocampus-independent cognitive processes. In addition, these results should stimulate further research on the relationship between the cAMP/PKA/CREB pathway and extinction of fear (Myers et al., 2006). Such studies may yield novel ways to ameliorate extinction (Isiegas et al., 2006), which may impact strategies for treatment of anxiety disorders (Hofmann, 2007).

Acknowledgments

Thanks are extended to Julia Ray, Ryong Cho, Kristin Brewer and Sabrina Copes for assistance in data collection. Stefan G. Hofmann is a paid consultant of Organon (Schering-Plough) for work unrelated to this study. This study was partially supported by NIMH grant MH-078308 to Dr. Hofmann and seed funding from the Boston University Department of Psychology.

References

Alberini, C.M., 1999. Genes to remember. J. Exp. Biol. 202, 2887–2891.
Barad, M., Bourtchouladze, R., Winder, D.G., Golan, H., Kandel, E., 1998. Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. Proc. Natl. Acad. Sci. U.S.A. 95, 15020–15025.
Barad, M., 2003. Later developments: molecular keys to age-related memory impairment. Alzheimer Dis. Assoc. Disord. 17, 168–176.
Blanchard, R.J., Griebel, G., Henrie, J.A., Blanchard, D.C., 1997. Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. Neurosci. Biobehav. Rev. 21, 783–789.
Bourtchouladze, R., Lidge, R., Catapano, R., Stanley, J., Gossweiler, S., Romashko, D., Scott, R., Tully, T., 2003. A mouse model of Rubinstein-Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. Proc. Natl. Acad. Sci. U.S.A. 100, 10518–10522.
Bouton, M.E., 2004. Context and behavioral processes in extinction. Learn. Mem. 11, 485–494.
Carlezon Jr., W.A., Duman, R.S., Nestler, E.J., 2005. The many faces of CREB. Trends Neurosci. 28, 436–445.
Cherry, J.A., Davis, R.L., 1999. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. J. Comp. Neurol. 407, 287–301.
Comery, T.A., Martone, R.L., Aschmies, S., Atchison, K.P., Diamantidis, G., Gong, X., Zhou, H., Kreft, A.F., Pangalos, M.N., Sonnenberg-Reines, J., Jacobsen, J.S., Marquis, K.L., 2005. Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. J. Neurosci. 25, 8898–8902.
Davis, R.L., Cherry, J., Dauwalder, B., Han, P.-L., Skoulakis, E., 1995. The cyclic AMP system and Drosophila learning. Mol. Cell. Biochem. 150, 271–278.
Davis, M., 2006. Neural systems involved in fear and anxiety measured with fear-potentiated startle. Am. Psychol. 61, 741–756.
Gong, B., Vitolo, O.V., Trinchese, F., Liu, S., Shelanski, M., Arancio, O., 2004. Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. J. Clin. Invest. 114, 1624–1634.
Gray, J.A., McNaughton, N., 2000. The Neuropsychology of Anxiety: An Enquiry into the Functions of the Septohippocampal System, second ed.. Oxford University Press, Oxford, UK.
Hofmann, S.G., 2007. Enhancing exposure-based therapy from a translational research perspective. Behav. Res. Ther. 45, 1987–2001.
Isiegas, C., Park, A., Kandel, E.R., Abel, T., Lattal, K.M., 2006. Transgenic inhibition of neuronal protein kinase A activity facilitates fear extinction. J. Neurosci. 26, 12700–12707.
Josselyn, S.A., Shi, C., Carlezon Jr., W.A., Neve, R.L., Nestler, E.J., Davis, M., 2001. Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. J. Neurosci. 21, 2404–2412.
Kanes, S.J., Tokarczyk, J., Siegel, S.J., Bilker, W., Abel, T., Kelly, M.P., 2007. Rolipram: a specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. Neuroscience 144, 239–246.
Kehne, J.H., Boulis, N.M., Davis, M., 1991. Effects of the phosphodiesterase inhibitor rolipram on the acoustic startle response in rats. Psychopharmacology (Berl.) 105, 27–36.
Kessler, R.C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K.R., Rush, A.J., Walters, E.E., Wang, P.S., 2003. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 289, 3095–3105.
Kim, J.H., Richardson, R., 2008. The effect of temporary amygdala inactivation on extinction and re-extinction of fear in the developing rat: unlearning as a potential mechanism for extinction early in development. J. Neurosci. 28, 1282–1290.
Krupa, D.J., Thompson, R.F., 2003. Inhibiting the expression of a classically conditioned behavior prevents its extinction. J. Neurosci. 23, 10577–10584.
Langton, J.M., Richardson, R., 2008. D-cycloserine facilitates extinction the first time but not the second time: an examination of the role of NMDA across the course of repeated extinction sessions. Neuropsychopharmacology 33, 3096–3102.
Laurent, V., Marchand, A.R., Westbrook, R.F., 2008. The basolateral amygdala is necessary for learning but not for relearning extinction of context conditioned fear. Learn. Mem. 15, 304–314.
LeDoux, J., 2007. The amygdala. Curr. Biol. 17, R868–R874.
Li, Y.F., Huang, Y., Amsdell, S.L., Xiao, L., O'Donnell, J.M., Zhang, H.T., 2009. Antidepressant- and anxiolytic-like effects of the phosphodiesterase-4 inhibitor Rolipram on behavior depend on cyclic AMP response element binding protein-mediated neurogenesis in the hippocampus. Neuropsychopharmacology 34, 2404–2419.
Meloni, E.G., Jackson, A., Gerety, L.P., Cohen, B.M., Carlezon Jr., W.A., 2006. Role of the bed nucleus of the stria terminalis (BST) in the expression of conditioned fear. Ann. N.Y. Acad. Sci. 1071, 538–541.
Monti, B., Berteotti, C., Contestabile, A., 2006. Subchronic rolipram delivery activates hippocampal CREB and arc, enhances retention and slows down extinction of conditioned fear. Neuropsychopharmacology 31, 278–286.
Morgan, M.A., Romanski, L.M., LeDoux, J.E., 1993. Extinction of emotional learning: contribution of medial prefrontal cortex. Neurosci. Lett. 163, 109–113.
Myers, K.M., Davis, M., 2007. Mechanisms of fear extinction. Mol. Psychiatry 12, 120–150.
Myers, K.M., Ressler, K.J., Davis, M., 2006. Different mechanisms of fear extinction dependent on length of time since fear acquisition. Learn. Mem. 13, 216–223.
Nibuya, M., Nestler, E.J., Duman, R.S., 1996. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J. Neurosci. 16, 2365–2372.
Overton, D.A., 1991. Historical context of state dependent learning and discriminative drug effects. Behav. Pharmacol. 2, 253–264.
Pandey, S.C., Zhang, H., Roy, A., Xu, T., 2005. Deficits in amygdaloid cAMP-responsive element-binding protein signaling play a role in genetic predisposition to anxiety and alcoholism. J. Clin. Invest. 115, 2762–2773.
Perez-Torres, S., Miro, X., Palacios, J.M., Cortes, R., Puigdomenech, P., Mengod, G., 2000. Phosphodiesterase type 4 isozymes expression in human brain examined by in situ hybridization histochemistry and[3H]rolipram binding autoradiography. Comparison with monkey and rat brain. J. Chem. Neuroanat. 20, 349–374.
Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33, 56–72.
Ramos, B.P., Birnbaum, S.G., Lindenmayer, I., Newton, S.S., Duman, R.S., Arnsten, A.F., 2003. Dysregulation of protein kinase a signaling in the aged prefrontal cortex: new strategy for treating age-related cognitive decline. Neuron 40, 835–845.
Randt, C.T., Judge, M.E., Bonnet, K.A., Quartermain, D., 1982. Brain cyclic AMP and memory in mice. Pharmacol. Biochem. Behav. 17, 677–680.
Reneerkens, O.A., Rutten, K., Steinbusch, H.W., Blokland, A., Prickaerts, J., 2009. Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. Psychopharmacology (Berl.) 202, 419–443.
Rescorla, R.A., 1997. Response-inhibition in extinction. Q. J. Exp. Psychol. B 50B, 238–252.
Rutten, K., Prickaerts, J., Blokland, A., 2006. Rolipram reverses scopolamine-induced and time-dependent memory deficits in object recognition by different mechanisms of action. Neurobiol. Learn. Mem. 85, 132–138.
Silvestre, J.S., Fernandez, A.G., Palacios, J.M., 1999a. Effects of rolipram on the elevated plus-maze test in rats: a preliminary study. J. Psychopharmacol. 13, 274–277.
Wachtel, H., 1983. Potential antidepressant activity of rolipram and other selective cyclic adenosine 3′,5′-monophosphate phosphodiesterase inhibitors. Neuropharmacology 22, 267–272.
Waddell, J., Dunnett, C., Falls, W.A., 2004. C57BL/6J and DBA/2J mice differ in extinction and renewal of extinguished conditioned fear. Behav. Brain Res. 154, 567–576.
Wang, H., Ferguson, G.D., Pineda, V.V., Cundiff, P.E., Storm, D.R., 2004. Overexpression of type-1 adenylyl cyclase in mouse forebrain enhances recognition memory and LTP. Nat. Neurosci. 7, 635–642.

Zeller, E., Stief, H.J., Pflug, B., Sastre-y-Hernandez, M., 1984. Results of a phase II study of the antidepressant effect of rolipram. Pharmacopsychiatry. 17, 188–190.
Zhang, H.T., Crissman, A.M., Dorairaj, N.R., Chandler, L.J., O'Donnell, J.M., 2000. Inhibition of cyclic AMP phosphodiesterase (PDE4) reverses memory deficits associated with NMDA receptor antagonism. Neuropsychopharmacology 23, 198–204.
Zhang, H.T., Huang, Y., Jin, S.L., Frith, S.A., Suvarna, N., Conti, M., O'Donnell, J.M., 2002. Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. Neuropsychopharmacology 27, 587–595.
Zhang, H.T., Zhao, Y., Huang, Y., Dorairaj, N.R., Chandler, L.J., O'Donnell, J.M., 2004. Inhibition of the phosphodiesterase 4 (PDE4) enzyme reverses memory deficits produced by infusion of the MEK inhibitor U0126 into the CA1 subregion of the rat hippocampus. Neuropsychopharmacology, 1432–1439.

Appendix

Appendix I - Summary in German

Summary in German

Es ist davon auszugehen, dass biologisch implementierte Mechanismen zur erfolgreichen Bewältigung von Gefahrensituationen einem positivem Selektionsdruck unterliegen und eine Vielzahl solcher Mechanismen im Laufe der Phylo- und Anthropogenese evolviert ist, um auch im menschlichen Genom konserviert zu werden. In der vorliegenden Konzeptualisierung werden solche Mechanismen mindestens einer von zwei generellen Strategien zugeordnet. In der sogenannten *proaktiven* Gefahrenbewältigung werden vor Auftreten eines konkreten Gefahrenreizes organismische (z.B. neuronale, zelluläre, endokrinologische, motivationale, kognitive, behaviorale) und damit auch extra-organismische (z.B. Auswahl von Situationen betreffende) Faktoren dahingehend moduliert, dass *spätere* Gefährdungen des Organismus (bzw. des Fortbestandes seiner Gene) maximal abgeschwächt werden. In der *reaktiven* Gefahrenbewältigung werden hingegen organismische und extra-organismische Faktoren dahingehend moduliert, dass *augenblickliche* Gefährdungen maximal abgeschwächt werden. Mechanismen der proaktiven Gefahrenbewältigung beinhalten unter anderem Aufmerksamkeitsverzerrungen gegenüber potentiellen Gefahrenindikatoren, Fehlervermeidung, peripherphysiologische Anpassungsprozesse, Unsicherheitsreduktion, Verhaltenshemmung, aversive und negative Affektivität und verminderte Antriebsmotivation. Die prominentesten Mechanismen der reaktiven Gefahrenbewältigung sind Kampf-, Flucht- und Todstelltendenzen. Während proaktive Gefahrenbewältigungsmechanismen in der vorliegenden Arbeit unter dem Begriff „Angst“ subsummiert werden, ist der reaktiven Gefahrenbewältigung der Begriff „Furcht“ zugeordnet.

In der vorliegenden Arbeit wird weiterhin davon ausgegangen, dass um diese teilweise genetisch vorprogrammierten Mechanismen im Verhalten des Individuums auf adaptive und situationsangepasste Weise implementieren zu können, multiple Organisationsebenen und deren Interaktionen erforderlich sind. Als Organisationsebenen werden dabei ausschließlich

intraindividuelle neurobiologische Ebenen (z.B. Molekülebene, Synapsen-Ebene, Netzwerkebene, Strukturebene, Systemebene, ZNS/PNS-Ebene, Gesamtsystemebene) betrachtet. So kann sich Angst beispielsweise auf die Molekülebene (z.B. Genexpression), Synapsen-Ebene (erhöhte Serotonin-Ausschüttung), Netzwerkebene (erhöhte neuronale Erregbarkeit), Strukturebene (erhöhte Aktivität in bestimmten Nuclei der Amygdala), Systemebene (Modulation fronto-striataler Kommunikation), PNS-Ebene (Erhöhung der Herzfrequenz) und Gesamtsystemebene (subjektives Erleben von Angst) auswirken. Dass sich darüber hinaus die als Furcht und Angst bezeichneten Gefahrenbewältigungststrategien auch auf extraindividuellen Ebenen manifestieren (z.B. Phänomene wie Massenpaniken oder kollektive Angst nach Naturkatastrophen) und entsprechend auf der Dimension reaktiv vs. proaktiv trennen lassen können sei hier nur der Vollständigkeit halber erwähnt.

Der Mehrebenenansatz zur Defintion von Furcht und Angst erlaubt, dass deren Phänotype von situativen Faktoren beeinflusst werden können und gestattet somit eine dynamischere Perspektive als herkömmliche Konzeptualisierungen. Beispielsweise kann ein intrazellulärer Botenstoff die Extinktion von Furcht fördern, falls diese Furcht an einen bestimmten Ort gekoppelt ist, aber er kann die Extinktion stören, falls die Furcht an einen Ton gekoppelt ist (zur Erklärung siehe Studie 4). Mit einer eindimensionalen Perspektive im Sinne von „Botenstoff fördert Furchtextinktion“ kann diese Dynamik nicht erklärt werden. Erst durch Hinzunahme einer weiteren Ebene (z.B. Strukturebene) kann diese Interaktion ermöglicht werden (z.B. Botenstoff in Hippokampus fördert Furchtextinktion, Botenstoff außerhalb des Hippokampus behindert Furchtextinktion). Zusammengenommen werden Angst und Furcht somit als pro- und reaktive Strategien mit hoher evolutionärer Relevanz betrachtet, die sich dynamisch auf unterschiedlichsten Organisationsebenen und deren Interaktionen manifestieren, um als (ultimates) Ziel den Fortbestand des Genoms vor Gefahren zu schützen.

In der vorliegenden Untersuchungsreihe bildet diese Mehrebenenperspektive das zentrale Rahmenmodell zur Integration von vier sehr unterschiedlich angelegten Studien (z.B. human- vs. tierexperimentell, pharmakologisch, molekulargenetisch) zur Neurobiologie von Furcht und Angst bzw. Furchtsamkeit und Ängstlichkeit. Dabei soll betont werden, dass es im Kern nicht um die Überprüfung des Modells geht, sondern darum, die einzelnen Studien in einen Gesamtzusammenhang einzuordnen. Nichtsdestotrotz hat die vorliegende Arbeit den Anspruch zu verdeutlichen, dass diese oder ähnliche Konzeptualisierungen von Furcht und Angst ein besonderes Potenzial haben um (a) die Modell- und Ergebnisdiversität aus der pharmakologischen, molekulargenetischen, lerntheoretischen, tierexperimentellen, strukturell/bildgebenden, und vielen weiteren Richtungen der Furcht- und Angstforschung zu integrieren und (b) die Relevanz unterrepräsentierter Forschungsfelder (z.B. Netzwerkmodellierung) re-evaluieren zu können. Die anhand des Rahmenmodells in der vorliegenden Arbeit integrierten Studien sollen im Folgenden kurz zusammengefasst werden.

Die erste Studie untersuchte Mechanismen neuroviszeraler Kopplung mittels einer von uns entwickelten Korrelationsmethode (sog. Cardio-Electroencephalographic-Covariance-Traces, CECTs) zur Erfassung intraindividueller Zusammenhänge zwischen Einzeltrial-EEG und chronotroper (die Schlagfrequenz betreffender) Herzaktivität. Dabei zeigte sich, dass die frontozentrale EEG-Amplitude um 300 ms nach einem Feedback-Stimulus in einer Glücksspielaufgabe vier Sekunden später einsetzende Veränderungen der Herzschlagfrequenz signifikant vorhersagen konnte. Darüber hinaus wurde diese Prädiktion bei negativen Feedback-Reizen durch Trait-Ängstlichkeit dahingehend moderiert, dass ängstlichere Probanden durch ein erhöhtes Ausmaß neuroviszeraler Kopplung charakterisiert waren. Diesen Moderationseffekt konnten wir jüngst in einem anderen Paradigma mit n = 180 Probanden replizieren, was dafür spricht, dass wir mit den CECTs einen Indikator für einen dynamischen (da von Feedback-Valenz abhängigen) ängstlichkeitsrelevanten Mechanismus identifizieren konnten.

In der zweiten Studie wurde die dopaminerge Grundlage neuronaler Fehlerverarbeitung mittels EEG, molekulargenetischen Assessments und einer pharmakologischen Challenge durch einen selektiven Dopamin-D_2-Rezeptorantagonisten (Sulpirid, 200 mg) untersucht. Grundlage hierfür waren vorherige Studien, die einen Zusammenhang der durch Performanzfehler evozierten ERP-Komponente Error-Related Negativity (ERN) mit Ängstlichkeit bzw. Angststörungen und Dopamin zeigen konnten. Anhand einer modernen EEG-Auswertungsmethode (Independent Component Analysis, ICA), konnten wir zeigen dass (a) der mit präfrontaler Dopaminverfügbarkeit assoziierte COMT Val158Met Polymorphismus die Independent Component ERN-Amplitude und Verhaltensmaße der Fehlerverarbeitung modulierte, und (b) Sulpirid die Richtung dieser Modulation umkehrte. Darüberhinaus gab es erste Hinweise darauf, dass der für D_2-Rezeptorendichte im Striatum prädiktive DRD2TaqIa Polymorphismus mit Sulpirid interagierte, um neuronale Korrelate der Fehlerverarbeitung und deren Zusammenhang zu Ängstlichkeit zu modulieren. Zusammengenommen sprechen die Ergebnisse der zweiten Studie somit für eine dopaminerge Grundlage interindividueller Unterschiede in der Fehlerverarbeitung, einem für die proaktive Gefahrenbewältigung hochrelevanten Mechanismus.

Die dritte Studie untersuchte zukunftsorientiertes Entscheidungsverhalten in Probanden mit Generalisierter Angststörung (Generalized Anxiety Disorder, GAD). Im Sinne einer proaktiven Gefahrenbewältigung gingen wir davon aus, dass GAD-Probanden in höherem Ausmaß als nicht ängstliche Kontrollprobanden zukunftsorientierte Entscheidungen im Iowa Gambling Task (IGT) treffen. Der IGT ist ein Instrument das ursprünglich zur Erfassung von Zukunftsinsensitivität bei Patienten mit Läsionen im ventromedialen präfrontalen Kortex konstruiert wurde und Entscheidungsverhalten unter Ambiguität (d.h. ohne explizites Wissen über die mit einzelnen Entscheidungen verbundenen Risiken) misst. Unsere Erwartungen bestätigend fanden wir, dass GAD-Probanden in zwei unterschiedlichen

Varianten des IGT mehr zukunftsorientierte Entscheidungen trafen als Kontrollprobanden, was dafür spricht, dass zukunftsorientiertes Entscheidungsverhalten einen weiteren angstrelevanten Gefahrenbewältigungsmechanismus widerspiegelt.

Die vierte Studie untersuchte tierexperimentell einen wichtigen Bestandteil einer intrazellulären Signaltransduktionskaskade, die sowohl für den Erwerb als auch für die Extinktion von Furcht von Bedeutung ist. Der Second Messenger cAMP (cyclic adenosine monophosphate) aktiviert intrazelluläre Vorgänge, die zur Expression von bestimmten Proteinen führen, die für synaptisches Lernen erforderlich sind. cAMP wird durch die cAMP-spezifische Phosphodiesterase 4 (PDE4) abgebaut. Frühere Studien in der Arbeitsgruppe um den Nobelpreisträger Eric Kandell konnten zeigen, dass rolipram, ein selektiver PDE4-Hemmer intrazelluläre cAMP-Verfügbarkeit steigert und Gedächtnisformation in vitro und in vivo steigern kann. Darauf aufbauend vermuteten wir, dass rolipram ebenfalls die Konsolidierung von Furchtextinktion potenzieren kann und somit als sog. „Extinction Booster“ in Frage kommen könnte. Solche Extinction Booster (z.B. D-Cycloserine) wurden im letzten Jahrzehnt intensiv beforscht da sie die Effektivität von Expositionstherapien bei Angststörungen steigern können. Die Hypothese, dass rolipram Furchtextinktion verbessert, testeten wir in einer Serie aus fünf einzelnen Experimenten bei denen Mäuse zunächst an einen Ton furchtkonditioniert wurden und das Ausmaß der Furcht dann vor und nach einer Extinktionsbehandlung über die furchtreizgetriggerte Verstärkung des Startle-Reflexes gemessen wurde. Im Gegensatz zu unseren Erwartungen zeigten unsere Ergebnisse jedoch deutlich, dass rolipram die Konsolidierung von Extinktionsinhalten dramatisch beeinträchtigte statt sie zu verbessern. Darüber hinaus dämpfte rolipram den Ausdruck von Furcht. Zusammengenommen sprechen die Ergebnisse aus Studie vier dafür, dass der cAMP-Kreislauf für den Ausdruck und die Extinktion von Furcht von Bedeutung ist, dass PDE4-Hemmer jedoch nicht uneingeschränkt Gedächtnisfunktionen verbessern, sondern die Extinktion von an explizite Reize konditionierter Furcht beeinträchtigen können.

Obgleich sich die Ergebnisse der vier Studien durch ihre Vielfältigkeit schwer direkt zueinander in Beziehung setzen lassen, können sie anhand des Rahmenmodells integriert werden. Wie aus Tabelle 2 ersichtlich handelt es sich sowohl bei der peripherphysiologischen Regulierung (Studie 1), als auch bei der Fehlerverarbeitung (Studie 2), der zukunftsorientierten Entscheidungsfindung (Studie 3) und der Reflexpotenzierung (Studie 4) um Mechanismen, die das ultimate Ziel haben, Gefahren für den Organismus zu reduzieren und neurobiologisch auf mehreren Ebenen dynamisch implementiert sind. Trotz des gemeinsamen Ziels, dürften die genauen Zusammenhänge der einzelnen Mechanismen zueinander relativ komplex sein und unter anderem in Abhängigkeit von Spezien, Individuen, Ebenen und situativen Bedingungen variieren. Wie auch in Tabelle 2 (s. Hauptteil) angedeutet, eröffnet das Rahmenmodell somit eine Vielzahl neuer Forschungsperspektiven.

Appendix II – Additional Remarks to Study 2

Additional Remarks to Study 2

Models on dopamine and error processing

The prominent reinforcement learning theory by Holroyd and Coles (D. Mueller et al., 2008) states that the commitment of an error (or the presentation of negative performance feedback) ideally triggers an internal negative prediction error ("events are worse than expected"), which is used for reinforcement learning in order to perform better in the future. In short, it is assumed that this prediction error is signaled by the mesencephalic dopamine system as a cessation in dopamine burst firing (cf. Stoltenberg & Vandever, 2010), which is carried to the anterior (mid-) cingulate cortex (AMC). The theory states that one function of the AMC is to select among the inputs of various available motor controllers (e.g., dorsolateral prefrontal cortex, amygdala) all of which may intend a different motor response. According to the theory, the AMC learns from the phasic dip in dopamine which motor controller not to choose in future similar situations. The theory further states that the error-related negativity (ERN, see below) occurs as a consequence of such a negative prediction error phasic dip in prefrontal cortex dopamine. Another relevant theory, the neurocomputational model by Frank (2009), focuses on basal ganglia – rather than prefrontal cortex – dopamine, and states that error processing is associated with No-Go signals mediated by dopamine D_2 receptors (Holroyd & Coles, 2002) in dorsal striatum cells. In sum, from both theories it can be derived that interindividual differences in error processing may be due to interindividual differences in dopamine (Frank, 2005; Schultz, 1998).

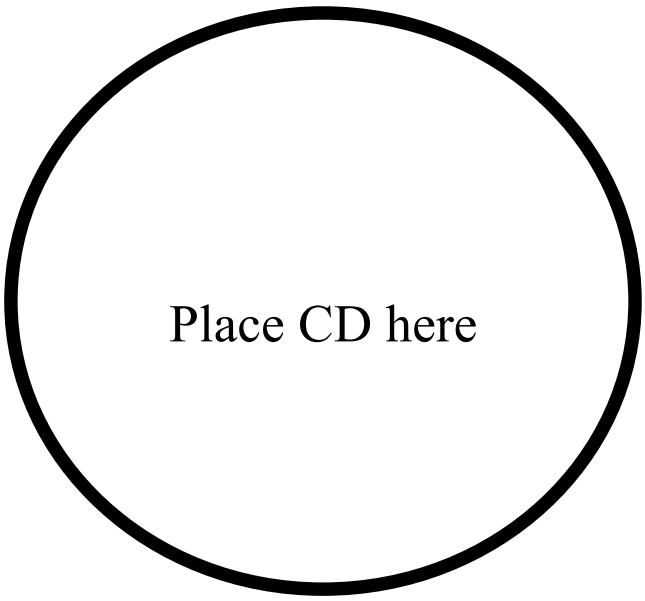
The principles of Independent Component Analysis (ICA)

To understand the principles of ICA, one can imagine a room (or a skull) in which several persons (or brain processes) who independently talk at the same time are recorded with several microphones (or EEG-sensors). Spatiotemporal ICA is a blind source separation technique that finds a way to linearly combine the information at all microphones such that

new time courses (i.e., independent component time courses) that are maximally independent from each other are generated. Assuming that the speakers all talked independently, each of these IC-time-courses reflects what one speaker said. In addition, by inspecting the relative weight that is given to each microphone in order to generate one IC time-course, one can model where in the room the speaker associated with that IC stood (i.e., if the weight is equal for all microphones he would have stood in the middle of the room; if it is high for one microphone he would have stood in proximity thereto, etc.). Similarly for EEG research, the scalp topography of ICs (reflecting how much each electrode contributes to one IC-signal) can be used to estimate the localization of a given IC/brain process.

ICA-algorithms are relatively complex and beyond the scope of this thesis (for an introduction the interested reader is referred to: Frank et al., 2007). However, the basic idea is that under some conditions the activity distribution of a sum of independent random variables tends toward gaussian distribution according to the Central Limit Theorem. Accordingly, the sum of two independent random variables will be more gaussian than any of the two random variables alone. In other words, if there were two mixtures (each reflecting the sum of two random variables), a linear combination of these mixtures that is maximally non-gaussian would reflect only one random variable rather than the sum of both random variables (i.e., the weight of the other random variable would become zero). This can be extended to multiple sources and mixtures, and ICA uses iterative algorithms to find such linear combinations with maximum non-gaussianity (or minimum mutual information).

Appendix III – Matlab scripts


Place CD here

Appendix IV - Curriculum Vitae

Curriculum Vitae

Erik Mueller, Dipl.-Psych.

Address

Gutenbergstraße 18
35032 Marburg
erik.mueller@staff.uni-marburg.de

Education

Since 10/09	Qualification training as clinical psychotherapist (Institut für Psychotherapie Ausbildung, Marburg)
Since 10/08	Doctoral candidate at Philipps-Universität Marburg (supervisor: Dr. J. Wacker)
2008	Diplom at Philipps-Universität Marburg (overall grade: „sehr gut“)
2008	Diplomarbeit at Harvard University: *Attentional shifts in Social Anxiety Disorder. An electrophysiological source localization study.* Supervised by D. Santesso, Harvard University and W. Rief, Philipps-Universität Marburg (grade: A/„sehr gut“)
2006-2007	Graduate student at the Pennsylvania State University (overall GPA: 4.0)
2005	Vordiplom at Philipps-Universität Marburg (overall grade: „sehr gut“)
2002	Abitur at Prälat-Diehl-Schule in Groß-Gerau, Germany

Occupations

Since 04/11	One Semester temporary replacement for Assistant Professor Dr. J. Wacker (Division for Differential Psychology and Psychological Diagnostics)
Since 10/09	University teaching assignment at Philipps-Universität Marburg (cognitive neuroscience)
Since 10/09	Psychotherapist at Psychotherapie Ambulanz Marburg
Since 10/08	Project leader of research project funded by the Deutsche Forschungsgemeinschaft, DFG WA 2593/2-1 and DFG WA 2593/2-2 (Personality and action monitoring: Molecular genetics and pharmacological investigation of dopaminergic mechanisms, Principal Investigator: Dr. Jan Wacker)

Internships & Work Experience

2010	University of California, San Diego: three month research fellowship at the Swartz Center for Computational Neuroscience (supervisor: Dr. S. Makeig)

2007	Harvard University: three month research internship at the Affective Neuroscience Laboratory (supervisor: Dr. D. Pizzagalli)
2007	Boston University: two month research internship at the Center for Anxiety and Related Disorders (director: Dr. D. Barlow)
2006	Salus Klinik Lindow, Lindow, Germany: Clinical internship
2002-2003	Sozialpsychiatrischer Verein in Groß-Gerau, Germany: Community Service

Awards

2011	DGPA Spring School 2011 stipend (Genes, Brain and Behavior)
2010	Jena Autumn School 2010 stipend (Anterior cingulate cortex)
2010	Society for Psychophysiological Research 2010 student poster award
2010	Dresden Spring School 2010 stipend (Molecular Genetics)
2010	Cover illustration for the journal *Neuroscience* (Vol. 166, 2)
2009	SPR Research-Training Award 2009 (Society for Psychophysiological Research) (covers 3 month fellowship in UCSD, San Diego, CA)
2009	fMRI-Spring School 2009 stipend (Section Biological Psychology and Neuropsychology of the German Psychological Association)
2007	"Outstanding Academic Achievement" (Pennsylvania State University International Program)
2006	"Exceptional Academic Achievement" (Pennsylvania State University International Program)
2006	Scholarship from the Marburg University-Pennsylvania State University-Exchange program
2006	Stipend from the German Academic Exchange Service for one semester study abroad (DAAD)

Publications

Mueller, E.M., Makeig, S., Stemmler, G., Hennig, J.,& Wacker, J. (submitted). Dopamine effects on human error processing depend on COMT VAL158MET genotype.

Mueller, E. M.*, Hofmann, S.G., & Cherry, J. (2010). The type IV phosphodiesterase inhibitor rolipram disturbs expression and extinction of conditioned fear in mice. *Neuropharmacology, 59,* 1 – 8.

Mueller, E. M., Stemmler, G., & Wacker, J. (2010). Single-trial EEG predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity. *Neuroscience, 166,* 491-500.

Mueller, E. M., Nguyen, J., Ray, W., & Borkovec, T. D. (2010). Future oriented decision-making in Generalized Anxiety Disorder is evident across different versions of the Iowa Gambling Task. *Journal of Behavior Therapy and Experimental Psychiatry, 41,*

165-171.

Mueller, E. M., Hofmann, S. G., Santesso, D. L., Meuret, A. E., Bitran, S., & Pizzagalli, D. A. (2009). Electrophysiological evidence of attentional biases in social anxiety disorder. *Psychological Medicine, 39*, 1141-1152.

Wacker, J., **Mueller, E. M.,** Hennig, J., & Stemmler, G. (under revision). How to consistently link extraversion and intelligence to the Catechol-O-Methyltransferase (COMT) Gene: On defining and measuring psychological phenotypes in neurogenetic research. *Journal of Personality and Social Psychology.*

Santesso, D. L., Meuret, A. E., Hofmann, S. G., **Mueller, E. M.,** Ratner, K. G., Roesch, E. B., & Pizzagalli, D.A. (2008). Electrophysiological correlates of spatial orienting towards angry faces: A source localization study. *Neuropsychologia, 46*, 1338-1348.

Published Abstracts & Conference Presentations

Mueller, E. M., Makeig, S., Delorme, A., Stemmler, G., & Wacker, J. (submitted). Interindividual differences in independent brain components - methodological considerations. Psychologie und Gehirn, June, 23 – 25, 2011, Heidelberg, Germany.

Panitz, C.**, Wacker, J., Stemmler, G., & **Mueller, E. M.** (submitted). Temporal coupling of cortical and cardiac activity: Testing the CECT-approach. Psychologie und Gehirn, June, 23 – 25, 2011, Heidelberg, Germany.

Mayer, I.**, Wacker, J., Stemmler, G., & **Mueller E. M.** (submitted). Anxiety predicts neurovisceral coupling – a CECT study. Psychologie und Gehirn, June, 23 – 25, 2011, Heidelberg, Germany.

Wacker, J., **Mueller, E. M.,** & Stemmler, G. (submitted). Umkehrung des Zusammenhangs zwischen Trait BAS und frontaler Asymmetrie durch Gabe eines Dopamin-D2-Rezeptorblockers. Psychologie und Gehirn, June, 23 – 25, 2011, Heidelberg, Germany.

Mueller, E. M., Mayer, I., Stemmler, G., Hennig, J., & Wacker, J. (submitted) Neurovisceral connectivity and brain activity following negative feedback – Two COMT-Val158Met dependent intermediate phenotypes for trait anxiety. Society for Psychophysiological Research, September, 13 – 18, 2011, Boston, USA.

Wacker, J., **Mueller, E. M.**, & Stemmler, G. Dopamine D2 receptor blockade reverses the association between trait BAS and frontal asymmetry. Society for Psychophysiological Research, September, 13 – 18, 2011, Boston, USA.

Mueller, E.M., Stemmler, G., Hennig, J., Wacker, J. (2011). 5HTTLPR and general condition influence experimentally induced negative affect and trait worry in healthy individuals – evidence for a diathesis-stress interaction. 7. Workshopkongress der DGPs-Fachgruppe Klinische Psychologie und Psychotherapie. June 2 – 4, 2011, Berlin, Germany.

Mueller, E.M., Hennig, J., Stemmler, G., Wacker, J. (2011). Dopaminergic polymorphisms

COMT and DRD2TaqIa predict learning from gains and losses, respectively. Poster presentation at DGPA Spring School 2011: Genes, Brain and Behavior, March 23 – 26, 2011, St. Goar, Germany.

Mueller, E.M., Makeig, S., Stemmler, G., Hennig, J.,& Wacker, J. (2010). Both trait anxiety associations and dopamine antagonist-induced reductions of the error-related negativity are moderated by the D2 receptor gene. *Psychophysiology, 47*, S47-S48.

Mueller, E.M., Makeig, S., Stemmler, G., Hennig, J.,& Wacker, J. (2010). The dopamine-antagonist sulpiride modulates frontomedial error signals depending on dopaminergic genotypes. Oral and poster presentation at Autumn School Jena 2010: Cognitive, Affective, and Nociceptive Functioning of the Anterior Cingulate Cortex", November 25-28, 2010, Jena, Germany.

Mueller, E.M. & Wacker, J. (2010). Sulpiride modulates the association of negative affect and error-related brain potentials depending on DRD2taq1 A polymorphism. Poster presentation at "Dresden Spring School 2010: From vulnerability to resilience: Molecular genetic persepctives", March 17-20, 2010, Dresden, Germany.

Mueller, E. M., Stemmler, G., Wacker, J. (2010). Studying the brain with the heart cardio-electroencephalographic covariance traces as an alternative to the event-related potential technique? Oral presentation at "52. Tagung experimentell arbeitender Psychologen (TeaP)", March 22-25, 2010, Saarbruecken, Germany.

Mueller, E. M., Nguyen, J., Ray, W., & Borkovec, T. D. (2009). Entscheidungsverhalten bei Generalisierter Angststoerung: Erhoehte Sensitivitaet fuer zukuenftige Verluste. *Zeitschrift für Klinische Psychologie und Psychotherapie, 38,* S50.

Mueller, E. M., Ahrens, B., Stemmler, G., Zangl, M., & Wacker, J. (2009). Single trial EEG amplitude predicts subsequent changes in heart rate within individuals – the cardio electroencephalographic covariance trace. *Psychophysiology, 46,* S85.

Mueller, E. M., Ahrens, B., Stemmler, G., Zangl, M., Wacker, J. (2009). Vorhersage intraindividueller Herzratenveränderungen durch Einzeltrial-ERP-Magnituden: Kardio-Elektroenzephalographische Kovarianz-Spuren. Poster presentation at "35. Arbeitstagung Psychophysiologie und Methodik (APM 2009)", June 11- June 13, 2009, Leipzig, Germany.

Mueller, E. M., Nguyen, J., Ray, W. J., Borkovec, T.D. (2009). The Iowa Gambling Task – Decision-making or punishment-learning? Poster presentation at "51. Tagung experimentell arbeitender Psychologen (TeaP)", March 29- April 01, 2009, Jena, Germany.

Mueller, E. M., Hofmann, S. G., Santesso, D. L., Meuret, A. E., Bitran, S., Pizzagalli, D. A. (2008). Attentional Shifts in Social Anxiety Disorder: An electrophysiological source localization study. Oral presentation at "50. Tagung experimentell arbeitender Psychologen (TeaP)", March 03-05, 2008, Marburg, Germany.

* article was re-published in Neuropharmacology, Virtual Special Issue of 4/6/2011 on Anxiety and Depression enclosing "outstanding contributions on the topic".

** student first author was supervised by E. M.

Supervised Theses

EEG-based prediction of heart rate in Panic Disorder and Depression (Christian Panitz, ongoing Diploma thesis)

Effects on dopamine antagonist on neurovisceral connectivity (Isabella Meyer, ongoing Diploma thesis)

Decision making after worry induction (Maja Erkic and Berta Skip, Bachelor thesis)

Personality and Feedback – an EEG investigation (Alexandra Hofmann, Christian Panitz and Sarah Utke, Bachelor thesis)

Invited to Review for

- Psychophysiology
- Biological Psychology
- Journal of Experimental Psychopathology
- Brain and Cognition

Appendix V: Relative contributions of E.M. Mueller to the included submissions

Relative contributions of E.M. Mueller to the included submissions

Study 1:

Mueller, E. M., Stemmler, G., & Wacker, J. (2010). Single-trial EEG predicts cardiac acceleration: A time-lagged P-correlation approach for studying neurovisceral connectivity. *Neuroscience, 166,* 491-500.

Relative contribution of EMM: 70%

Study 2:

Mueller, E.M., Makeig, S., Stemmler, G., Hennig, J.,& Wacker, J. (submitted). Dopamine effects on human error processing depend on COMT VAL158MET genotype.

Relative contribution of EMM: 60%

Study 3:

Mueller, E. M., Nguyen, J., Ray, W., & Borkovec, T. D. (2010). Future oriented decision-making in Generalized Anxiety Disorder is evident across different versions of the Iowa Gambling Task. *Journal of Behavior Therapy and Experimental Psychiatry, 41,* 165-171.

Relative contribution of EMM: 80%

Study 4:

Mueller, E. M., Hofmann, S.G., & Cherry, J. (2010). The type IV phosphodiesterase inhibitor rolipram disturbs expression and extinction of conditioned fear in mice. *Neuropharmacology, 59,* 1 – 8.

Relative contribution of EMM: 50%

Appendix VI: Erklärung

Erklärung

Ich versichere, dass ich meine Dissertation

From the Cell to the Brain – Fear and Anxiety across the Levels of Neuroscience

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg, 22. April 2010

Erik Müller
AG Differentielle Psychologie und Psychologische Diagnostik
Gutenbergstr. 18
35037 Marburg

Tel.: 06421-895157
Mail: erik.mueller@staff.uni-marburg.de