Fronto-striatal brain circuits involved in the pathophysiology of schizophrenia and affective disorders: FMRI studies of the effects of urbanicity and fearful faces on neural mechanisms of reward processing and self-control

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen Doktorgrades

„Doctor rerum naturalium“

der Georg-August-Universität Göttingen

im Promotionsprogramm Biologie

der Georg-August University School of Science (GAUSS)

vorgelegt von

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Göttingen, 2016

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Date of thesis submission: 10.03, 2016

Date of oral examination: 21.04.2016

Statement of Originality

I hereby declare that this thesis has been written independently with no other sources and aids than quoted in the text.

Göttingen, 07.03. 2016

Bernd Krämer

Preface

The present work is a publication-based dissertation based on two original manuscripts. The first manuscript is submitted for publication in the journal “Human Brain Mapping” and the second one is published in the journal “Neuropsychobiology”.

Paper 1

Krämer, B., Diekhof, E. K., Gruber, O. 201X Effects of city living on the mesolimbic reward system – an fMRI study; Submitted to Human Brain Mapping, Manuscript ID HBM-16-0186, Date Submitted 18-Feb-2016, currently under review

Paper 2

Krämer, B., Gruber, O., 2015. Dynamic Amygdala Influences on the Fronto-Striatal Brain Mechanisms Involved in Self-Control of Impulsive Desires. Neuropsychobiology 72, 37–45. doi:10.1159/000437436

The experiments were performed at University Medical Center Göttingen, Department of Psychiatry and Psychotherapy. The studies were supervised regarding design, statistical analysis and publication by Professor Dr. Oliver Gruber. The author of this dissertation had an essential role regarding (a) the development of study design, (b) the development of the experimental design, (c) the statistical analysis and interpretation of data, and (d) the preparation for publication of manuscripts.

The following text provides the common frame of both studies and describes the theoretical background and the goals of the dissertation. Finally, the main results of both papers are summarized and discussed considering the common frame of this work.

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Abbreviations

ACC	anterior cingulate cortex
ANS	autonomic nervous system
avPFC	anteroventral prefrontal cortex
BLA	basolateral amygdala
BOLD	blood-oxygen-level-dependent
DA	dopamine
DC	desire context
DLPFC	dorsolateral prefrontal cortex
DMPFC	dorsomedial prefrontal cortex
DRD	desire-reason-dilemma
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
fMRI	functional magnetic resonance imaging
FWE	family-wise error
FWHM	full-width at half maximum
GLM	general linear model
GWAS	genome-wide association studies
HPA	hypothalamus-pituitary-adrenal
hrf	hemodynamic response function
ICD	International Statistical Classification of Disease
MDD	major depressive disorder
MHC	major histocompatibility complex
MNI	Montreal Neurological Institute
MPFC	medial prefrontal cortex
NAc	nucleus accumbens
OFC	orbitofrontal cortex
PFC	prefrontal cortex
pgACC	pregenual anterior cingulate cortex
PPI	psychological-physiological-interaction
RC	reason context
SSRI	selective serotonin reuptake inhibitors
svc	small volumes correction
VMPFC	ventromedial prefrontal cortex
VS	ventral striatum
vSub	subiculum of the hippocampus
VTA	ventral tegmental area

1 Introduction

This thesis is concerned with two studies which have the common pursuit to improve the investigation of pathomechanisms underlying schizophrenic and affective disorders. They were motivated by the fact, that the diagnosis of mental disorders is a complex process and despite advances still error prone (Freedman et al., 2013). For an example, the most frequent initial symptom of schizophrenia and depression is depressive mood and the disorders can be distinguished when positive symptoms start appearing (Häfner et al., 2013). If there were disease specific biological features (biomarkers) detectable with a clinical test, then there would be an opportunity to improve the diagnosis and to initiate specific treatment early to prevent the onset of the disorder (Weickert et al., 2013). A better understanding of disorders pathophysiology is one factor contributing to identification of possible biomarkers. There are promising results from research in neuroimaging which highlight a relation between alterations in brain structure and function and mental disorders but the search for reliable biomarkers is an ongoing process (Frey et al., 2013; Savitz et al., 2013; Weickert et al., 2013).

Such studies are important because mental disorders do not only affect patients' wellbeing but, in addition will change hers/his life. When after a first episode a chronical course of the disorder evolves social decline is often an additional outcome. Loss of employment, loss of spouse and social isolation worsen individuals situation (Häfner et al., 2013). The individual burden has also a huge impact for the society because about 38 million people were affected by affective and psychotic disorders in Europe in the year 2010 (Gustavsson et al., 2011). The accumulated cost reached 207 billion euro, whereby the greatest amount was allocated to indirect cost, 137 billion; cost associated with patients' production losses. This explains the demand for neuroscientific research to contribute to the improvement of mental disorders diagnostics and therapy.

The following introduction provides a short overview about the factors contributing to the development of mental disorders, the main brain regions affected by them and the observed changes in neural processing. This should illustrate the scientific background for the goals and hypothesis of this thesis. Additionally, a short summary of the general methods applied in the experiments will be provided.

1.1 Factors contributing to the development of mental disorders

The search for biomarkers is supported by models which describe how biomedical, social, psychological and behavioral factors contribute to the development and manifestation of mental disorders (Engel, 1977). These so called diathesis-stress or vulnerability-stress models (Ingram and Luxton, 2005) describe the predispositional factors (vulnerability), like genetic variation, obstetric complications , the contributing factors, like stress, the maintaining and coping factors and their interaction. But it is a common understanding that a complete description of the underlying pathological processes is not possible for most mental disorders (DSM-5).

1.1.1 Genetic factors in the development of mental disorders

A genetically determined vulnerability for psychiatric disorders is supported by family-, twin- and adoption-studies. Heritability values vary between 81% for schizophrenia, 75% for bipolar disorder (BD) and 37% for major depressive disorder (MDD) (Sullivan et al., 2012). The common environment contributes to the liability of schizophrenia with an effect of 11% (Sullivan et al., 2003) and in MDD the individual-specific environment has an effect of 63% and the common environment has only a minimal effect (Sullivan et al., 2000). This illustrates that the development of mental disorders is influenced by genetic and environmental factors.

To identify risk genes which are related to mental disorders several genome-wide association studies (GWAS) were performed. The recent one for schizophrenia identified several genetic variations, single nucleotide polymorphism (SNP), involved in dopaminergic, glutamatergic neurotransmission and neurodevelopment which reached genome-wide significance (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Also for bipolar disorder several genes were identified (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011) which could be involved in e.g. memory (Erk S et al., 2010) and emotion regulation (Cichon et al., 2011). But up to now the search for gene loci that exceed genome-wide significance for MDD still continues (Flint and Kendler, 2014).

The genetic architecture of mental disorders is extremely complex, e.g. for schizophrenia it is estimated that 6300 – 10200 SNPs contribute to its etiology and these SNPs collectively account for about 50% of the heritability (Ripke et al., 2013a). Therefore, it is unlikely that a

unimodal genetic biomarker could be detected which could reliable improve diagnosis and therapy.

1.1.2 Psycho-social factors in the development of mental disorders

Influences of the individual and the common environment constitute another risk factor. Distress during development, e.g. childhood loss or severe childhood trauma may create an enduring cognitive vulnerability that could contribute to the development and maintenance of schizophrenic and affective disorders (Garety et al., 2007; Gotlib and Joormann, 2010; Roiser et al., 2012).

Also stressful life events have the opportunity to trigger a mental disorder. It is reported that 50% to 80% of depressed persons experienced a major life event preceding the onset of depression, e.g. loss of partner (Cohen et al., 2007).

There is also an influence of the urban environment. The latest German mental health survey showed significant increased prevalence rates of affective disorders (13.9% vs. 7.8%) and psychotic disorders (5.2% vs. 2.5%) for people living in cities with more than 500k inhabitants compared to people in rural areas with less than 20k inhabitants (Jacobi et al., 2014). This confirms the findings of a previous study (Dekker et al., 2008) and is corroborated by recent meta-analyses which revealed a higher risk for schizophrenia (Vassos et al., 2012) and higher prevalence rates for mood and anxiety disorders (Peen et al., 2010) for people living in cities.

In summary, during the pathogenesis of mental disorders interaction of genes and environment lead to functional changes in brain regions and related brain networks. The next chapter describes how these changes affect several cognitive functions.

1.2 Fronto-striatal brain circuits and the pathophysiology of schizophrenic and affective disorders

Hypotheses regarding a biological foundation of mental disorders derived from observations that pharmacological interventions relieve or worsen the symptoms of mental disorders. In schizophrenic patients antipsychotics reduced positive symptoms by blocking dopamine receptors and drugs that increased dopamine (DA) activity worsens the positive symptoms. Therefore an abnormal low dopamine activity is responsible for the negative and high DA

activity for the positive symptoms (Davis et al., 1991). Also in MDD a deficiency of central noradrenergic and/or serotonergic systems describes a pathophysiological mechanism because serotonin reuptake inhibitors (SSRI) treatment restores normal function in depressed patients. But these observations could not explain the full pathophysiological picture. About 30% of depressed patients fail to respond to SSRI treatment (Willner et al., 2013) and DA dysfunction is not confined to schizophrenia itself and is detected in patients with other psychotic disorders (Howes and Murray, 2014).

But both transmitter systems are core components of several behavioral processes. The mesolimbic DA projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) shell and the medial prefrontal cortex (MPFC) thus play major roles in reward learning (Price and Drevets, 2012). Behavioral studies showed that antidepressants treatment causes a positive bias in emotional face processing and functional magnetic resonance imaging (fMRI) studies showed that the neural effects that are congruent with the concomitant behavioral changes. The amygdala response to masked fearful faces was attenuated and the activity of the fusiform gyrus to presentation of happy faces increased (Harmer et al., 2009). In summary, a common observation for schizophrenic and affective disorders is a disturbed activity in brain regions involved in emotion, reward and stress processing, especially in the amygdala the mesolimbic DA system and areas of the prefrontal cortex (PFC) (Howes and Kapur, 2009; Savitz and Drevets, 2009; Treadway and Zald, 2011).

The following passages describe the observed changes for schizophrenic and affective disorders with the focus on reward, emotion and stress processing and the limbic and mesolimbic dopamine system.

1.2.1 Reward processing and its abnormalities in mental disorders

Unpredicted reward or cues associated with reward (expected reward) elicit burst firing patterns of VTA DA neurons (Schultz et al., 1997) and increase the DA release in the NAc, sometimes referred as ventral striatum (VS). Additionally the NAc integrates contextual input from the ventral subiculum of the hippocampus (vSub), emotional information from the basolateral amygdala (BLA), and behavioral control information from PFC (Haber and Knutson, 2009; Sesack and Grace, 2009) enabling the amygdala to coordinate reward-seeking (approach) and fear-related (avoidance) behaviors via its differential regulation of

NAc output (Gill and Grace, 2011). Therefore the mesolimbic DA projections from the VTA to the NAc shell and the MPFC play a major role in learning associations between operant behaviors or sensory stimuli and reward (Price and Drevets, 2012).

This process is disturbed in schizophrenia. Patients display an increased response to expected and a reduced response to unexpected rewards in the VS (Morris et al., 2012). This is a deviation from the normal activation pattern which is increased in response to unexpected rewards only. Also the activity in ventromedial prefrontal cortex (VMPFC) is reduced in response to reward and increased to unrewarded trials (Schlagenhauf et al., 2009).

Also in MDD anhedonic symptoms are linked with attenuated activity of the mesolimbic DA system (Price and Drevets, 2009). Subjects with MDD have significantly weaker responses to gains in the VS (Pizzagalli et al., 2009). Furthermore, the reward learning seems to be impaired which relies on the functional integrity of the amygdala, the hippocampus, the VTA, the VS and the medial prefrontal network.

Manic patients show an altered activation pattern in VTA and NAc in response to expectation and omissions of reward (Abler et al., 2007) and hypomania is associated with stronger reward related striatal activity (O'Sullivan et al., 2011). Also prefrontal regions fail to downregulate reward related activity in the NAc and if reduced reward related VS activation was found, this observation is likely related to anhedonia symptoms of the included sample (Trost et al., 2014).

1.2.2 Stress processing and its abnormalities in mental disorders

Psychological stress stimulates the hypothalamus-pituitary-adrenal (HPA) axis via amygdala and hypothalamus leading to cortisol release. Increased cortisol levels further stimulate the amygdala providing positive feedback to the HPA axis. Cortisol stimulation of the hippocampus inhibits the HPA axis limiting the HPA axis activity. Additionally, the dorsal medial prefrontal cortex (DMPFC) exerts negative feedback over the HPA axis enabling emotional self-regulation (Ulrich-Lai and Herman, 2009).

Psychosocial stress acting on the HPA axis is seen as an additional factor driving the development and manifestation of schizophrenic and affective disorders. Schizophrenic

patients exhibit higher cortisol levels, which causes structural changes in amygdala and hippocampus. The resulting altered hippocampal activity contribute to dysregulation of stress processing (Walker et al., 2008). Because the vSub of the hippocampus controls the burst firing of VTA dopamine neurons via NAc and ventral pallidum pathways, it is proposed that changes in hippocampal activity can affect the mesolimbic DA system. A stress induced dysfunction in this circuit leads then to an increased dopamine baseline and lays the foundation that minor salient or even non salient events can gain attention (Grace, 2010).

That stress contributes to the development of MDD and the hyperactivity of the HPA axis in patients is described since the late 1950s (Marques et al., 2009) but HPA axis abnormalities are seen in 35 to 65% of depressed individuals only (Lucassen et al., 2014). This effect might be related to aversive early-life experiences because depressed patients without such experiences showed a normal HPA axis responses to stress (Nemeroff and Vale, 2005). Also an impaired corticosteroid receptor function might be another key mechanism in the pathogenesis of depression (Ising et al., 2005).

The role of stress as a causal factor in the manifestation of bipolar disorder is established but the exact mechanisms by which stress exerts its effects on the brain remains largely unknown. A comprehensive theoretical framework that fully characterizes the role of stress in BD pathophysiology is not yet available (Brietzke et al., 2012).

1.2.3 Emotion processing and its abnormalities in mental disorders

The amygdala role in stimulus processing is seen as relevance detector tuning cognitive and social processes to give priority to relevant events (Phelps, 2009). It is activated by presentation of emotional facial expressions (Fusar-Poli et al., 2009) and other behavioral significant stimuli. The hippocampal formation is required for episodic and semantic memory and its retrieval (Rolls and Kesner, 2006).

When processing fearful facial expressions schizophrenic patients (exhibiting positive symptoms) display reduced activity in the MPFC, the amygdala and the hippocampal formation. Also a higher activity in the hippocampal formation is observed when patients with paranoia symptoms process neutral stimuli (Goghari et al., 2010). During affect processing the connectivity of the PFC and the amygdala seems to be abnormally reduced (Frangou, 2014).

Depressed patients show increased amygdala activity in response to negative stimuli, e.g. sad faces and a negative bias when processing happy faces accompanied by alterations in pregenual anterior cingulate cortex (pgACC), dorsolateral prefrontal cortex (DLPFC) and hippocampal activity (Price and Drevets, 2012).

In BD increased amygdala activation in response to affective faces during mania but not always during depression is reported by meta-analyses (Chen et al., 2011; Strakowski et al., 2012).

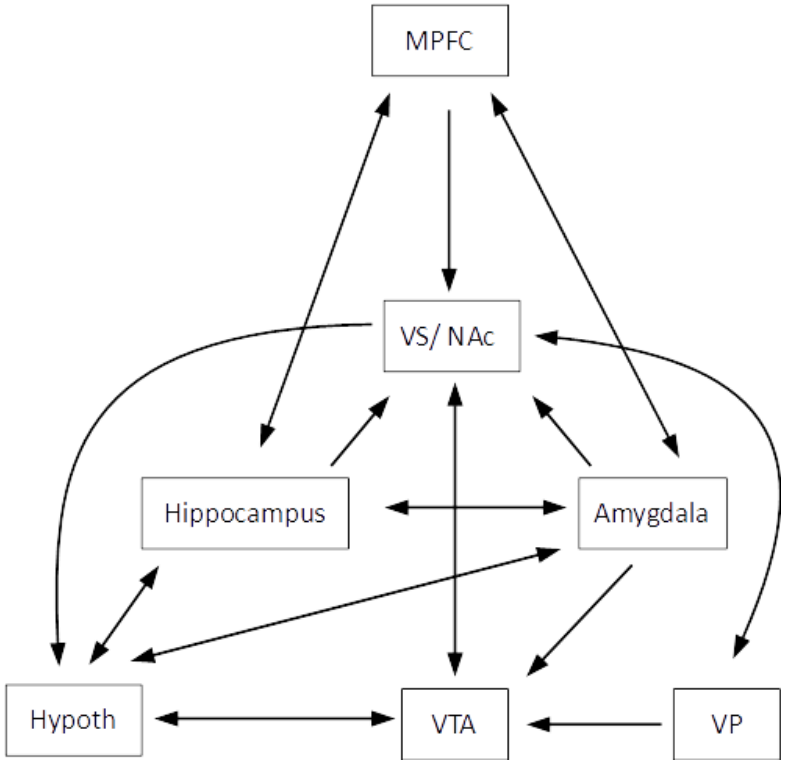

Figure 1: Brain regions and interconnections

A schematic illustration of the brain regions involved in the pathophysiology of schizophrenic and affective disorders and its interconnections. Presented information was extracted from literature (Haber and Knutson, 2009; Sesack and Grace, 2009; Ulrich-Lai and Herman, 2009; Yetnikoff et al., 2014). MPFC medial prefrontal cortex, Hypoth hypothalamus, NAc nucleus, accumbens VP ventral pallidum, VS ventral striatum, VTA ventral tegmental area.

1.3 General methodological bases for the experiments

1.3.1 Established DRD paradigm for the functional MRI investigation

Both studies of this thesis project are based on the previously introduced desire-reason dilemma (DRD) paradigm (Diekhof et al., 2012b). It is a delayed-matching-to-sample task with a desire context (DC) and a reason context (RC). During scanning two out of six target colors were presented at the beginning of each block. Subjects had to accept all probes that matched the target colors, and to reject all other probe colors. Successful performance of this task will be rewarded. A contingency between two additional color probes and an additional bonus will be established before the experiment. In the DC the acceptance of the so called bonus colors will yield in an additional reward; in the RC an acceptance of the bonus color will result in the loss of the reward for current experimental block. For details see methods section and figure 1 in the publication of study one. In a previous study it was shown how interaction between anteroventral prefrontal cortex (avPFC), NAc and VTA mediate human goal-directed behavior (Diekhof and Gruber, 2010).

1.3.2 Established scores for urbanicity rating

The effects of the place of birth on the risk of schizophrenia were reported by Mortensen et al. for a Danish population (Mortensen et al., 1999). To model the effects of the urban environment they classified subjects urbanicity according to the number of inhabitants in their residency (1: less than 10k, 2: between 10k and 100k, 3: more than 100k). This approach was adopted by Lederbogen et al. (Lederbogen et al., 2011) and extended by the differentiation between current urbanicity (CU), representing the urbanicity score for the place of current residency and early life urbanicity, representing an accumulated score for the first 15 years of life. The rating for study one followed this approach and the subjects CU were scored according to the number of inhabitants in their residency (1: less than 10k, 2: between 10k and 100k, 3: more than 100k). Also their early life scores were calculated for the first 15 years of life whereby every year of residence was multiplied with the value of the related residency.

1.3.3 Functional magnetic resonance imaging

The differences in regional brain activation elicited by the experimental paradigms relies on fMRI which enables in vivo observation of functional changes with a relatively high

spatiotemporal resolution (Logothetis, 2008). Differences in blood oxygenation elicited by neural activity are depicted by the blood-oxygen-level-dependent (BOLD) contrast (Ogawa et al., 1990). As described by Woolrich et al. inference of fMRI experiments involves several steps (Woolrich et al., 2009). A vector representing the temporal onset of the experimental conditions will be convolved with a canonical hemodynamic response function (hrf) in order to produce a predicted hemodynamic response for each condition. Then a general linear model (GLM) will be fitted to the data of each voxel separately for every single subject. Linear t-contrasts will be defined for assessing differential effects elicited by the experimental conditions and visualized in statistical maps. After that, group effects will be regressed with second-level random effects model using single subject's contrasts images (3D representation of contrast parameters estimates). Inference on second level statistics incorporates corrections for multiple testing, family wise error correction (FWE), for the whole brain or for single regions in the case that a priori hypotheses are available, small volume correction (svc) (Worsley et al., 1996).

1.3.4 Functional connectivity analysis

The functional connectivity between brain regions will be analyzed with psychological-physiological-interaction (PPI) analysis (Friston et al., 1997). The process-specific changes of functional interactions between brain regions will be regressed with a GLM including one regressor representing the BOLD signal time course in a given brain region and another regressor representing the experimental manipulation. Random effects analysis will be performed with second-level GLM using single subjects' contrast images. PPI analyses represent an accepted 'gold standard' for functional connectivity analysis that has been widely used after its first description.

1.4 Goals and hypotheses

1.4.1 Usage of an existing fMRI paradigm to examine the influence of the risk factor urbanicity

Living in an urban environment increases the risk to be affected by a psychiatric disorder (Dekker et al., 2008; Jacobi et al., 2014). It has been shown with fMRI that there is a link between the population density of residency and differences in individual's stress processing. Subjects living in a more urban environment had a higher amygdala activity and

subjects grown up in the city had more activity in the pgACC when they performed a stress task. Such effects of city living could not be observed when the subjects performed a working memory or an emotional face matching task (Lederbogen et al., 2011).

As described in the introduction there is evidence that chronic stress could also affect the mesolimbic DA system, and if reward and acute stress were combined changes in the reward circuit were observed (Kumar et al., 2014; Porcelli et al., 2012). Therefore, it could be assumed that urban living could influence the function of the reward circuit. Such an influence was not observed by Lederbogen et al. but this might be related to the applied stress paradigm which contains no rewarding component. Its stress induction based on i) arithmetical calculations, ii) individual increased task speed and iii) feedback in a way that the subject under examination had the impression to be a low performer (Dedovic et al., 2005).

To analyze urbanicity related influences on the mesolimbic DA system and in the limbic system a reward imaging paradigm seems to be the more promising approach. Therefore, the DRD paradigm should be applied to analyze the reward processes of subjects living in cities and in a rural environment. If there could be an influence of urbanicity detected with the application of the DRD paradigm it might be possible to identify subjects at risk in a prodromal phase which could improve the diagnosis and treatment of schizophrenic and affective disorders.

1.4.2 Further development of an existing fMRT paradigm to target the pathomechanisms of schizophrenia and affective disorders

As described above schizophrenic and affective disorders are associated with changes in brain regions involved in emotion, reward and stress processing and self-control. The basic understanding of function and interaction in these brain regions derived from research with rodents and non-human primates (Ghashghaei et al., 2007; Haber et al., 2006; Sesack and Grace, 2009) and fMRI studies extended the understanding about the involved neuro-mechanisms in humans which are summarized in the following.

The NAc (O'Doherty, 2004) and the VTA (Adcock et al., 2006) are activated by presentation of reward and particularly during decisions for immediate or high rewards. Regions of the DLFC modulate value information in the VMPFC when subjects exert behavioral self-control

(Hare et al., 2009). Also inhibitory influences of the avPFC on the mesolimbic dopamine system enable more flexible and self-controlled human decisions by decoupling behavior from more automatic impulsive desires that are mediated by the mesolimbic dopamine system (Diekhof et al., 2012b). Additionally, emotions like fear also affect human decisions via activation of the amygdala (Bechara et al., 2003; De Martino et al., 2006; Hartley and Phelps, 2012; Seymour and Dolan, 2008) and there is evidence that the NAc regulate goal-directed behavior by integrating the information from these regions (Haber and Knutson, 2009).

It is expected that neuroimaging studies might identify brain activation or connectivity patterns that could aid differential diagnosis of mood disorders or guide treatment selection (Frey et al., 2013). But it is argued that there are currently no brain imaging biomarkers that are clinically useful for establishing diagnosis or predicting treatment outcome in mood disorders (Savitz et al., 2013). With the application of the DRD paradigm it was possible to show how interaction between the avPFC, NAc and VTA mediate human goal-directed behavior(Diekhof and Gruber, 2010). As described above patients with schizophrenic and affective disorders exhibit different activation patterns in the DA system and in the amygdala as compared to healthy controls. An extension of the DRD paradigm by emotional fearful expression could provide an opportunity to contribute to the improvement of diagnosis and treatment selection because this targets the functional pathomechanisms of schizophrenia and affective disorders.

2 Summary of the original publications

Within the scope of this thesis the results of two research projects led to two publications which will be summarized in the following. The experiments were approved by University Medical Center Goettingen ethics committee and written informed consent was obtained before investigation from participants.

2.1 Effects of city living on the mesolimbic reward system – an fMRI study

The objective of this study was to investigate if living in big cities could affect the mesolimbic reward system and densely connected cortical and subcortical structures by application of the DRD paradigm. 147 Caucasian (91 females) right-handed healthy subjects provided current and early life urbanicity information, performed the DRD paradigm and were included in the study. A detailed description of current and early life urbanicity scores is available in the supplemental material of the publication, table S1. For the statistical analysis on single subject level the GLM comprised 3 regressors (i.e., target-stimuli, non-target-stimuli, conditioned reward stimuli), both for the DC and for the RC. Also, regressors for the cues and the feedback for either successful goal completion or overall goal failure were included in the model.

Urbanicity effects were assessed by a second level full-factorial analysis based on single subject contrast images for the conditions when reward stimuli were accepted in DC and rejected in RC resulting in the factors urbanicity (low vs. high) and task (DC vs RC). The statistical threshold for the factorial analysis (F-test) was set to $P<0.05$ family-wise error (FWE) corrected for the whole brain. Post-hoc t-tests were applied to determine statistical differences between the high and the low urbanicity groups. For brain regions with a priori hypotheses based on the literature, correction for multiple comparisons was performed using FWE correction for small volumes (Worsley et al., 1996). The coordinates for the amygdala were reported for an interaction of stress and glucocorticoid receptors and stress and amygdala activity (Geuze et al., 2012), the one for the pgACC was reported for the interaction of stress and reward (Treadway et al., 2013) and the one for the left ventral tegmental area (VTA) was taken from a recent meta-analysis (Diekhof et al., 2012a).

For the factorial analysis the subjects with score 1 and 2 were included in a low CU group (15), and with score 3 in a high CU group (132). The analysis revealed a main effect of urbanicity in the right amygdala, the left VTA, the right pgACC, left orbital frontal gyrus and the left medial orbital gyrus, and a main effect of task in the left VTA, hypothalamus, NAc, and pgACC. A full listing of main effects can be found in manuscript's supplementary tables S2 and S3. Subsequent post-hoc t-tests showed that subjects living in the city presented reduced activation of the left VTA (t_{290}=3.26, P_{FWE}<0.05 svc) during acceptance of additional reward in the DC. They also showed a reduced suppression of left VTA activity in the desire-reason dilemma situation (contrast DC-RC; t_{290}=1.83, P_{unc}=0,034), see also figure 2 of the manuscript. Overall subjects living in the city showed significantly reduced modulation of VTA activity in terms of both bottom-up activation elicited by conditioned reward stimuli and top-down suppression in the dilemma situation, see also figure 3 of the manuscript.

These findings are in good accordance with extensive literature about the influence of cortico-subcortical networks on the VTA. The VTA receives a multitude of afferents originating from mPFC, amygdala, hypothalamus and other brain regions involved in the regulation of the stress response (Ulrich-Lai and Herman, 2009; Yetnikoff et al., 2014). Further, it is known that VTA dopamine neurons respond to several forms of stress (Marinelli and McCutcheon, 2014). Finally, recent research with rodents found that chronic mild stress attenuates VTA dopamine activity via an amygdala-ventral pallidum pathway (Chang and Grace, 2014).

Furthermore, amygdala activation was higher in people living in cities in both experimental conditions (DC: left: t_{290}=2.76, P_{FWE}<0.05 svc; right t_{290}=3.68, P_{FWE}<0.05 svc; RC: right: t_{290}=2.65, P_{FWE}<0.05 svc), figure 4 of the manuscript. A similar effect was observed by Lederbogen et al. (Lederbogen et al., 2011) utilizing the Montreal Stress Imaging Task (MIST (Dedovic et al., 2005)). Such findings are supported by human and animal research. For rodents it was shown that chronic stress alters amygdala's neuronal properties (Liu et al., 2014; Rosenkranz et al., 2010) and its morphology (Joëls et al., 2007). Additionally a recent human study found an interaction between glucocorticoid receptor number and stress and amygdala activity (Geuze et al., 2012) exactly there where the DRD paradigm elucidated an current urbanicity related effect.

Influence of current urbanicity was also found in two regions of the vmPFC. This regions are proposed to integrate memory, social cognition, emotion, reward and other functions (Roy et al., 2012). Higher activation for city dwellers was found in the left medial orbital gyrus (DC: t_{290}=4.92, P_{FWE}<0.05, RC: t_{290}=3.41, P_{unc}<0.001) which has a role in reward evaluation (Diekhof et al., 2012a). Also they had higher activations in the right pgACC (DC: t_{290}=2.67, P_{FWE}<0.05 svc, RC: t_{290}=3.78, P_{FWE}<0.005 svc). This region contributes to the regulation of emotional conflict (Etkin et al., 2011) and is involved in the inhibition of HPA responses to psychogenic stressors (Ulrich-Lai and Herman, 2009).

In two regions involved in the regulation of the stress response (Ulrich-Lai and Herman, 2009) the subjects with high urbanicity score displayed functional alterations which are only uncorrected significant but are worth to mention. The left hippocampal activation was enhanced (DC: t_{290}=2.63, P_{unc}=0.005, [-22;-24;-12]) and in the hypothalamus (t_{290}=2.16, P_{unc}<0.05) the suppression in the desire-reason dilemma was decreased, figure 5 of the manuscript.

The GLM is relatively robust against unequal sample sizes but the size of both groups differed quite a lot. To assure that the different sample size has no limiting influence a rigid approach was chosen (Quinn and Keough, 2002) – analysis of a sample matched for size, sex, age and early life urbanicity score. The analysis confirmed the findings of the full sample with less power, but with still significant results, see tables S2 and S3 of the manuscript. This was also observed for the post-hoc t-test. The reduced modulation of VTA activity (manuscript figure 3) and the decreased suppression of hypothalamic activity (manuscript figure 5) showed the same extend for the matched sample.

So there is confidence to have revealed effects of current urbanicity on limbic function and the mesolimbic dopamine system with the additional benefit that the results were related to city living only.

There were no urbanicity effects in NAc observed and a search for behavioral effects found no significant differences in response time and error rates between the two groups.

The first study provided evidence for an influence of an environmental risk factor on cortico-subcortical networks involved in reward and emotion processing. Dysfunctions of these brain networks are involved in the development of schizophrenic and affective disorders.

2.2 Dynamic amygdala influences on fronto-striatal brain mechanisms involved in self-control of impulsive desires

The aim of the second study was to investigate dynamic functional interactions between amygdala, NAc and prefrontal cortex that underlie the influences of emotions, desires and rationality on human decisions because quite little is known about dynamic functional interactions between these brain circuits that underlie reward processing (Haber and Knutson, 2009; Schultz, 2002), self-controlled pursuit of long-term goals (Hare et al., 2009; McClure et al., 2004; Peters and Büchel, 2010) and emotions (Bechara, 2005; De Martino et al., 2006; Seymour and Dolan, 2008).

For this purpose, the DRD paradigm was extended by an affective component. The experimental stimuli consisted of 960 colored squares. One half of the stimuli showed a gray ellipse representing non-emotional stimuli and the other half showed an emotional face in its center. The images of 114 different identities (50% males and 50% females) from ADFES (van der Schalk et al., 2011), Ekman (Ekman and Friesen, 1976), KDEF (Goeleven et al., 2008; Lundqvist et al., 1998), NimStim (Tottenham et al., 2009) and RaFD (Langner et al., 2010) image data sets were presented in a random sequence. Supplementary figure S1 shows the detailed stimulus layout and figure 1 of the original paper (Krämer and Gruber, 2015) the affective DRD paradigm.

Seventeen Caucasian (8 females) right-handed healthy volunteers were included in the study and nine additional subjects were excluded due to head movements of more than 3mm. The repeated measures ANOVA of the reaction times included the three factors emotion (fearful face versus no face), reward (reward stimuli versus non-reward stimuli), and task context (DC versus RC, i.e. acceptance (approach) vs. rejection (avoidance) of reward stimuli). Post-hoc paired tests (two-tailed significance) were performed to assess the behavioral effects of the additionally presented fearful faces when i) accepting reward stimuli in the DC, ii) accepting non-reward stimuli in the DC, iii) rejecting reward stimuli in the RC, iv) rejecting non-reward stimuli in the RC, and also the differential effects of fearful faces when v) accepting reward vs. accepting non-reward stimuli in the DC, vi) rejecting reward vs. rejecting non-reward stimuli in the RC.

This analysis revealed main effects of reward ($F_{(1, 16)}$ =26.27, P<0.001) and task context ($F_{(1, 16)}$=15.37, P=0.001), an interaction effect of emotion x task ($F_{(1, 16)}$=15.04, P=0.001) and a triple interaction between emotion, reward and task context ($F_{(1, 16)}$=5.61, P<0.05). Fearful faces generally increased avoidance tendency to non-reward stimuli leading to significantly slower acceptance (t_{16}=3.08, P<0.01) and faster rejection of these stimuli (t_{16}=4.02, P=0.001). Presentation of a conditioned reward stimulus in the DC significantly counteracted these effects of fearful faces by facilitating approach behavior, i.e. accelerating acceptance of stimuli despite the presence of fearful faces (t_{16}= 2.5, P<0.05).

The Statistical analyses of the fMRI data used a general linear model (GLM) including the conditions according to the three factors emotion (fearful face versus no face), reward (reward stimuli versus non-reward stimuli) and task context (desire versus reason context). Statistically significant effects were determined using a primary search criterion of p<0.005 uncorrected. For a priori regions statistical inference was based on a significance threshold of p < 0.05, corrected for multiple comparisons using family-wise error (FWE) correction for small volumes based on a priori hypotheses (Worsley et al., 1996). The coordinates therefore derived from the literature: Amygdala (Fusar-Poli et al., 2009), avPFC and NAc (Diekhof and Gruber, 2010).

The modulation of reward-related activity by fearful faces was investigated by using interaction contrasts comparing both bottom-up activation and top-down modulation of the reward system during presentation of fearful faces with the corresponding contrasts without fearful faces. In the DC, when the conditioned reward stimulus was accepted a significant interaction of fear and reward was observed in the right VTA (t_{16}=3.05, P_{FWE} <0.05, svc) and the right NAc (t_{16}=3.13, P_{FWE} <0.05, svc) In the RC, top-down suppression of reward related VTA activation was reduced by presentation of fearful faces (left: t_{16}=3.54, P_{FWE} <0.05, svc; right: t_{16}= 2.35, ns.).

The behavioral results showed that reward counteracted the avoidance tendency of fearful faces in DC. In correspondence with this, the functional connectivity analyses of the fMRI data (PPI) revealed an increased positive coupling between the amygdala (which was activated by the fearful faces) and the NAc (which was activated by the reward stimulus) (t_{16}=4.91, P=0.005, svc.). This was not observed in the absence of reward.

Further PPI analyses revealed that the increased positive functional coupling between the amygdala and the NAc found in the DC turned into a decreased functional coupling in the RC (t_{16}=5.58, P=0.001, svc.), which coincides with the reduced approach facilitation by reward stimuli in this dilemma situation. In a similar way, the effects of fearful faces on the functional couplings of the avPFC with both the amygdala (t_{16}=3.27, P<0.05, svc.) and the NAc (t_{16}=4.02, P<0.05, svc.) were reversed in the RC. Results section of the original paper and its figure 3 provides a more detailed description (Krämer and Gruber, 2015). Also, results showing the replication of previous DRD experiments (Diekhof et al., 2012b) and replication of amygdala activation by fearful faces (Fusar-Poli et al., 2009) are available there.

Up to now this seems to be the first *in vivo* neuroimaging study showing that emotional signals from the amygdala and goal-oriented information from prefrontal cortices interact in the nucleus accumbens to guide human decisions and reward-directed actions. Avoidance tendency increased by presentation of fearful faces was counteracted by simultaneous presentation of conditioned reward stimuli. This was accompanied by an increased functional connectivity between amygdala and nucleus accumbens and associated with increased activation of the NAc. The amygdala-accumbens coupling was not increased when a fearful face was presented alone suggesting that additional reward-related dopaminergic input from the VTA to the NAc was necessary to elicit this effect. This notion is consistent with recent findings from animal studies showing that dopamine supplied by the VTA may enhance limbic influences on decision-making (Grace et al., 2007) by modulating NAc responses to amygdala inputs (Faure et al., 2008; Johnson et al., 1994) and is in line with optogenetic stimulation of glutamatergic, i.e. excitatory connections from the amygdala to the NAc in the rodent (Stuber et al., 2011). This finding is also consistent with the results from another recent study showing that task independent presentation of fearful faces accelerated probabilistic reward learning (Watanabe et al., 2013).

Overall, these findings are consistent with substantial animal-experimental evidence demonstrating that emotional signals from the amygdala and goal-oriented information from prefrontal cortices directly interface in and are integrated by the NAc (Sesack and Grace, 2009).

In correspondence with the literature (Fusar-Poli et al., 2009) the neutral faces in the experiment activated the amygdala (left: t_{16}=6.39, P_{FWE}<0.001, svc; right t_{16}=5.82, P_{FWE}<0.001, svc) and when conditions with fearful faces were directly compared to conditions with neutral faces, no significant activation could be observed in the amygdala. The literature provides different kinds of speculations about possible reasons for these activations of emotion-processing areas by “neutral” faces. For instance, it has been posed into question whether faces can be emotionally “neutral” at all (e.g. (Carvajal et al., 2013; Lee et al., 2008)). On the other hand, facial expressions represent a very important source of social information, amygdala activation by so-called “neutral” faces may be attributed to very fast and raw evaluation processes that act upon emotionally salient cues such as human faces (e.g. (LeDoux, 1995; Todorov and Engell, 2008; Vuilleumier, 2005)). Therefore, the no-face condition was chosen as the more appropriate control condition.

This is some kind of limitation because the statistical comparisons between fearful faces and no-face conditions are unable to disentangle the processing of faces and the processing of emotions. It could not be determined whether the dynamic functional interactions of the amygdala with nucleus accumbens and prefrontal cortex are related to emotional processes or, more generally, to the processing of human faces independent of their emotional expressions. However, in this context it is very important to note that the broader scientific literature clearly documents that an attempt to disentangle face and emotional processing (e.g. in the amygdala) has no reasonable chance because even so-called “neutral” faces elicit activation in emotion-processing brain areas particularly in the amygdala (e.g.(Derntl et al., 2009; Fusar-Poli et al., 2009; Said et al., 2011)).

In summary the second work provided an experimental paradigm that for the first time enabled the in vivo investigation of the interaction between amygdala, Nac und avPFC when emotional-, reward related information and self-controlled pursuit of long-term goals guide human decisions and reward-directed actions.

3 Discussion

Aim of this thesis was to investigate the patho-mechanisms involved in schizophrenic and affective disorders, because improving their diagnosis and therapy requires a better understanding of the functional changes in affected fronto-limbic and fronto-striatal brain regions. Commonly observed features of these disorders are a disturbed reward and emotion processing and constraints in self-control.

The influence of a risk factor (urbanicity) contributing to the development of mental disorders on amygdala and mesolimbic dopamine system was illustrated in the first study. In comparison to the subjects from less urban areas city dwellers showed an altered activation and modulation capability of the midbrain (VTA) dopamine system. Additionally, they displayed altered responses in other brain regions involved in reward processing, and in the regulation of stress and emotions such as amygdala, hypothalamus, orbitofrontal and pregenual anterior cingulate cortex.

It is the first time that an effect of city living on the VTA, the OFC and pgACC is shown in humans. As discussed in detail in the manuscript of study one, the findings are supported by animal research which show that several forms of stress could alter VTA activity (Marinelli and McCutcheon, 2014; Ulrich-Lai and Herman, 2009; Yetnikoff et al., 2014). The finding of study one that subjects with high urbanicity score had higher pgACC activation is corroborated by another recent human fMRI study which found an interaction of stress and reward in the pgACC (Treadway et al., 2013). This region is involved in the integration of reward and emotion (Roy et al., 2012) and depressed patients show an altered activity in this region during face processing (Price and Drevets, 2012).

That subjects living in the city had an altered amygdala activation was previously observed when they performed a stress paradigm (Lederbogen et al., 2011). The application of the DRD paradigm revealed a similar effect which is very plausible because the amygdala is also involved in reward processing (Murray, 2007).

The urbanicity related changes observed in the amygdala are located in an area where an interaction with stress and an influence of stress on glucocorticoid receptor number was

found previously (Geuze et al., 2012). Because the manifestation of MDD is associated with stressful experience dysfunction of the HPA axis is seen as one of the contributing key elements. The glucocorticoid receptor (GCR) is an important regulator of the HPA axis negative feedback and there is preliminary evidence for a role of its genetic variation in the genetic vulnerability of MDD (Claes, 2009). Also a role of the GCR in psychosis of depression is reported (Schatzberg et al., 2014) but overall the latest mega analysis still failed to identify a significant association between any genetic variation and MDD (Ripke et al., 2013b).

It is plausible that the findings of the first experiment could be interpreted as support for the position that urbanicity is a risk factor contributing to development of mental disorders. It shows that cortico-subcortical networks affected by related mental disorders display functional changes which are in line with current models of depression and schizophrenia. But worldwide epidemiological research results regarding urbanicity effects are inconsistent. Several reports, especially from Europe, document a relationship between risk for mental illness and city living (Dekker et al., 2008; Jacobi et al., 2014; Peen et al., 2010; Vassos et al., 2012) but latest US surveys do not find this relationship (Breslau et al., 2014; McCall-Hosenfeld et al., 2014). This is an issue which could not be easily resolved due to methodical differences of European and US surveys. Therefore, future functional investigations in this area demand for control of possible confounds.

The importance of the DRD paradigm, which allows the detailed investigation of dopaminergic reward circuit functioning, is indicated by two recent reviews that demonstrate the central role of dopamine in the pathogenesis and pathophysiology of schizophrenia (Howes and Murray, 2014) and depression (Pizzagalli, 2014). The first, an integrated social developmental model of schizophrenia proposes that genetic liability, developmental factors and subsequent stressors on the dopamine system determine the trajectory towards psychosis (Howes and Murray, 2014). In their detailed review they describe how gene variation, neurodevelopmental hazards like obstetric complications, the influence of childhood adversity and social stress on the stress response disrupt the development of and sensitizes the dopamine system. Then social adversity and subsequent stress lead to dysregulated dopamine system. Finally, they state that in the view of dopamine's role in reward learning its dysfunction could account for the negative symptoms.

The other one reviewed the roles of anhedonia, dopamine and stress in depression and suggested that the effects of acute and chronic stress lead to a dysfunction in the mesolimbic dopaminergic pathways and might subserve disrupted reinforcement learning and lack of reactivity to pleasurable stimuli seen in depression. Finally, it was stated that several questions regarding the pathophysiology of depression are unanswered and require further attention. Beneath the focus on the DA pathways several other key regions implicated in the regulation of emotion and stress responses had been associated with depression, including the PFC, amygdala and hippocampus (Pizzagalli, 2014).

The new findings of the second study are i) the increased functional connectivity between amygdala and nucleus accumbens that facilitated the approach of immediate reward when emotional information was present and ii) the increased functional interactions of the anteroventral prefrontal cortex with amygdala and nucleus accumbens that were associated with rational decisions in dilemma situations. This was achieved by an extension of the DRD paradigm with fearful emotional faces. Also the effects of previous experiments with the DRD paradigm were replicated(Diekhof et al., 2012a; Diekhof and Gruber, 2010).

Therefore, the new affective DRD paradigm will provide an improved opportunity to investigate the pathomechanisms of schizophrenic and affective disorders because it activates the involved brain regions and elucidate the functional connectivity between them. Patients with major depressive disorder show a reduced responsivity to gains in caudate, nucleus accumbens and anterior cingulate cortex (Pizzagalli et al., 2009). The activity in ventral striatum during reward processing is abnormally elevated and the prefrontal control of the VS is disturbed in bipolar disorder (Caseras et al., 2013). Additionally, in response to fearful faces activity in the amygdala is increased (Kim et al., 2012). In schizophrenia the ventral striatal responses to expected reward was exaggerated and blunted in response to unexpected rewards (Morris et al., 2012). Patients with positive symptoms show also a reduced activity in amygdala and hippocampus when processing fearful faces (Goghari et al., 2010). During affect processing the connectivity of the PFC and the amygdala seems to be abnormally reduced (Frangou, 2014).

It was beyond the scope of this thesis to evaluate the paradigm in clinical trials with patients affected by relevant disorders. It could be expected that application of the affective DRD

paradigm will generate new insights in differences between patients and healthy controls in terms of brain activation and of functional connectivity, because the interaction between limbic and mesolimbic dopamine system during decision making could be explored. It is proposed that dysfunctional connectivity in this and other networks is a core symptom of described disorders (Gong and He, 2015; Pettersson-Yeo et al., 2011).

With the studies it was shown that the activity in the amygdala, which is hypothesized as an integration region of cognition and emotion (Pessoa, 2008), is influenced by a risk factor contributing to development of mental disorders and that the amygdala interacts with cortical regions and the mesolimbic dopamine system to coordinate goal directed behavior. Additionally, the amygdala is involved in the regulation of the stress response. Current findings provide only little additional evidence how reward, emotion and stress processing interact but it could be assumed that future application of the affective DRD paradigm and inclusion of information about genetic variation (genetic imaging) will provide additional insight in the underlying neuro-mechanisms of schizophrenic and affective disorders.

In the future, studies like the ones performed within this thesis project will also become relevant for the development of biomarkers which could improve the diagnosis of mental disorders. Research in this area is compared with the search for a needle in the haystack (Atluri et al., 2013). There are several biomarker candidates for psychiatric disorders which require replication in real life cohorts because when replicated at all, they have been replicated in small cohorts in identical research environments. Due to the complex etiology of mental disorders it might be more promising to apply multi-modal approaches including biochemical, cognitive, electrophysiological, genetic and neuroimaging tests (Scarr et al., 2015). Within this, both DRD paradigms provide a cognitive and neuroimaging test which suitability as a one of many multimodal tests have to be investigated in further longitudinal studies. A recent application of the DRD paradigm showed that disturbed top-down control of the mesolimbic-reward signals might be a trait marker of bipolar disorder (Trost et al., 2014) but only in small cohort in a research environment.

4 Original articles and manuscripts

4.1 Effects of city living on the mesolimbic reward system – an fMRI study

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Keywords

fmri; neuroimaging; stress; dysregulation;

Abstract

Based on higher prevalence rates of several mental disorders for city dwellers, social stress effects of urban living have been proposed as an environmental risk factor contributing to the development of mental disorders. Recently, it was shown that amygdala activation in response to a cognitive-social stressor differs between city dwellers and rural residents. It is known that chronic social stress also affects brain regions involved in reward processing. Further, stress-related dysregulation of the mesocorticolimbic dopamine system is thought to contribute to onset and manifestation of psychiatric disorders. Therefore, we compared the functional magnetic resonance imaging data of 147 healthy subjects living either in cities or in less urban areas acquired during performance of the desire-reason-dilemma (DRD) paradigm, which permits an investigation of the reward circuit.

Compared with subjects from less urban areas, city dwellers showed an altered activation and modulation capability of the midbrain (VTA) dopamine system. City dwellers also revealed exaggerated responses in other brain regions involved in reward processing, and in the regulation of stress and emotions such as amygdala, hypothalamus, orbitofrontal and pregenual anterior cingulate cortex.

These results provide further evidence for the influence of human habitat-related social stress on cortico-subcortical networks involved in reward and emotion processing. As dysregulation of these brain networks represents a core process in the pathophysiology of several psychiatric disorders, the observed differences between subjects living in cities and in less urban areas further contribute to our understanding of the pathomechanisms by which environmental risk factors may alter healthy brain function.

Highlights

- City living affects human brain function in cortical and subcortical regions involved in stress and reward processing
    - Altered regulation of midbrain dopamine system
    - Increased neural responses in the limbic system
- Urban environment may increase the risk to develop mental disorders via dysregulatory effects on the mesolimbic dopamine system and the limbic system

Introduction

Mental and behavioral disorders affect approximately 20 - 25% of all people at some time during their lives (World Health Organization et al., 2001). Urbanization and accompanying effects of social stress are seen as one of the risk factor contributing to the development of mental illness. This is corroborated by recent meta-analyses which revealed a higher risk for schizophrenia (Vassos et al., 2012) and higher prevalence rates for mood and anxiety disorders (Peen et al., 2010) for people living in cities. Recently it has been shown with functional magnetic resonance imaging (fMRI) that there is a link between the population density of residency and differences in individual's stress processing. Subjects living in a more urban environment had a higher amygdala activity and subjects grown up in the city had more activity in the pregenual anterior cingulate cortex (pgACC) when they performed a stress task. Such effects of city living could not be observed when the subjects performed a working memory or an emotional face matching task (Lederbogen et al., 2011). In summary this study provided evidence for a link between a social risk factor, urbanicity, and social stress processing.

How stress could contribute to the development of schizophrenia is illustrated by a current review. On the background of an increased genetic vulnerability, neuro- and socio-developmental hazards the influence of subsequent stress may lead to a dysregulation of the dopamine system and to the development of psychosis (Howes and Murray, 2014). Regarding depression another review suggests that stress induces anhedonic behavior by causing dysfunction of the mesolimbic dopamine pathways (Pizzagalli, 2014).

The limbic regions regulating the autonomic nervous system (ANS) and hypothalamic-pituitary-adrenocortical (HPA) axis responses to stress, i.e. the amygdala, the hippocampus and the medial prefrontal cortex (mPFC), are (at least partially) also involved in emotion, memory and reward processing (Ulrich-Lai and Herman, 2009).

From these findings, one may hypothesize that a higher degree of urban living as a proxy for chronic social stress could act via functional changes in the limbic system, especially MPFC, hippocampus and amygdala on the mesolimbic dopamine system contributing to its dysregulation. The recently established Desire-Reason-Dilemma (DRD) paradigm reliably

activates the reward circuit and interacting prefrontal areas (Diekhof et al., 2012b; Diekhof and Gruber, 2010). Therefore, its application might elucidate urbanicity related functional changes in these brain regions. To test this hypothesis, we used residency information and imaging results from 147 subjects performing a Desire-Reason-Dilemma (DRD) Paradigm.

Methods

Subjects

147 Caucasian right-handed healthy volunteers (91 females), age 24 (-5, +7), without history of psychiatric or neurological disorder, provided current and early life urbanicity information and were included in the study. Approval from Göttingen University Medical Center ethics committee and written informed consent were obtained before investigation.

Urbanicity scores

Subjects current urbanicity (CU) were scored according to the number of inhabitants in their residency (1: less than 10k, 2: between 10k and 100k, 3: more than 100k). Also their early life scores were calculated for the first 15 years of life whereby every year of residence was multiplied with the value of the related residency, see table S1. For the factorial analysis the subjects with score 1 and 2 were included in a low CU group (15), and with score 3 in a high CU group (132). Due to the imbalance between low and high CU score subjects an additional balanced sample with equal group size and subjects matched for sex, age and early life urbanicity score were defined, 15 with low, 15 high CU score.

Task

For the experiment we used the previously introduced desire-reason dilemma (DRD) paradigm (Diekhof et al., 2012b). Experimental stimuli consisted of colored squares. Before scanning a contingency between two colors (green and red) and a reward (10 bonus points) was established. During scanning, participants performed a delayed-matching-to-sample task whereby two out of six target colors were presented at the beginning of each block. Subjects had to accept all probes that matched the target colors, and to reject all other probe colors. Successful performance of this task was the superordinate task goal and yielded in 50 points per block. Two different block types were performed. In the Desire Context (DC), indicated by a capital “B” (for ”Bonus”) presented before the target set (Figure 1, top row), the participants were allowed to also accept ”bonus” reward colors (10 points) that were instrumentally conditioned before the experiment. By contrast, in the Reason

Context (RC), indicated by a capital ”Z” (for ”Ziel” = “target” in German) presented before the target set (Figure 1, bottom row), the conditioned reward stimuli had to be rejected as well if they did not match one of the target colors, thereby creating the desire-reason dilemma (leading to top-down suppression of reward signals elicited by the conditioned stimuli (Diekhof et al., 2012b; Diekhof and Gruber, 2010)). Two blocks of one type were followed by two blocks of the other type, whereby one type of block had the length of 4 and the other one the length of 8 (i.e. probe stimuli). The sequence between blocks with 8 and with 4 trials varied pseudo randomly. Erroneous acceptance of conditioned reward stimuli in RC led to loss of the 50 points in the corresponding block. Subjects received monetary compensation for the participation and depending on performance they could double the amount received. Each trial lasted 1900 ms and the interval between the stimuli was 300ms. If the subject did not respond within 900 ms trial timed out and the block aborted. Detailed trial timing is shown in figure 1.

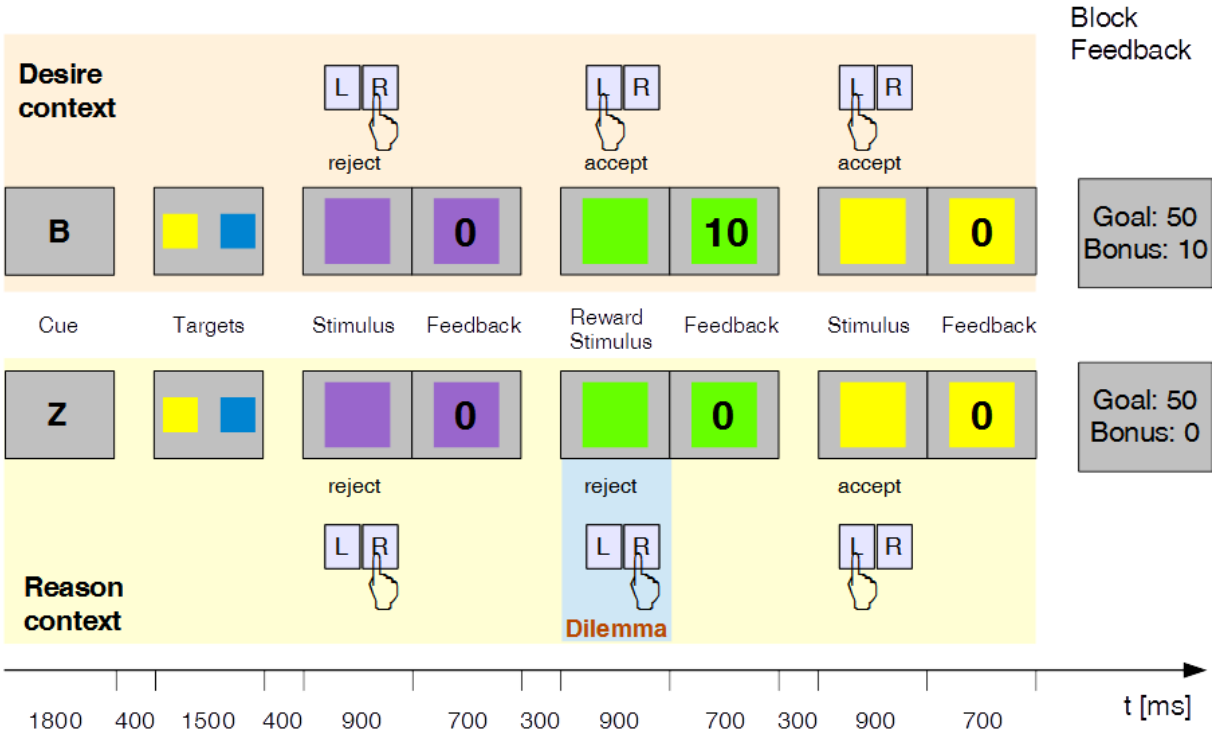

Figure 1. The desire-reason dilemma paradigm. Participants performed a delayed-matching-to-sample task with two target colors presented at the beginning of each block. Successful performance of this task was the superordinate goal and yielded 50 points per block. In the Desire Context (DC; top row) the participants were allowed to also accept reward colors (e.g. green) in order to gain additional 10 bonus points. These colors were instrumentally conditioned before the experiment. By contrast, in the Reason Context (RC; bottom row) these stimuli had to be rejected if they did not

match one of the target colors. Erroneous acceptance of conditioned reward stimuli in a RC block led to loss of the 50 points.

fMRI data acquisition

The experiment was performed on a 3T MRI scanner (Siemens TRIO) equipped with an 8-channel head coil. Head motion was restricted by small cushions. A high-resolution T1-weighted anatomical scan (3D-MPRAGE, voxel size 1x1x1 mm^3) was obtained for each subject. Functional images were acquired using a T2*-sensitive echo planar imaging (EPI) sequence (voxel size, 3x3x3mm^3; gap, 20%; interscan interval, 1.9 s; echo time, 30 ms; flip angle, 70°; field of view, 192 mm) parallel to the anterior commissure–posterior commissure plane in ascending direction. During two sessions a total of 370 image volumes were acquired.

fMRI Analysis

Functional imaging data preprocessing and analysis was performed with Statistical Parametric Mapping SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). The realigned and unwarped functional images were slice time corrected, normalized to MNI space and saved with a spatial resolution of 2x2x2mm^3. Smoothing utilized a 6-mm full-width at half maximum (FWHM) isotropic Gaussian kernel. The high-resolution anatomical image was segmented and co-registered with the mean EPI image.

Statistical analyses used a general linear model (GLM), which comprised 3 regressors (i.e., target-stimuli, non-target-stimuli, conditioned reward stimuli), both for the DC and for the RC. The block cues, the target cues and the block feedback for either successful goal completion or overall goal failure were also modeled as independent regressors, which resulted in a total of 11 onset regressors. Incorrect trials and trials in which the conditioned reward stimulus was not collected in the DC were excluded from the analyses. A vector representing the temporal onset of stimulus presentation was convolved with a canonical hemodynamic response function (hrf) in order to produce a predicted hemodynamic response to each experimental condition. Linear t-contrasts were defined for assessing differential effects elicited by the experimental conditions. All statistical analyses of the single subject data included a high-pass filter with 128s cut off and an autoregressive AR(1) model to account for serial correlations in fMRI time series.

The effects of urban living were assessed by a second level 2x2 full-factorial analysis. The model included the first level contrast images of the subjects with high and low urbanicity score (factor Urbanicity) for the conditions when reward stimuli were accepted in DC and rejected in RC (factor Task). Because the size of both groups differed we performed an additional full-factorial analysis with a sample matched for sex, age and early life urbanicity score. The statistical threshold for the factorial analysis (F-test) was set to $P<0.05$ FWE corrected for the whole brain. Post-hoc t-tests were applied to determine statistical differences between the high and the low urbanicity groups.

For brain-regional a priori hypotheses based on the literature, correction for multiple comparisons was performed using family-wise error (FWE) correction for small volumes with 4 mm spheres (Worsley et al., 1996). As stated in the introduction we expected that urbanicity as a proxy of chronic mild stress affects the function of the limbic system and the mesolimbic dopamine system. Therefore, we applied for the small volume correction MNI coordinates which had been shown to be related with stress and reward processing. For the amygdala we used left [-20, -2, -16]; right [20, -2, -20]. Both coordinates were reported for an interaction of stress and glucocorticoid receptors and stress and amygdala activity (Geuze et al., 2012). The coordinate for the pgACC [0, 50, 4] was reported for the interaction of stress and reward(Treadway et al., 2013) and the one for the left ventral tegmental area (VTA) [-4, -16, -14] we took from a recent meta-analysis (Diekhof et al., 2012a). The beta values for the experimental conditions were extracted from 2 mm boxes using marsbar (Brett et al., 2002).

Results

Behavioral results

Behavioral effects were assessed with a repeated measures ANOVA. There were no significant differences in response time and error rates between the two groups.

fMRI results

The factorial analysis for the full sample of 147 subjects revealed a main effect of urbanicity in the right amygdala, the left VTA, the right pgACC, left orbital frontal gyrus and the left

medial orbital gyrus, and a main effect of task in the left VTA, hypothalamus, nucleus accumbens, and pgACC. Urbanicity x Task interaction effects were found in right amygdala ($Z_{1,290}$=2.0, P_{unc}<0.05), left VTA ($Z_{1,290}$=1.5, P_{unc}<0.1), right pgACC ($Z_{1,290}$=1.34, P_{unc}<0.1) and hypothalamus ($Z_{1,290}$=1.86, P_{unc}<0.05). A full listing of main effects can be found in supplementary tables S2 and S3. Subsequent Post-hoc t-tests showed that subjects living in the city presented reduced activation of the left VTA (t_{290}=3.26, P_{FWE}<0.05 svc) during acceptance of additional reward in the DC, figure 2A. They also showed a reduced suppression of left VTA activity in the desire-reason dilemma situation (contrast DC-RC; t_{290}=1.83, P_{unc}=0,034), figure 2B. Thus, overall subjects living in the city showed significantly reduced modulation of VTA activity in terms of both bottom-up activation elicited by conditioned reward stimuli and top-down suppression in the dilemma situation (figure 3).

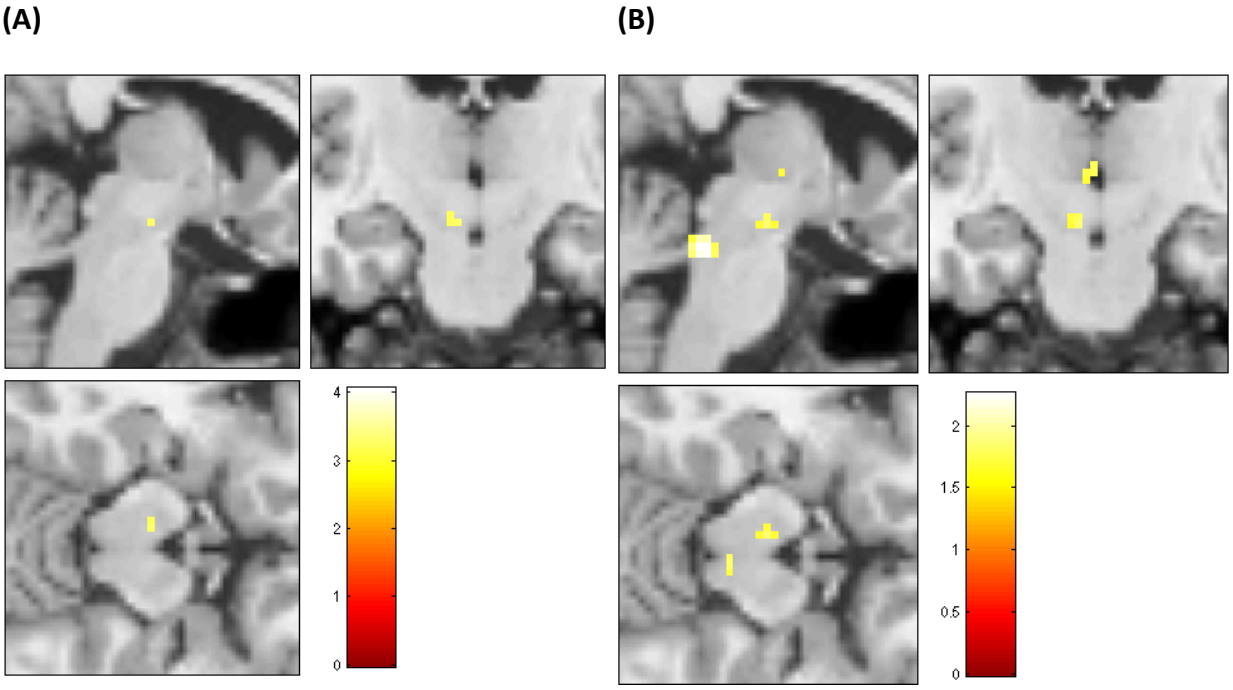

Figure 2. Relationship between city living and modulation of VTA activity. A) Reduced bottom-up activation of left VTA by conditioned reward stimuli in high versus low urbanicity subjects [-4;-16;-14], t_{290}=3.26 P_{FWE}=0.009 svc in DC; image shown at P<0.001. **B)** Reduced top-down modulation of left VTA activity in high versus low urbanicity subjects in the desire-reason dilemma. [-4;-16;-14], t_{290}=1.83 P_{unc}=0.034; image shown at P<0.05.

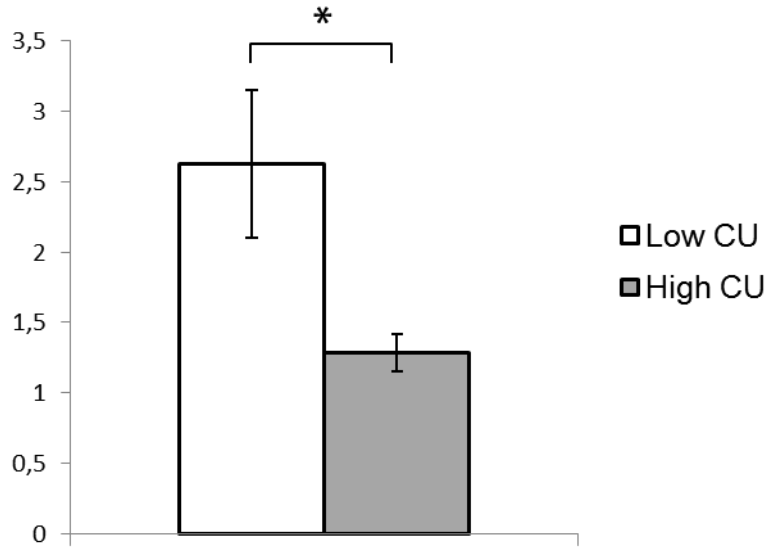

Figure 3. Significantly reduced modulation capability of left VTA in subjects with high current urbanicity (CU) scores. Bars show the mean effects of bottom-up activation by conditioned reward stimuli in the DC and top-down suppression of these reward signals in the desire-reason dilemma, * P < 0.005, error bars denote standard error.

Furthermore, in the subjects with high CU scores we also observed increased activations in brain regions with dense connections to the human reward system such as amygdala, hippocampus, hypothalamus, orbitofrontal and pregenual anterior cingulate cortex. Amygdala activation was higher in people living in cities in both experimental conditions (DC: left: t_{290}=2.76, P_{FWE}<0.05 svc; right t_{290}=3.68, P_{FWE}<0.05 svc; RC: right: t_{290}=2.65, P_{FWE}<0.05 svc), figure 4, reflecting the significant main effect reported above. Additionally, we found higher activations of the left medial orbital gyrus (DC: t_{290}=4.92, P_{FWE}<0.05, RC: t_{290}=3.41, P_{unc}<0.001) and the right pgACC (DC: t_{290}=2.67, P_{FWE}<0.05 svc, RC: t_{290}=3.78, P_{FWE}<0.005 svc). In addition, left hippocampal activation was also enhanced although only at an uncorrected significance level (DC: t_{290}=2.63, P_{unc}=0.005, [-22;-24;-12]). Finally, in the hypothalamus the subjects with high urbanicity scores showed decreased suppression in the desire-reason dilemma (t_{290}=2.16, P_{unc}<0.05), figure 5.

Subsequent factorial analysis with the matched sample confirmed the findings of the full sample analysis. The main effects of urbanicity and task were smaller, less regions survived the P<0.05 correction for the whole brain but the activations in regions of interest were significant when corrected for small volume, see tables S2 and S3. This was also observed for

the post-hoc t-test. The reduced modulation of VTA activity (figure 3) and the decreased suppression of hypothalamic activity (figure 5) showed the same extend for the matched sample. Significant differences in reward-related brain activations between subjects with high and low urbanicity score for the full and the matched sample are depicted in table 1.

We also looked for urbanicity effects in nucleus accumbens (NAc) where we only observed a significant general task-related effect (left: t_{290}=9.52, P_{FWE}<0.05, [-10, 10, 0]; right: t_{290}=8.79, P_{FWE}<0.05, [12, 14, 0]), but there was no difference between the two groups (high vs. low CU) in that region at P<0.05 unc.

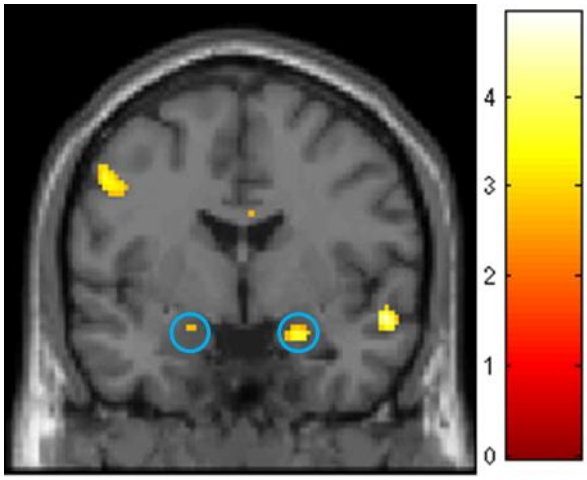

Figure 4. Effects of city living on amygdala activity in the desire context (DC). T-map shows higher amygdala activation, blue circles, (left [-20;-2;-16] t_{290}=2.76, P_{FWE}=0.032 svc; right [22;-2;-18] t_{290}=3.64, P_{FWE} =0.002) for subjects with high versus low urbanicity score. Crosshair at 0 -2 -18; image shown at P<0.005.

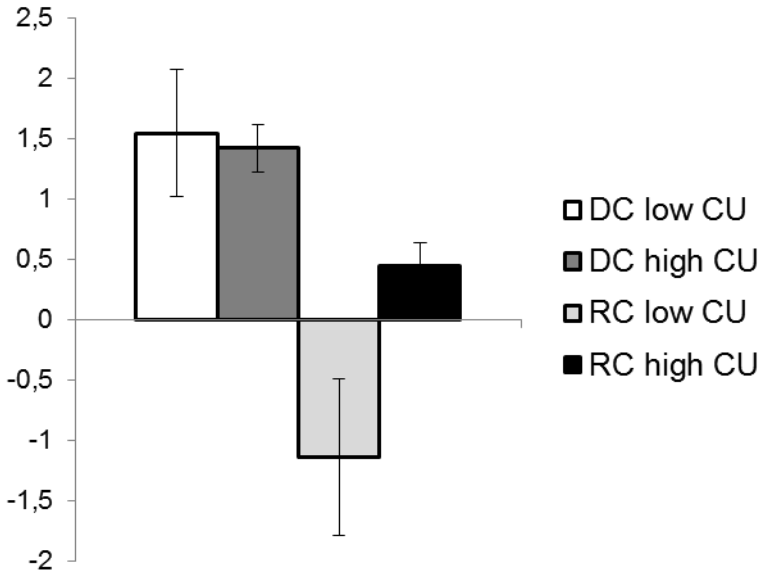

Figure 5. Reduced suppression of hypothalamic activity in city dwellers in the desire-reason dilemma situation. While both rural residents and city dwellers exhibit similar hypothalamic activation in the desire context (DC; white and dark gray bar on the left), suppression of this activation in the reason context (RC) is significantly reduced in the city dwellers (black bar) in comparison with subjects living in more rural environments (light gray bar). Bars depict contrast estimates for hypothalamus at MNI [0;-12; 0], error bars denote standard error.

Table 1 Significant differences in reward-related brain activations between subjects with high (H) and low (L) urbanicity scores listed for the full and the matched sample. DC, desire context; RC, reason context.

	DC H>L				RC H>L			
Sample	**Full**		**Matched**		**Full**		**Matched**	
Region	**MNI**	**t-val.**	**MNI**	**t-val.**	**MNI**	**t-val.**	**MNI**	**t-val.**
L Amygdala	-20;-2;-16	2.76*						
R Amygdala	22;-2;-18	3.68*	20;-2;-18	3.19*	22;-2;-18	2.65*	22;-4;-18	
R Pregenual Cingulate Gyrus	2;52;4	2.67*			2;48;6	3.78*	2;50;6	3.44*
L Medial Orbital Gyrus	-12;52;-18	4.92	-10;52;-18	4.92				

Statistical effects presented at P<0.05, FWE corrected for whole brain; * FWE corrected for small volume P<0.05

Discussion

The objective of this study was to investigate if living in big cities is associated with variation of brain function in the mesolimbic reward system and densely connected cortical and subcortical structures. The DRD paradigm has been established as an instrument to assess the prefrontal modulation of the reward system when humans have to choose between immediate and long term rewards (Diekhof et al., 2012b; Diekhof and Gruber, 2010). When applied to elucidate effects of city living we found that subjects living in cities showed a reduced activation and modulation capability of the midbrain (VTA) dopamine system, and that activation in brain regions involved in stress and reward processing such as amygdala, hypothalamus, orbitofrontal and pregenual anterior cingulate cortex, were increased in these subjects.

These findings are in good accordance with extensive literature about the influence of cortico-subcortical networks on the VTA. The VTA receives a multitude of afferents originating from mPFC, amygdala, hypothalamus and other brain regions involved in the regulation of the stress response (Ulrich-Lai and Herman, 2009; Yetnikoff et al., 2014). Further, it is known that VTA dopamine neurons respond to several forms of stress (Marinelli and McCutcheon, 2014). Finally, recent research with rodents found that chronic mild stress attenuates VTA dopamine activity via an amygdala-ventral pallidum pathway (Chang and Grace, 2014).

The application of the DRD paradigm revealed higher amygdala activation for city dwellers. A similar effect was previously reported by Lederbogen et al. (Lederbogen et al., 2011) using a task which induces social stress during arithmetical calculations, the Montreal Stress Imaging Task (MIST (Dedovic et al., 2005)). Such findings are supported by human and animal research. Experiments with rodents demonstrated that chronic stress alters amygdala's neuronal properties (Liu et al., 2014; Rosenkranz et al., 2010) and its morphology (Joëls et al., 2007). A recent human study found an interaction between glucocorticoid receptor number and stress and amygdala activity (Geuze et al., 2012) exactly there where the DRD paradigm elucidated an current urbanicity related effect.

Also the activity in two regions of the ventral-medial prefrontal cortex (vmPFC) was influenced by city living. Based on empirical evidence a recent review (Roy et al., 2012)

concluded that the vmPFC is involved in the integration of memory, social cognition, emotion, reward and other functions. One of its regions showing a very strong difference between city dwellers and town/rural residents is the medial-orbital prefrontal cortex (mOFC) which has a role in reward evaluation (Diekhof et al., 2012a). The other one, the pgACC contributes to the regulation of emotional conflict (Etkin et al., 2011) and is involved in the inhibition of HPA responses to psychogenic stressors (Ulrich-Lai and Herman, 2009).

Just like for the above mentioned limbic forebrain regions we also observed an urbanicity effect for the hypothalamus, one of the main regions regulating the stress response (Ulrich-Lai and Herman, 2009). Although this effect only reached an uncorrected significance level, the finding still suggests that city living may also affect brain regions involved in the regulation of the stress response.

The GLM is relatively robust against unequal sample sizes but the size of the high and low urbanicity group differed quite a lot. To assure that the different sample size did not confounded the results a rigid approach was chosen (Quinn and Keough, 2002) and a sample matched for size, sex, age and early life urbanicity score analyzed. This analysis confirmed the findings of the full sample with reduced statistical power, but with still significant results. The present study is limited by the fact that the sample was drawn from a population of university students mainly living in the city. Further work is required with samples which provide an improved fit with the population spectrum, i) a better match for age and education ii) additional subjects from rural areas and iii) subjects from bigger cities >500k.

All in all using an established paradigm for the investigation of the reward circuit, we observed urbanicity-related differences in brain function not only within the reward circuit itself (Haber and Knutson, 2009), but also in closely connected brain circuits involved in emotion (Etkin et al., 2011) and stress regulation.

Living in cities has been shown to be a risk factor contributing to the development of psychiatric diseases (Peen et al., 2010; Vassos et al., 2012). With the current research we show an influence of city living on the mesolimbic dopamine system. Several reviews have shown how alterations in the dopamine system may contribute to development of mental disorders (e.g. Heinz et al., 1994; Howes and Kapur, 2009). In particular, it has been proposed that stress influence on afferent structures of the dopamine system such as

hippocampus and amygdala may lead to its dysregulation (Belujon and Grace, 2015). In the present study, the DRD paradigm enabled us to provide further evidence of how living in an urban environment may increase the risk to develop mental disorders via dysregulatory effects on the mesolimbic dopamine system and the limbic system.

Acknowledgements

We would like to thank Mohammad Al-Bayati for the sampling of urbanicity information and Maria Keil for assistance in MR data acquisition.

Author Contributions

O.G and B.K designed the experiment, E.D and O.G. designed the DRD-paradigm, E.D. and M.K. conducted the experiment, B.K and O.G analyzed the data and wrote the paper.

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Supplementary Information

Table S1 Subject characteristics

Current Urbanicity	N	Early life score Mean±SD	Age Mean±SD
1	5	31±13.9	23.2±2.6
2	10	26±9.7	25.2±2.2
3	132	31±11.5	23.8±2.4
Total	147	30.7±11.5	23.9±2.4

Table S2 Brain regions showing a main effect (F-contrast) of urbanicity/ city living

Sample	Full		Matched	
Region	**MNI**	**Z-value**	**MNI**	**Z-value**
R Amygdala	22;-2;-18	4,27*	22;-2;-18	3,43*
L VTA	-4;-16;-14	2,78*		
R Pregenual Anterior Cingulate Gyrus	2;50;6	4,04*	2;52;6	3,41*
L Lingual Gyrus	-18;-80;-6	5,36	-18;-84;-4	5,26
L Precuneus	0;-56;28	5,45		
L Inferior Temporal Gyrus	-46;-54;-8	5,10		
R Angular Gyrus	58;-54;42	4,81		
L Middle Temporal Gyrus			-58;-30;-4	5,02
R Superior Temporal Gyrus	56;-2;-12	5,24		
L orbital part Inferior Frontal Gyrus	-34;32;-4	5,92		
R Posterior Orbital Gyrus	36;32;-10	4,82		
L Medial Orbital Gyrus	-12;52;-18	5,65	-8;52;-18	5,34

Statistical effects presented at FWE corrected for whole brain $P<0.05$; * FWE corrected for small volume $P<0.05$.

Table S3 Brain regions showing a main effect (F-contrast) of task

Sample	**Full**		**Matched**	
Region	**MNI**	**Z-value**	**MNI**	**Z-value**
L Supramarginal Gyrus	-52;-44;52	5.96		
L Superior Colliculus	-6;-30;-10	5.19		
R Superior Colliculus	6;-26;-14	5.09		
L Posterior Thalamus	-4;-26;0	4.79		
L VTA	-4;-14;-14	5.06	-4;-14;-16	3.22**
Hypothalamus	0;-14;4	4.89		
L Hypothalamus	-6;-2;-8	5.11		
L Caudate	-12;8;12	5.53		
R Nucleus Accumbens	10;14;-2	5.61	10;14;-2	4.55**
L Nucleus Accumbens	-10;14;-2	5.47	-10;14;0	4.36**
L Anterior Insula	-32;14;-12	5.04		
Right Caudate	12;16;8	5.67		
R Anterior Insula	36;16;-10	5.52		
L Superior Frontal Gyrus	-18;30;54	4.93		
R Pregenual Anterior Cingulate Gyrus	4;46;10	5.62		

Statistical effects presented at FWE corrected for whole brain P<0.05; ** FWE corrected for small volume P<0.05.

4.2 Dynamic amygdala influences on fronto-striatal brain mechanisms involved in self-control of impulsive desires

Original Paper

Neuropsychobiology

Neuropsychobiology 2015;72:37–45
DOI: 10.1159/000437436

Received: February 12, 2015
Accepted after revision: July 6, 2015
Published online: August 28, 2015

Dynamic Amygdala Influences on the Fronto-Striatal Brain Mechanisms Involved in Self-Control of Impulsive Desires

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Key Words
Functional magnetic resonance imaging · Neuroimaging · Neurofunctional connectivity magnetic resonance imaging · Reward system · Self-control · Approach · Avoidance

Abstract
Human decisions are guided by a variety of motivational factors, such as immediate rewards, long-term goals, and emotions. We used functional magnetic resonance imaging to investigate the dynamic functional interactions between the amygdala, the nucleus accumbens, and the prefrontal cortex that underlie the influences of emotions, desires, and rationality on human decisions. We found that increased functional connectivity between the amygdala and the nucleus accumbens facilitated the approach of an immediate reward in the presence of emotional information. Further, increased functional interactions of the anteroventral prefrontal cortex with the amygdala and the nucleus accumbens were associated with rational decisions in dilemma situations. These findings support previous animal studies by demonstrating that emotional signals from the amygdala and goal-oriented information from prefrontal cortices interface in the nucleus accumbens to guide human decisions and reward-directed actions.

Introduction

Human decisions are guided by a variety of motivational factors, such as immediate rewards [1], long-term goals [2–4], and emotions like fear [5]. These different motivational factors are processed by partially distinct neural circuits in the human brain.

On the one hand, regions of the mesolimbic dopamine system like the nucleus accumbens (NAc) and the ventral tegmental area (VTA) are activated by the presentation of a reward [6, 7] and particularly during decisions for immediate or high rewards. On the other hand, recent functional neuroimaging studies in humans have shown that regions of the dorsolateral prefrontal cortex modulate value signals in the ventromedial prefrontal cortex when subjects exert behavioral self-control [8]. Also inhibitory influences of the anteroventral prefrontal cortex (avPFC) on the mesolimbic dopamine system enable more flexible and self-controlled human decisions via decoupling behavior from more automatic impulsive desires that are mediated by the mesolimbic dopamine system [9]. Finally, emotions like fear also affect human decisions via activation of the amygdala [5, 10–12].

So far, however, quite little is known about the dynamic functional interactions between these brain circuits that underlie reward processing [1, 13], self-controlled

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pursuit of long-term goals [3, 4, 8], and emotions [2, 5, 12]. Here, we investigate the dynamic neurofunctional mechanisms that underlie the effects of emotional contexts on decision making in situations in which a superordinate goal contradicts the proximal reward bias. To manipulate the emotional context, we used fearful emotional facial expressions which have been shown to strongly influence attention and perception, memory, and other cognitive functions [14]. In particular, we focused on how the dynamic interplay between the key brain regions involved in reward processing (NAc), self-control of impulsive desires (avPFC), and emotional face processing (amygdala) mediates subjects' decisions.

Methods

Subjects

Seventeen Caucasian, right-handed, healthy volunteers (8 females) aged 25 ± 4 years without a history of psychiatric or neurological disorders were included in this study. Nine additional subjects were excluded due to head movements of more than 3 mm. Approval from Göttingen University Medical Center Ethics Committee and written informed consent were obtained before investigation.

Stimuli

Experimental stimuli consisted of 960 colored squares, i.e. 480 with a gray ellipse and 480 with an emotional face in the center. Squares were filled with 6 different colors, whereby red and green represented conditioned reward stimuli (see Task). Online supplementary figure S1 (for all online suppl. material, see www.karger.com/doi/10.1159/000437436) shows the stimulus layout. Images of 114 different identities (50% males and 50% females) from ADFES [15], Ekman [16], KDEF [17, 18], NimStim [19] and RaFD [20] image data sets were presented in a random sequence. Stimuli were presented and responses were recorded using Presentation V14 (Neurobehavioral Systems, Berkeley, Calif., USA).

Task

For the experiment, we adapted the previously introduced desire-reason dilemma (DRD) paradigm [9] and added emotional facial expressions in the center of the color stimuli for some of the experimental conditions (online suppl. fig. S1). Fearful faces were used as an experimental factor, whereby neutral faces and colored stimuli without faces served as control conditions. The decision to include these two options of control conditions in our experimental design was based on clear evidence from the broader literature that the so-called 'neutral' faces may not represent an appropriate control condition because they themselves produce activation in emotion-processing brain areas such as the amygdala [e.g. 21–23].

Before scanning, a contingency between 2 colors, green and red, and a reward, 10 bonus points, was established. During scanning, participants performed a delayed matching-to-sample task with 2 target colors presented at the beginning of each block. Subjects had to accept all probes that matched the target colors and reject all other probe colors. Successful performance of this task was the superordinate task goal and yielded 50 points per block. Two different block types were performed in a pseudo-randomized order. In the desire context (DC), indicated by a capital 'B' (for 'bonus') above the target set (fig. 1, top), the participants were allowed to also accept bonus reward colors (10 points) that were instrumentally conditioned before the experiment. By contrast, in the reason context (RC), indicated by a capital 'Z' (for 'Ziel' or 'target' in German) above the target set (fig. 1, bottom), the conditioned reward stimuli had to be rejected as well if they did not match one of the target colors, thereby creating the DRD (leading to top-down suppression of reward signals elicited by the conditioned stimuli) [9, 24]. Erroneous acceptance of conditioned reward stimuli led to loss of the 50 points in the corresponding block. Block length pseudo randomly varied between 5 and 7 trials (i.e. probe stimuli) per block. Each trial lasted 2 s and the interval between the stimuli was 300 ms. Stimuli with and without a face were presented for 1 s. If the subject did not respond within 1 s, the trial timed out and the block aborted. Block feedback was given only for incorrect or timed out trials. At the end of the task, subjects received a summary feedback. The detailed trial timing is shown in figure 1.

Behavioral Data Analyses

Statistical analyses of the behavioral data were done using SPSS for Windows (version 20.0; IBM). A repeated-measures ANOVA was performed with the 3 factors emotion (fearful face vs. no face), reward (reward stimuli vs. nonreward stimuli), and task context (DC vs. RC). The results of Mauchly's tests of sphericity were not significant; therefore, sphericity was assumed. Post hoc paired tests (2-tailed significance) were performed to assess the behavioral effects of the additionally presented fearful faces when: (i) accepting reward stimuli in the DC, (ii) accepting nonreward stimuli in the DC, (iii) rejecting reward stimuli in the RC, and (iv) rejecting nonreward stimuli in the RC; they were also performed to assess the differential effects of fearful faces when: (v) accepting reward versus accepting nonreward stimuli in the DC and (vi) rejecting reward versus rejecting nonreward stimuli in the RC. Error trials and missed bonus trials were excluded from the analysis.

Functional Magnetic Resonance Imaging Data Acquisition

The experiment was performed on a 3-Tesla MRI scanner (Siemens TRIO) equipped with an 8-channel head coil. Head motion was restricted by small cushions. A high-resolution T1-weighted anatomical scan (3D-MPRAGE; voxel size, $1 \times 1 \times 1$ mm^3) was obtained for each subject. Functional images were acquired using a T2*-sensitive echo planar imaging sequence (voxel size, $3 \times 3 \times 3$ mm^3; gap, 20%; interscan interval, 1.9 s; echo time, 30 ms; flip angle, 70°, and field of view, 192 mm) parallel to the anterior commissure-posterior commissure plane in an ascending direction. During 4 sessions, a total of 1,640 image volumes were acquired. Before the functional scan, a B0 field map was recorded using a gradient-echo sequence.

Functional Magnetic Resonance Imaging Analysis

Functional imaging data preprocessing and analysis were performed using Statistical Parametric Mapping SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). The data were realigned and unwarped with the voxel displacement map generated by the FieldMap toolbox of SPM from B0 field map data. The high-resolution anatomical image was segmented with the 'new seg-

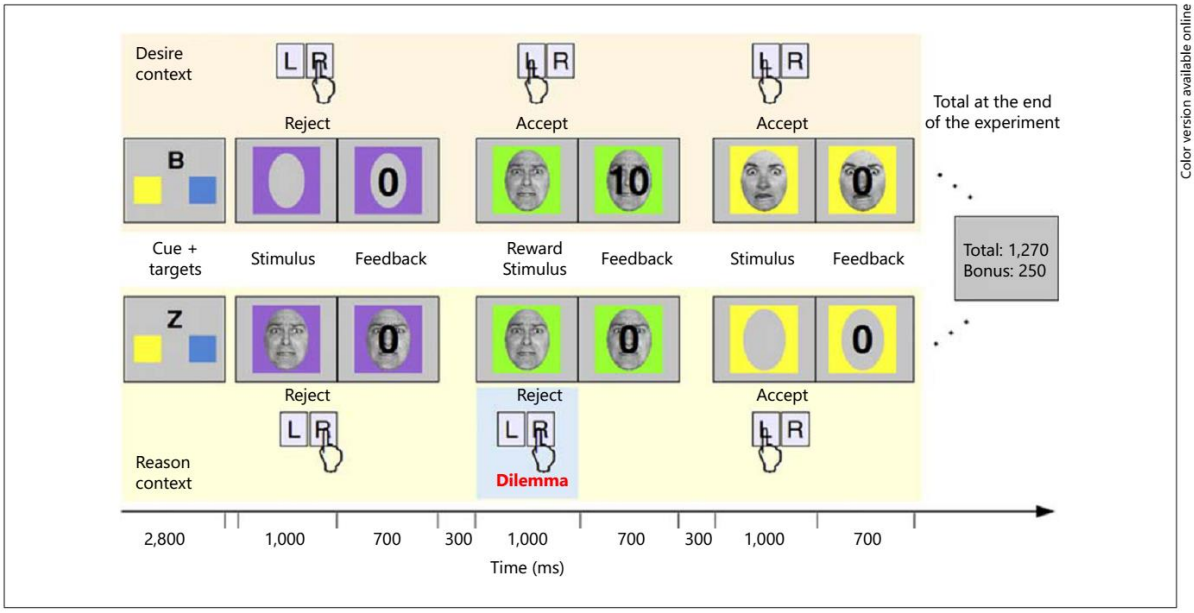

Fig. 1. Affective DRD paradigm. Participants performed a delayed matching-to-sample task with 2 target colors presented at the beginning of each block (colors refer to the online version only). Successful performance of this task was the superordinate goal and yielded 50 points per block. In the DC (top) the participants were allowed to also accept reward colors in order to gain an additional 10 points. These colors were instrumentally conditioned before the experiment. By contrast, in the RC (bottom) these stimuli had to be rejected if they did not match one of the target colors. Erroneous acceptance of conditioned reward stimuli in an RC block led to loss of the 50 points.

ment' routine generating white and grey tissue class images and coregistered with the mean echo planar imagining image. Structural templates and individual flow fields were created with the DARTEL toolbox of SPM. Then the slice time-corrected data were spatially normalized to MNI space with the DARTEL toolbox routine using individual flow fields. Functional images were smoothed using a 6-mm full width at half maximum (FWHM) isotropic Gaussian kernel and saved with a spatial resolution of 2 × 2 × 2 mm^3.

It is known that amygdala neurons respond to a variety of stimuli and that even neutral faces, i.e. faces without an overt emotional expression, may activate the amygdala [22]. For our data analyses regarding dynamic functional interactions of the amygdala on the fronto-striatal brain mechanisms involved in human decisions for or against an immediate reward, we used experimental conditions with fearful faces because they had been shown to produce very strong and most reliable amygdala activation [25]. The no-face condition was chosen as the more appropriate control condition because our findings (see below for a detailed discussion) also clearly supported the prevalent view that so-called neutral faces, due to their still largely unexplained own effects on amygdala activation, may not represent an appropriate control condition for studies interested in the modulatory effects of amygdala activation.

Statistical analyses used a general linear model including the conditions according to the 3 factors emotion (fearful face vs. no face), reward (reward stimuli versus nonreward stimuli), and task context (DC vs. RC). Error trials were excluded from the analysis. Missed bonus trials were included in the general linear model but not further analyzed due to their small number. A vector representing the temporal onset of stimulus presentation was convolved with a canonical hemodynamic response function in order to produce a predicted hemodynamic response to each experimental condition. Linear t contrasts were defined to assess the differential effects elicited by the experimental conditions.

To test for amygdala activation produced by the presentation of fearful faces, we compared conditions with nonreward stimuli and fearful faces to conditions with nonreward stimuli without faces.

In order to assess functional interactions between the amygdala, the NAc, and the avPFC, we performed several psychophysiological interaction (PPI) analyses [24]. PPI analyses represent an accepted 'gold standard' for functional connectivity analysis that has been widely used after its first description and allows examination of dynamic, i.e. process-specific, changes in functional interactions between brain regions. PPI analyses require one regressor representing the signal time course in a given volume of interest (VOI) and one regressor representing the psychological variable of interest. In our study, individual BOLD signal time courses were generally extracted from VOI around the local activation maxima of the contrast (i.e. the psychological variable) effects determined

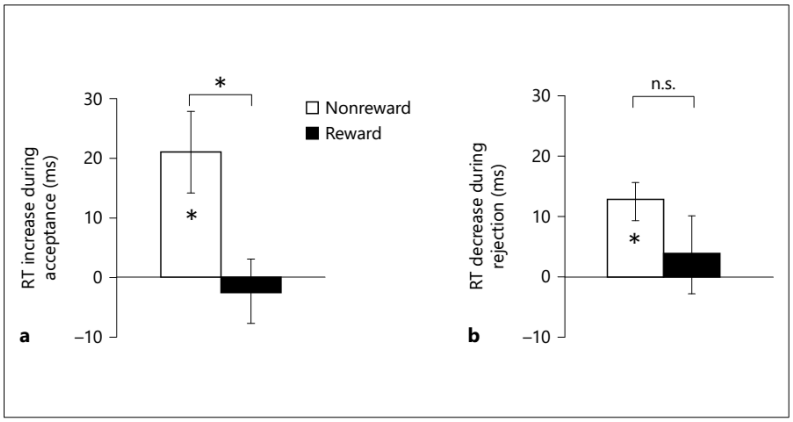

Fig. 2. Triple interaction effect of the emotion × reward × task context. Bars depict the RT differences evoked by presentation of fearful faces during acceptance (desire context; **a**) or rejection (reason context; **b**) of nonreward or reward stimuli. Fearful faces generally increased the avoidance tendency (see white bars in **a** and **b**), reward counteracted this effect (see differences between white and black bars in both **a** and **b**), and task context again increased the avoidance tendency with regard to reward stimuli in the RC [reduced difference between the white and black bars in **b** (RC) compared to **a** (DC); see text for details]. Data are presented as means ± SEM. * $p < 0.05$. n.s. = Not significant.

in the second-level group analyses. To account for possible interindividual functional-neuroanatomical differences, the box dimension for all VOI was set to $12 \times 12 \times 12$ mm^3. For PPI analyses assessing functional interactions of the amygdala, a VOI around the local activation maximum within the amygdala in the contrast between fearful-face and no-face conditions in the absence of a reward was determined (MNI coordinates: –24, –6, –18). For PPI analyses assessing functional interactions of the NAc, VOI were centered at (12, 12, 6), which was the second-level local activation maximum of the NAc in response to reward stimuli in the no-face condition.

First, in order to assess functional interactions between the amygdala and the NAc during concurrent presentation of a reward stimulus and a fearful face in the DC, the psychological vector consisted of the comparison between reward stimuli with versus without a fearful face.

A similar PPI analysis was also performed for the analogous contrast with nonreward stimuli (again with vs. without a fearful face) in order to test whether the observed amygdala-NAc interactions were specifically related to the additional presence of a reward stimulus.

Changes in functional connectivity between the amygdala, the NAc, and the avPFC depending on the task context related to the DRD were assessed via PPI analyses again with the amygdala and the NAc as seed regions. The psychological vector was defined by the interaction contrast comparing the effects of fearful faces on reward-related brain activation between the DC and the RC. Finally, for illustration purposes only (fig. 3), we also performed PPI analyses using corresponding contrasts against the implicit baseline.

All statistical analyses of the single-subject data included a high-pass filter with a 128-second cut off and an autoregressive AR(1) model to account for serial correlations in functional magnetic resonance imaging (fMRI) time series. Group effects were assessed by second-level random-effect analyses based on single-subject contrast images. Statistically significant effects were determined using a primary search criterion of $p < 0.005$ (uncorrected if not otherwise stated). For a priori regions, statistical inference was based on a significance threshold of $p < 0.05$ corrected for multiple comparisons using family-wise error (FWE) correction for small volumes based on a priori hypotheses [26] derived from the literature for the amygdala [22], the avPFC, and the NAc [24]. For small volume correction (svc), spheres with a 5-mm radius were applied. PPI β values for the illustration were extracted at second-level local activation maximum coordinates.

Results

Behavior

Seventeen subjects (8 females) underwent fMRI while performing an adapted version of the previously introduced DRD paradigm [9], with emotional facial expressions being added in the center of the color stimuli (for details, see Methods). The overall error rate was 3%, and the subjects failed to accept the reward stimulus in 7.6% of the corresponding trials.

Analysis of reaction time (RT) data used ANOVA and included the 3 factors emotion (fearful face vs. no face), reward (reward stimuli vs. nonreward stimuli), and task context [DC vs. RC, i.e. acceptance (approach) vs. rejection (avoidance) of a reward stimuli]. This analysis revealed main effects of reward [$F_{(1, 16)} = 26.27$, $p < 0.001$] and task context [$F_{(1, 16)} = 15.37$, $p = 0.001$], an interaction effect of emotion × task [$F_{(1, 16)} = 15.04$, $p = 0.001$], and, most importantly, a triple interaction between emotion, reward, and task context [$F_{(1, 16)} = 5.61$, $p < 0.05$]. Fearful faces generally increased the avoidance tendency toward nonreward stimuli, leading to a significantly slower acceptance ($t_{16} = 3.08$, $p < 0.01$) and a faster rejection of these stimuli ($t_{16} = 4.02$, $p = 0.001$; see white bars in fig. 2a, b).

Presentation of a conditioned reward stimulus in the DC significantly counteracted these effects of fearful faces by facilitating approach behavior, i.e. accelerating the

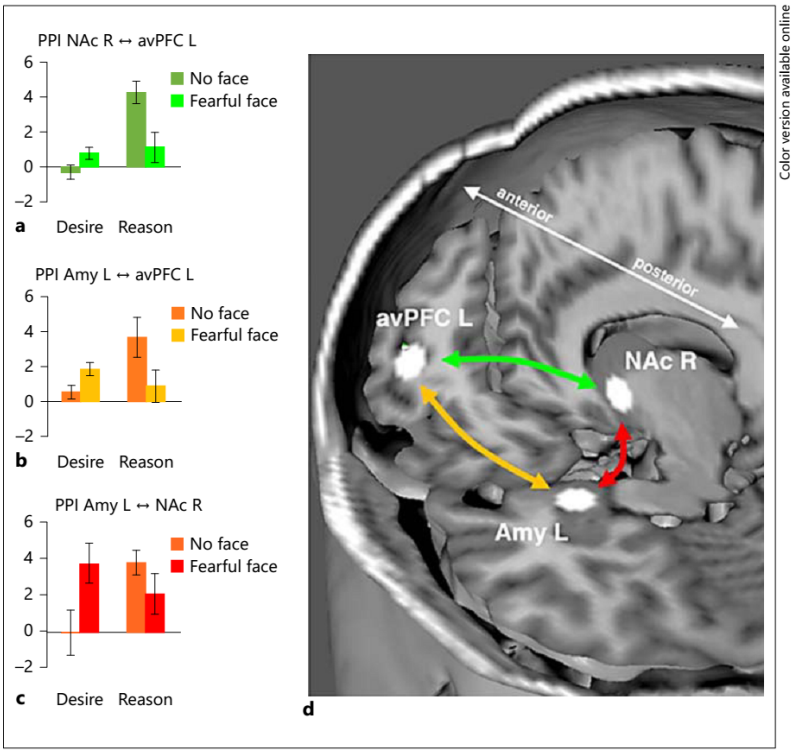

Fig. 3. Dynamic functional interactions between the amygdala (Amy), the NAc, and the avPFC during decisions in the affective DRD paradigm (**a–d**). Fearful faces increased the functional coupling between the amygdala and the NAc during presentation of conditioned reward stimuli in the DC (**c**, left, red arrow). This effect was reversed in the RC (**c**, right) and associated with decreased positive/increased negative prefrontal coupling to both the NAc (**a**, right, green arrow) and the amygdala (**b**, right, yellow arrow). Bars depict the β values for the different experimental conditions. For details, see Methods. Data are presented as means ± SEM. L = Left; R = right.

acceptance of stimuli despite the presence of fearful faces (fig. 2a, RT_{diff} = 23 ms, t_{16} = 2.5, $p < 0.05$).

Similarly, in the RC reward stimuli also counteracted the avoidance tendency that was increased by fearful faces, although this effect was not significant (fig. 2b). Importantly, as indicated by the triple interaction effect in our behavioral data, this counteracting effect of reward was significantly smaller in the RC ('avoidance task') compared to the DC ('approach task'; fig. 2a, b).

Imaging

The aim of this neuroimaging experiment was to investigate the dynamic effects of amygdala activity on the fronto-striatal brain mechanisms involved in self-control of impulsive desires. Presentation of fearful faces has been shown to be a powerful tool to elicit reliable amygdala activation [25]. In order to assess specific effects of fearful facial expressions, fearful faces have often been contrasted with so-called neutral faces in previous experiments. However, there is strong evidence that neutral faces themselves may produce amygdala activations [21–23] and may therefore cancel out amygdala activity when contrasted with fearful faces. This clearly raises doubts regarding the validity of so-called neutral faces as the control condition in experiments that are interested in the functional interplay between emotion-processing areas and other brain systems involved in human decision-making. For this reason, in the present experiment we implemented two different control conditions, i.e. conditions with neutral faces and conditions without faces, in order to be able to assess the effects of varying amygdala activity on fronto-striatal brain mechanisms independently of these potential confounds that so-called neutral faces may introduce.

First, we analyzed the effects of both fearful and neutral faces on amygdala activation by comparing them with the no-face condition. Both fearful faces (left: t_{16} = 6.02, p_{FWE} = 0.001, svc; right: t_{16} = 6.01, p_{FWE} = 0.001, svc, see online suppl. fig. S2A and online suppl. table S1) and neutral faces (left: t_{16} = 6.39, $p_{FWE} < 0.001$, svc; right: t_{16} = 5.82, $p_{FWE} < 0.001$, svc, see online suppl. fig. S2B and online suppl. table S2) led to significant bilateral amygdala activation compared to conditions without faces, confirming prior findings [22]. By contrast, when conditions with

Table 1. Modulation of reward-related NAc and VTA activation by additional presentation of fearful faces

Region	MNI coordinates (t value)			
	without faces	with fearful faces	reduced by fearful faces	increased by fearful faces
NAc L	–16, 8, –4 (3.36)*	–10, 4, –2 (3.91)*		–16, 16, –2 (3.08)
NAc R		16, 8, –4 (4.89)**		16, 10, –4 (3.29)*
VTA L		–8, –16, –12 (2.51)	–2, –20, –12 (3.60)	
VTA R	8, –24, –16 (3.20)*	12, –16, –14 (2.97)*		12, –16, –14 (3.21)*

Statistical effects are presented at $p < 0.005$, uncorrected. * $p < 0.05$, svc; ** $p < 0.005$, svc; for details, see Methods. L = Left; R = right.

Table 2. Modulation of top-down suppression of reward signals in NAc and VTA by additional presentation of fearful faces

Region	MNI coordinates (t value)			
	without faces	with fearful faces	reduced by fearful faces	increased by fearful faces
NAc L	–10, 12, 2 (6.21)**	–12, 2, –2 (6.72)*		–14, 4, –6 (3.47)
NAc R	12, 6, 0 (4.00)*	14, 8, –2 (4.67)*		
VTA L	–2, –20, –10 (6.53)**	–8, –16, –12 (4.24)*	–8, –16, –6 (3.54)*	
VTA R	12, –22, –10 (4.47)*	4, –22, –22 (4.56)**	8, –16, –6 (2.35)	

Statistical effects presented at $p < 0.005$, uncorrected. * $p < 0.05$, svc; ** $p < 0.005$, svc; for details, see Methods. L = left; R = right.

fearful faces were directly compared to conditions with neutral faces, no significant activation could be observed in the amygdala; as already suggested by earlier studies, amygdala activation was eliminated in this contrast. For this reason, and because the modulatory effects of amygdala activation on functional connectivity were the main focus of our study, the conditions with neutral faces were discarded from further analyses, which instead relied on conditions with fearful faces compared to conditions without faces as the more appropriate control condition in this context.

Replicating our prior findings [9, 24], presentation of conditioned reward stimuli elicited reliable bottom-up activation of the right VTA ($t_{16} = 3.2$, $p_{FWE} < 0.05$, svc) and the left NAc ($t_{16} = 3.36$, $p_{FWE} < 0.05$, svc) in conditions without presentation of faces (table 1). Likewise, we were able to replicate our prior findings of significant suppression of reward-related activity in the NAc (left: $t_{16} = 6.21$, $p_{FWE} < 0.005$, svc; right: $t_{16} = 4.0$, $p_{FWE} < 0.05$, svc) and the VTA (left: $t_{16} = 6.53$, $p_{FWE} < 0.005$, svc; right: $t_{16} = 4.47$, $p_{FWE} < 0.05$, svc) when the same conditioned reward stimuli were presented in the DRD situation (table 2).

Successful manipulation of amygdala activity in our experiment was confirmed by comparing conditions with fearful faces to conditions without faces. These contrasts showed reliable bilateral amygdala activation both in the absence (left: $t_{16} = 6.02$, $p_{FWE} = 0.001$, svc; right: $t_{16} = 6.01$, $p_{FWE} = 0.001$, svc; see online suppl. fig. S2a and online suppl. table S1) and in the presence of conditioned reward stimuli (left: $t_{16} = 5.14$, $p_{FWE} = 0.001$, svc; right: $t_{16} = 4.48$, $p_{FWE} < 0.005$, svc; see online suppl. table S3).

Based on these findings and replications of successful activation of reward-related (VTA and NAc) and emotion-related (amygdala) brain regions, we then investigated the modulation of reward-related activity by fearful faces. This was done by using interaction contrasts comparing both bottom-up activation and top-down modulation of the reward system during presentation of fearful faces with the corresponding contrasts without fearful faces (tables 1, 2). In the DC, when the conditioned reward stimulus was accepted a significant interaction between fear and reward was observed in the right VTA ($t_{16} = 3.21$, $p_{FWE} < 0.05$, svc) and the right NAc ($t_{16} = 3.29$, $p_{FWE} < 0.05$, svc; see table 1). In the RC, top-down suppression of reward-related VTA activation was reduced by the presentation of fearful faces (left: $t_{16} = 3.54$, $p_{FWE} < 0.05$, svc; right: $t_{16} = 2.35$, n.s.; table 2).

As shown in behavioral results, reward counteracted the avoidance tendency of fearful faces in DC by facilitating approach behavior. In correspondence with this, our functional connectivity (PPI) analyses of the fMRI data revealed increased positive coupling between the amygdala (which was activated by the fearful faces) and the NAc (which was activated by the reward stimulus) (t_{16} = 4.91, p = 0.005, svc; fig. 3c, left, online suppl. fig. S3). This effect was not observable in the absence of a reward, suggesting that additional reward-related dopaminergic input from the VTA to the NAc was necessary to increase positive amygdala-accumbens functional connectivity.

Further PPI analyses revealed that the increased positive functional coupling between the amygdala and the NAc found in the DC turned into decreased functional coupling in the RC (t_{16} = 5.58, p = 0.001, svc; fig. 3c, right, online suppl. fig. S4), which coincides with the reduced approach facilitation by reward stimuli in this dilemma situation. In a similar way, the effects of fearful faces on the functional couplings of the avPFC with both the amygdala (t_{16} = 3.27, p < 0.05, svc; fig. 3b, left vs. right) and the NAc (t_{16} = 4.02, p < 0.05, svc; fig. 3a, left vs. right) were reversed in the RC.

Discussion

To our knowledge, this is the first in vivo neuroimaging study to show that emotional signals from the amygdala and goal-oriented information from prefrontal cortices interact in the NAc to guide human decisions and reward-directed actions. While presentation of fearful faces generally increased the avoidance tendency, simultaneous presentation of conditioned reward stimuli counteracted this effect by facilitating approach behavior. This behavioral finding was accompanied by increased functional connectivity between the amygdala and the NAc and was associated with increased activation of the NAc. Notably, amygdala-accumbens coupling was not increased when a fearful face was presented alone, suggesting that additional reward-related dopaminergic input from the VTA to the NAc was necessary to elicit this effect. This notion is consistent with recent findings from animal studies showing that dopamine supplied by the VTA may enhance limbic influences on decision-making [27] by modulating NAc responses to amygdala inputs [28, 29]. Thus, in line with a recent optogenetic demonstration of glutamatergic, i.e. excitatory, connections from the amygdala to the NAc in the rodent [30], the increased functional connectivity between the amygdala and the NAc in the DC may represent an adaptive neural mechanism facilitating the approach of an immediate reward especially in the presence of negative emotional information. This finding is also consistent with the results of another recent study showing that task-independent presentation of fearful faces accelerated probabilistic reward learning [31].

Compared to the DC, behavioral data in the RC indicated reduced approach facilitation towards conditioned reward stimuli, suggesting that the third factor in our experiment, i.e. task context, moved the pendulum back towards a higher avoidance tendency and devaluation of the immediate reward. Again, our neurofunctional connectivity data provide evidence for possible neural mechanisms associated with this triple interaction effect in the behavioral data. In comparison to the DC, the effects of fearful faces on the functional couplings of the avPFC with both the amygdala and the NAc were reversed in the RC, suggesting increased prefrontal control [2] of both NAc [24] and amygdala activity [12, 32]. In a similar way, the increased positive functional coupling between the amygdala and the NAc in the DC turned into decreased functional coupling in the RC, indicating that amygdala-NAc signaling (that facilitates approach behavior) was reduced in the DRD situation.

Overall, these findings are consistent with substantial animal-experimental evidence demonstrating that emotional signals from the amygdala and goal-oriented information from prefrontal cortices directly interface in and are integrated by the NAc [33]. More specifically, it has been proposed that the interactions between specific sets of amygdalar, prefrontal, and NAc neurons may be critical to flexible adaption of behavioral responses and strategies to environmental conditions [34]. On a mesoscopic level, there is converging evidence for a rostrocaudal 'affective keyboard' organization in the NAc [35], with rostral and caudal NAc subregions mediating appetitive (approach) and fear (avoidance) behaviors, respectively. Thus, by playing the right melody on the affective keyboard in the NAc – depending on the situational (here: desire vs. reason) context – the convergent amygdala and prefrontal inputs may be a central adaptive and integrative neural mechanism to guide goal-oriented behavior and reward-directed actions [36].

The present study has some limitations that need to be considered. First, statistical comparisons between fearful faces and no-face conditions as used in the present study are unable to disentangle the processing of faces and the processing of emotions. Thus, we cannot determine whether the dynamic functional interactions of the amygdala with the NAc and the prefrontal cortex are related to emo-

tional processes or, more generally, to the processing of human faces independently of their emotional expressions. However, in this context it is very important to note that the broader scientific literature clearly documents that (at least when using face stimuli) this attempt to disentangle face and emotional processing (e.g. in the amygdala) has no reasonable chance because even so-called neutral faces elicit activation in emotion-processing brain areas, particularly in the amygdala [e.g. 21–23]. There have been different kinds of speculations in the literature about the possible reasons for these activations of emotion-processing areas by neutral faces. For instance, it has been posed as a question whether faces can be emotionally neutral at all [e.g. 37, 38]. On the other hand, since facial expressions represent a very important source of social information in the evolution of human beings, amygdala activation by so-called neutral faces may be attributed to very fast and raw evaluation processes that act upon emotionally salient cues such as human faces [e.g. 39–41]. Taken together, these findings clearly raise doubts on the validity of so-called neutral faces as the control condition in experiments that are interested in the functional interplay between emotion-processing areas and other brain systems involved in human decision making. Since, in fact, the goal of our study was to investigate dynamic functional interactions between the amygdala, the NAc, and the prefrontal cortex during human decisions, it was reasonable for our study design to both include experimental conditions that most reliably activate the amygdala (i.e. fearful faces) and exclude control conditions (i.e. so-called neutral faces) that may reduce these effects due to their still largely unexplained effects on amygdala activation. Further, as amygdala activation by presentation of fearful faces is usually attributed to the processing of emotions, we are confident that the new findings of our current study on functional interactions between the human amygdala, the NAc, and the prefrontal cortex provide important and reliable insight into the brain mechanisms involved in the complex processes of human decision making.

A second limitation of our current study is that we had to accept an image resolution of $3 \times 3 \times 3$ mm^3, which does not deliver an optimal amygdala signal, in favor of full brain coverage for the connectivity analyses. Modern multiplexed imaging sequences [42] could provide an alternative solution in future experiments. Finally, because of substantial head movements, we had to exclude 9 subjects, which reduced the sample size and the statistical power. Therefore, most reported regions are not significant at the most conservative level of whole-brain correction for FWE. Nevertheless, the regions for which we had a priori hypotheses showed clearly significant effects with FWE-correction for small volume. Thus, we are very confident that the results reported in this study are reliable.

In summary, our findings add to the growing understanding of how brain mechanisms may mediate the influence of desires, emotions, and rationality on human decisions. They provide direct insight into the functional interplay between the amygdala, the prefrontal cortex, and the NAc in the adaptive regulation of the balance between 'impulsive' and 'reflective' systems [2] in decision making. Recent evidence indicates a major role of these brain mechanisms also in social interactions [43, 44]. In the future, targeted investigations of these neural mechanisms may foster our understanding of the pathomechanisms that underlie brain disorders with affective, impulsive, and addictive symptoms.

Acknowledgements

We would like to thank A. Fischer, O. Langner, and N. Tottenham for granting access to their sets and databases of facial expressions.

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Supplementary Material for: Dynamic amygdala influences on fronto-striatal brain mechanisms involved in self-control of impulsive desires

Supplementary figures

Figure S1. **Stimulus layout used for the paradigm.** (**A**) Without face. (**B**) With fearful face. (**C**) Neutral face.

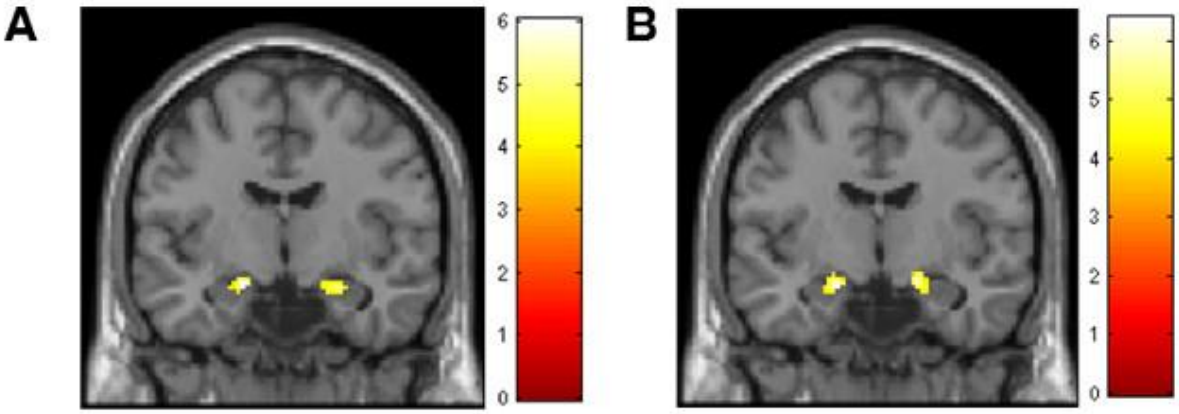

Figure S2. Bilateral amygdala activation elicited by faces. (A) Fearful faces, (left [-20,-8,-14]: t_{16}=6.02, P=0.001, svc; right [20,-6,-16]: t_{16}=5.65, P=0.001, svc). (B) Neutral faces, (left [-20,-8,-14]: t_{16}=6.53, P_{FWE}<0.001, svc; right [16,-8,-14]: t_{16}=5.65, P_{FWE}=0.001) Depicted is the statistical comparison of non-reward stimuli with vs. without face.

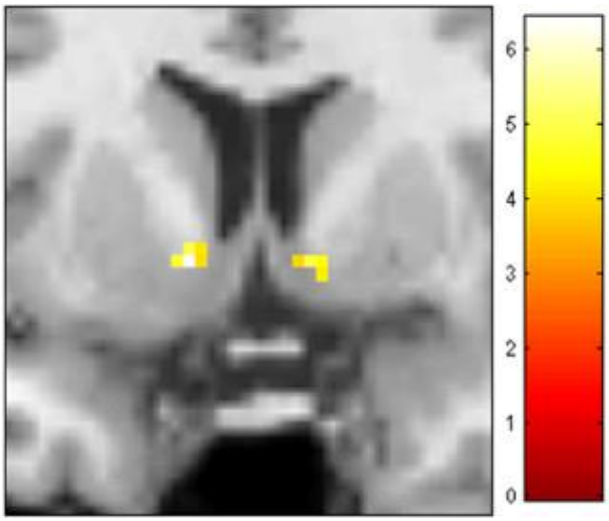

Figure S3. Enhanced amygdala – NAc functional connectivity. Functional coupling of the left amygdala (seed region in this analysis) with bilateral nucleus accumbens (left [-10, 8,-6]: t_{16}=6.42, P<0.001, svc; right [10, 10,-8]: t_{16}=4.91, P=0.005, svc) significantly increased when fearful faces were paired with conditioned reward stimuli in the Desire Context.

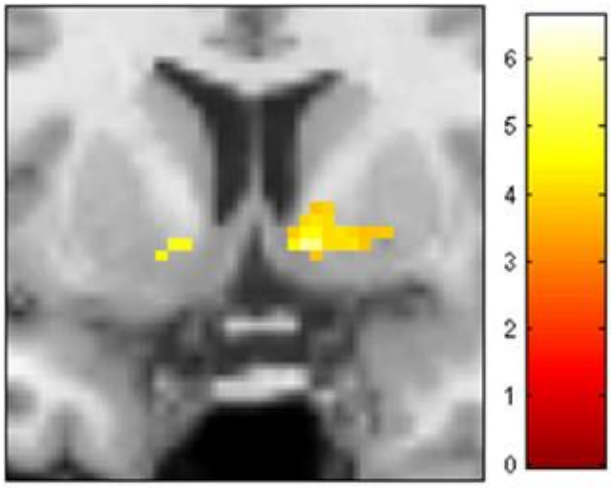

Figure S4. **Reduced amygdala - NAc functional connectivity.** Functional coupling between the left amygdala (seed region) and bilateral nucleus accumbens (left [-10, 8,-6]: t_{16}=4.64, P<0.01, svc; right [10, 8,-6]: t_{16}=5.58, P=0.001, svc) significantly decreased when fearful faces were presented while reward stimuli had to be rejected in the Desire-Reason Dilemma.

Supplementary tables

Table S1: Brain activations elicited by fearful faces (in the absence of reward)

Region	MNI coordinates			t-value	
L Amygdala	-18	-8	-16	6,02	**
R Amygdala	24	-6	-18	6,01	**
R Fusiform Gyrus	38	-50	-22	12.03	
L Inferior Occipital Gyrus	-24	-96	6	9.26	
L Hippocampus	-18	-34	-4	7.94	
R Hippocampus	18	-30	-6	6.29	
R Inferior Frontal Orbital Gyrus	42	30	-14	6.28	
L Superior Temporal Pole	-32	4	-24	5.77	
L Gyrus Rectus	-2	60	-16	5.46	
L Medial Superior Frontal	-4	52	38	5.32	
L Frontomarginal Gyrus	-44	50	-12	4.85	
L Superior Temporal Pole	-46	6	-22	4.80	
L Posterior Cingulate Gyrus	-8	-48	12	4.56	
L Medial Superior Frontal	-2	62	26	4.53	

Activations presented at p<0.001, uncorrected >10Voxel; *p<0.05 FWE-corrected for small volume, **p<0.005 FWE-corrected for small volume, details see methods section

Table S2 Brain activations elicited by neutral faces (in the absence of reward)

Region	MNI coordinates			t-value	
L Amygdala	-18	-8	-18	6,39	+
R Amygdala	16	-8	-14	5.82	+
R Fusiform Gyrus	36	-48	-22	11.94	
R Hippocampus	20	-32	-2	9.82	
L Fusiform Gyrus	-36	-52	-16	9.62	
L Cerebellum	-10	-38	-10	6.14	
L Hippocampus	-20	-32	0	5.83	
L Superior Parietal Lobe	24	-62	50	4.84	

Activations presented at p<0.001, uncorrected >10Voxel; +p<=0.001 FWE-corrected for small volume, details see methods section

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6 Acknowledgements

This doctoral thesis would not have been possible without the support of the University Medical Center Göttingen, Department of Psychiatry and Psychotherapy, Center of Translational Research, its members and the volunteers who participated in the experiments.

First of all, I would like to thank my supervisor Prof. Dr. Oliver Gruber for the opportunity to work in his group, his support, his patience and the guidance through the pitfalls of neuroimaging.

Also, I would like to thank the members of my thesis committee, Prof. Dr. Birgit Kröner-Herwig and Prof. Dr. Michael Waldmann for their assistance.

During the thesis project current and former colleagues of the Center of Translational Research supported me with their expertise; they shared their time and cookies, provided motivation and administrative guidance creating a creative and genial working environment. For this and the opportunity to know them, if would like to thank Tobias Melcher, Esther K. Diekhof, Katja Brodmann, Roberto Goya Maldonado, Maria Keil, Eiko Lajcsak, Anja Richter, Sarah Trost, Claudia Wolf and Sarah Wolter.

Additionally, I would like to thank the team of the MR research unit, Peter Dechent, Britta Perl, Ilona Pfahlert and Carsten Schmidt-Samoa, for their support during the experiments.

During the up and downs of the work I was backed up by my family and friends who motivated and endured me. There are more than I can mention, but this are the close ones: Susanne, Christine, Simone, Edmund, and my parents in law, also Karin, Simone, Thorsten, Malte and Mathis. And my parents - they passed away long before I started with the thesis - they would have been thought that I would have been crazy but also would have been proud.

Finally, there is thank that could not be expressed in words, my wife Ulla shared every emotion with me, dedicated herself to my plans and showed constant support and enormous patience during these years. Without her this thesis would never had happened.

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1973 - 1980	Diplom Ingenieur Informationsverarbeitung Universität Gesamthochschule Siegen, Abt. Gummersbach, Fachrichtung: Elektrotechnik

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	Different networks and capacity limits for articulatory and non-articulatory mechanisms of verbal Short Term Memory, Bernd Krämer, Sabrina Trapp, Karsten Mueller, Stephan Konrad, Joeran Lepsien, Oliver Gruber
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	Amygdala activation by fearful faces modulates the fronto-striatal reward system, Bernd Krämer, Oliver Gruber
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