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NEURAL CORRELATES OF RISK AND EMOTION PROCESSING

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presented by

UWE HERWIG

Prof. Dr. med., M.A., Universities of Ulm, Göttingen, Kaiserlautern/Witten-Herdecke

born on *05.06.1966*

citizen of
Germany

accepted on the recommendation of

Prof. Dr. phil. Michael Siegrist
Prof. Dr. phil. Birgit Kleim

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Content

General introductory remarks

Background

Coping with environmental events is essential for survival. Our brain developed through evolution to cope with as well as to avoid diverse threats and hazards, and to approach salutary conditions as healthy nutrition. The identification of risky or unhealthy conditions is deeply rooted in the organism's central nervous information processing system. Necessary identification steps are the perception and identification of distinct patterns indicating a possible hazardous incident. Therefore, an evaluation of the perceived information is necessary and, based on the result of the evaluation, the selection of an adequate action. We would not have come to this point of evolution if our brain did not provide a highly effective risk detection and anticipation, and strategies to cope with the situation.

However, in everyday life we are often confronted with ambiguous stimuli or with stimuli of which the reaction cannot simply be based on evolutionary learned patterns. Then, decisions are made with a certain grade of uncertainty resulting in a choice under risk. Thus, decisions can result in a disadvantageous outcome for the person in case of the "wrong" decision or in joy or satisfaction after the right one. This represents an emotional impact and such risk processing is subsumed as being closely related with emotion processing (e.g. Bach et al. 2009, Bach and Dayan 2017; Mohr et al. 2010a; Kohno et al. 2017).

From a categorical point of view, risk processing can be seen as e a sub-speciality of emotion processing such as fear or reward processing. However, one may also regard emotion processing in its principles as a calculation whether a perceived external or internal information means risk or chance for the organism. Nonetheless, investigating risk processing, which is to date only partially understood, bears potential to improve risk coping and decision making under potentially threatening conditions in the frame of known emotion processing principles.

Basic aspects of risk and emotion processing

Evaluating risk on the individual level, psychologically involves directed attention onto the respective item, an emotional signalling, a rational cognitive consideration of the items' meaning as well as a pro-contra estimation of potential reaction strategies (Slovic et al. 2004). The latter particularly takes place, when there is no imminent threat but time to cognitively evaluate the best way of coping with the stimulus, which is mostly the case. This psychological procedure is close to decision making regarding the evaluation of actions towards future events or personal development, which involves risky components.

On the procedural level, people are generally not fully aware of the principals behind the process of risk estimation or of decision-making. Lay person that are not familiar with for instance the scientific and statistical background of certain risky conditions may adopt a more affect- or emotion-based estimation when faced with the necessity of evaluating a risk or a benefit (Loewenstein et al. 2001;

Slovic et al. 2002). They may stronger rely on previous experiences, trust, narratives or metaphors than on sound knowledge (Fischhoff et al. 1982; Sjöberg 1998). Furthermore, they may use somatic signals associated with the emotional impact (Damasio, 1996), related to a hazard, as a cue for intuitively estimating the risks (Slovic et al. 2004). Professionals that deal with decisions bearing risks are often trained to follow algorithms leading to an evaluated estimation or decision. Although it has been acknowledged since a long time that feelings contribute to risk estimation (Slovic et al. 2004), the attentional consideration of feelings or emotional signals based on knowledge about the underlying neural principals is often just considered as attending to a "gut feeling". A strong weight is also given to a rational approach of dealing with risks.

Of course, this process of risk estimation always bears the possibility to act or decide in a way that is later recognized as wrong or unnecessary. This may be due to external developments that were not predictable, or due to individual risk processing characteristics. For instance, decisions might base on insufficient knowledge or prejudices. On the individual level, a classic example is represented by avoiding certain experiences due to pathological fears, as seen in phobias or fear- and rage-driven action in cases without real threat. A possible loss of impulse control might be a consequence.

In this context, it is to consider that our information processing is formed throughout lifetime and particularly by early psychosocial experiences. Disturbing conditions in the past development may lead to interpretations and reactions that may not be adequate for coping with the current situation. This takes place for all potentially risky environmental stimuli, as also for instance for food items.

Being aware of the individual mental processes, whether emotional or cognitive, may bear a potential to ameliorate sustainable risk management on a meta-level. Knowing about features and principles of general and also the individual central nervous information processing underlying risk evaluation may be one component for a profound analysis of one's own processes of risk estimation. Furthermore, presuming the potential of intentionally influencing mental information processing based on knowledge about the underlying neural circuits and on knowledge about one's individual risk processing features may lead to improved decisions and coping with risks.

A basic example of advantageous or disadvantageous decisions bearing also potential risks for health is present in the selection of food items. Food intake may cause pleasant and unpleasant feelings, and can such be considered as being associated with emotion processing (Rolls 2005; Ziauddeen et al. 2012; Morton et al. 2014). A feature of tasty but unhealthy nutrition consists of appetitive emotions occurring with impaired impulse control and self-guiding related to respective food stimuli, despite knowledge of a possible disadvantageous health value (Glanz et al. 1998).

Many people, for instance those with obesity, diabetes or other nutrition related conditions have to control eating certain foods of their desire. Evaluating and choosing healthy food on an individual and situation based level is especially important, as it controls impulses that potentially lead to unhealthy nutrition. In everyday life, many people do not actively reflect on whether the food they eat is healthy, but more on whether it is tasty (Glanz et al. 1998). However, consciously reflecting about the

healthiness of a food item can influence eating behaviour. Identifying the brain regions involved in the evaluation of food healthiness might help to understand which cognitive strategies are utilized to promote salutary nutrition.

The concept of threat involves the identification of emotional significance, the generation of an affective state, and a subsequent behaviour; they both engage overlapping neural structures and functions (Phillips et al. 2003; Mohr et al. 2010). Considering the developmental aspects of forming a risk processing system emphasizes the fact that coping with risks is rooted in the very first stages of organisms dealing with the environment. Such, it is evident that algorithms and strategies to behave onto environmental challenges or changes are deeply implemented in our mental systems (Bach and Dayan 2017). In modern times, however, the environmental threats, prevalent until a few centuries ago, are no longer the main threats to most people, particularly in modern urban societies.

Instead of the natural and evolutionary established threats we now increasingly face threats that are qualitatively different, more technical, and in some cases less tangible. This might require an adaptation of our perception, evaluation and reaction to these threats: In modern societies, we regularly do no longer have to fear for instance snakes, spiders and predators, but should rather be cautious in motor traffic, when facing guns as well as when handling tools like knives. This should be considered when dealing with risks or decision-making. Such, disentangling the principles of risk evaluation regarding modern and evolutionary threats including neural aspects adds knowledge to a distinct model of risk processing.

Neural correlates of risk and emotion processing

The neural underpinnings of risk evaluation are to date only partially understood. A central role is ascribed to pattern detection in sensory areas, emotional evaluation in limbic areas as the amygdalae, memory retrieval from the hippocampus, cognitive-emotional integration in the anterior cingulate and different prefrontal regions and decision making and action initiation in the left dorsolateral prefrontal cortex (LeDoux 2000, Tamietto et al. 2011, figure 1.1).

A particular light is shed onto emotional processing as a core process in the course of evaluation and action initiation onto external events. Several studies provided a profound investigation and discussion on the relation of risk processing and emotions regarding the neurobiological backgrounds (Bach et al. 2009; Mohr et al. 2010a; Kohno et al. 2017). Brain regions as the bilateral anterior insula, striatum, dorsomedial and posterior thalamus, midbrain, dorsomedial and dorsolateral prefrontal cortex (DM/DLPFC), and right parietal cortex are found to be involved in risk processing (Mohr et al. 2010a; Kohno et al. 2017). Lay-person may recruit more emotion-associated brain areas as the insula, amygdala and thalamic regions during risk processing than people dealing with certain risks professionally (Anderson et al. 2003; Vorhold et al. 2007; a.o.).

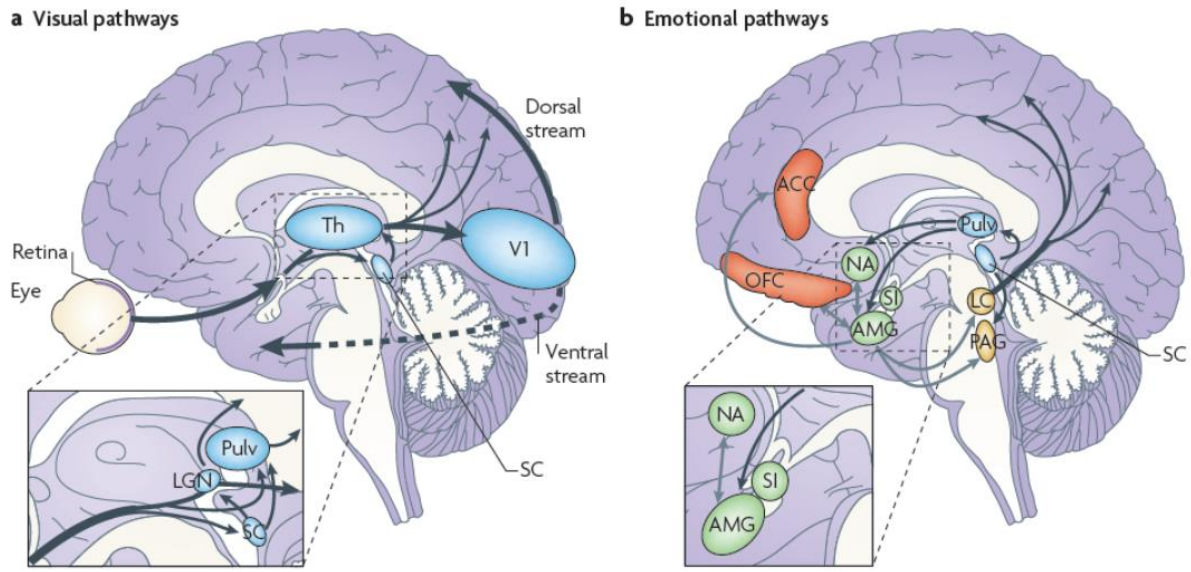

Fig. 1.1 Synopsis of neural systems involved in visual perception and emotion processing (Fig. adopted from Tamietto et al. 2011). Th Thalamus, V1 primary visual cortex, AMG amygdala, NA nucleus accumbens, SI substancia innominate, OFC orbitofrontal cortex, ACC anterior cingulate cortex, Pulv Pulvinar, LC Locus coeruleus, PAG periaqueductal grey, SC superior colliculus.

Neural aspects of the perception of threats and associated negative emotions have been studied extensively (LeDoux 2000; Phan et al. 2002; Pessoa 2008; Pessoa and Adolphs 2010). The current model posits that a network of cortical and subcortical regions, including the amygdala, orbitofrontal cortex, anterior insula, anterior cingulate cortex, and inferotemporal visual cortex, plays a central role in the perception and identification of threatening stimuli (Sabatinelli et al. 2005; Pessoa 2008). While the amygdala was initially thought to be involved primarily in the perception of threatening (or more general, emotionally negative) stimuli, the concept of this subcortical region has progressed to a more general function of significance detection and processing (Sander et al. 2003; Williams 2006; Pessoa and Adolphs 2010). According to this concept, the amygdala should be activated when encountering any stimuli that convey a biological significance for the individual, which can either be of positive or negative valence (Sergerie et al. 2008).

A special case of risk and emotion processing exists in the evaluation of food, which can be considered more closely in order to understand the underlying principles (Morton et al. 2014; Suzuki et al. 2017). Earlier studies on the neural processing of the visual presentation of food stimuli in healthy subjects showed heightened activation in insular regions and orbitofrontal cortex (OFC), when hungry (Porubska et al. 2006) and not hungry (Killgore et al. 2003; Simmons et al. 2005). These results are consistent with reports of gustatory representation in the same areas (Kringelbach et al. 2004; Rolls 2001). Furthermore, the OFC integrates different sensory modalities such as gustation and olfaction (Rolls 2001).

Fuhrer et al. (2008) analysed brain activity during the presentation of food items compared to other stimuli. They found stronger activation of the medial prefrontal, insular, anterior cingulate and striatal regions when participants were presented with food stimuli. Siep et al. (2009) reported activity in reward-associated brain regions, the amygdala and OFC, when assessing high versus low caloric nutrition. Rolls (2008) discussed a central involvement of the orbitofrontal and anterior cingulate cortex in the multimodal representation of food particularly associated with reward. These suggestions were also supported by Frank et al. (2010). The medial and lateral prefrontal cortex areas are also involved in value based decision-making (Deco et al. 2013; Dixon and Christoff 2014), and the OFC provides a major role in the evaluation of food (Suzuki et al. 2017).

Following these findings, general regions of interest to be considered are the medial and dorsolateral prefrontal cortex, anterior/subgenual cingulate gyrus, orbitofrontal cortex, anterior insula, amygdala, ventral striatum, medial thalamus and midbrain.

Mental control of emotion processing

On the neurobiological level, several mental interventions have been shown to be able to guide and control emotion processing. This has been done in previous own work for cognitive interventions as '*reality check*' upon the presentation of emotional pictures (Herwig et al. 2007, figure 1.2) as well as using emotion introspection ('*emospection*') as a mindfulness associated intervention for emotion regulation (Herwig et al. 2010). In both cases, the application of the mental intervention led to activation of mid- and dorsolateral prefrontal brain regions as well as a to a down-regulation of amygdala activity.

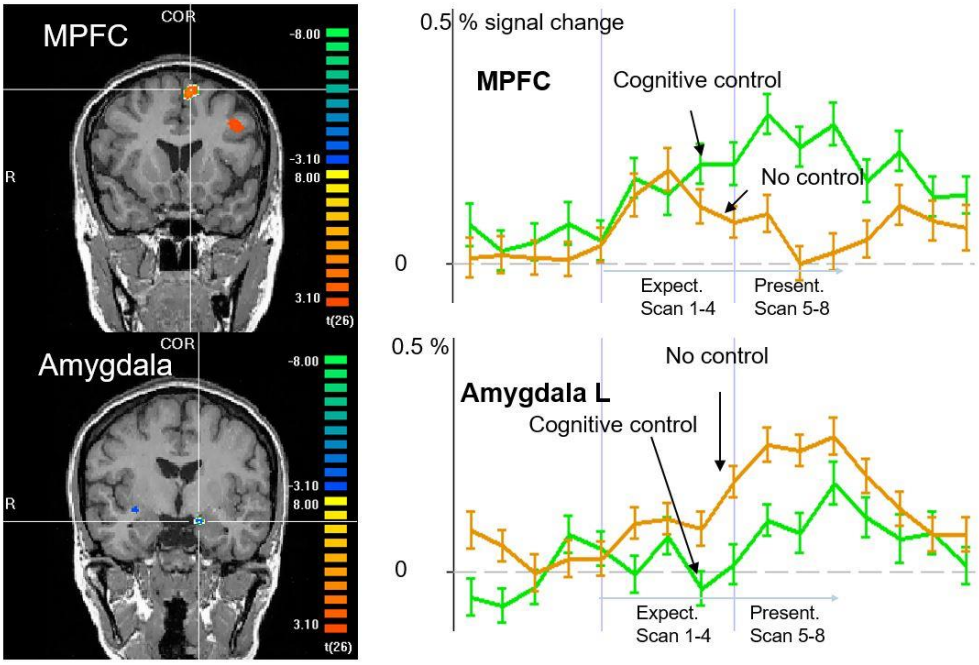

Fig. 1.2 Amygdala down-regulation through a cognitive control intervention, "reality check" along with activation in the medial prefrontal cortex (MPFC), adapted from Herwig et al. 2007.

These, and other studies, have shown that distinct mental interventions lead to aimed emotion regulation effects also on the neurobiological level. This can potentially also be applied in risk evaluation subsuming known neurobiological targets for the interventions.

Generally, neurobiological models of emotion regulation in healthy subjects encompass a top-down effect of mental emotion regulating strategies. These strategies are accompanied by an activation of particularly prefrontal regions, resulting in a reduction of activity in emotion processing brain regions as the amygdalae and the insular cortex (reviews: Bishop 2007; Ochsner, Gross 2007; Kohn et al. 2014, Buhle et al. 2014; Frank et al. 2014).

Psychotherapeutic mental interventions aim at gaining control over affective symptoms and at reducing disabling mood states and emotional reactions. On the neurobiological level, psychotherapeutic strategies are supposed to target a reduced and thus "normalized" activity of the amygdala (Koole 2009, Brühl et al. 2014). Associated psychotherapeutic interventions are elements in cognitive behavioral therapy (Beck 2008). The respective neurobiological background was also proposed in the concept of a "neuropsychotherapy" by Klaus Grawe (Grawe 2004).

Individualized methods to strengthen emotion regulation in the frame of psychotherapy could improve the outcome in these disorders. Real-time functional magnetic resonance imaging (rt-fMRI) could provide such a method for, first, investigating neurobiological basics of distinct short mental emotion regulating interventions and, second, for a personalized training by giving direct feedback on the efficacy of the applied mental intervention.

Thereby, gaining insight into the neural changes associated with successful psychotherapeutic interventions can guide mental activity in the frame of rt-fMRI by improving the interventions targeted at pathological activation patterns in psychiatric disorders (Johnston et al. 2011; Linden et al. 2012; Brühl et al. 2014).

Methodological approach

An established method to assess activated brain regions associated with psychological functions exists in functional magnetic resonance imaging (fMRI; Poldrack et al. 2017). FMRI is a non-invasive method to locate activation in brain regions with relatively high spatio-temporal resolution (Logothetis 2001, 2008). FMRI provides an indirect measure of brain activation by detecting changes in the blood oxygenation level (BOLD signal) associated with neural activity.

It is assumed that the activation of a region leads to the need of oxygen consumption with: first, deoxygenation of the blood, and second, reflexive hyper-oxygenation due to greater blood flow to the region that is reflected in the BOLD signal (Jäncke 2005). The difference between the oxygenated and deoxygenated iron in the haemoglobin leads to different signals following radio frequency impulses in a strong stable electromagnetic field. These signals are recorded and calculated as signal changes over time related to the space of which they result.

We performed the imaging with 3 Tesla whole-body scanners equipped with a head coil. Initially, for each subject T1* weighted anatomical volumes were acquired (matrix size 256 × 256; slice thickness 1mm) for later coregistration with the fMRI. T2* weighted functional MR images were obtained using echoplanar imaging in an axial orientation. Image size for fMRI was 64 × 64 pixels, with study specific fields of view. Study specific stimuli in order to elicit functional neuronal activation within the experimental tasks were presented with LCD video goggles (Resonance Technologies, Northridge, CA). FMRI data were analyzed using BrainVoyager Software (Brain Innovation, Maastricht, The Netherlands).

Preprocessing of the functional scans included motion correction, slice scan time correction, high frequency temporal filtering, and removal of linear trends. Functional images were superimposed on the 2D anatomical images and incorporated into 3D data sets through trilinear interpolation. For group analyses, the individual 3D data sets were transformed into Talairach space (Talairach 1988) and then spatially smoothed with an 8 mm Gaussian kernel. The volume time courses of the groups were pooled. Three-dimensional statistical parametric maps were calculated for the groups with separate subject predictors using a general linear model and a random effects analysis. These maps finally represent the results.

Rationales

The basic rational for this study series was to gain more profound understanding of the neural correlates of risk processing in distinct domains. We selected the domains of risk-evaluation of different items in society (such as guns etc., presented in words) and the field of nutrition (estimating of whether food is healthy or not). We further examined differences in neural activation on modern or evolutionary threatening stimuli. We generally hypothesized the involvement of emotion processing circuits within all domains, and aimed to differentially describe the associated neural circuits.

The first study addressed neurobiological correlates of estimating environmental conditions as being of high or low risk. We hypothesized that evaluating the degree of risk of various hazards for society, in case of high risk, would recruit brain areas involved in different aspects of emotion processing despite not addressing any imminent personal meaning.

The second study aimed at elucidating neural activity associated with the attribution of healthiness to food items. We expected differential evaluation of food healthiness to be associated with the activation of brain areas related to emotion processing, especially in more primordial brain regions such as the midbrain, amygdala and ventral striatum regarding an emotional connotation, and in the insula and OFC regarding the viscero-sensitive interoception. A cognitive approach, however, would involve higher cortical regions such as medial prefrontal and dorsolateral prefrontal cortex regions.

The third study should differentiate neuronal processing between modern and evolutionary content of presented threatening pictures. We proposed that the affective pictures will engage a network of brain regions comprising the amygdala, orbitofrontal cortex, anterior insula, anterior cingulate cortex,

inferotemporal visual cortex as well as medial thalamus and midbrain. We thereby suggested differences in the activation of emotion processing circuits between evolutionary and modern stimuli. The evolution of a fear processing module in response to threats such as snakes and spiders implies that evolutionary threatening stimuli might be associated with a stronger activation particularly in evolutionary older regions as the amygdala, thalamus and midbrain than the modern stimuli, which are supposed to evoke stronger activation in cortical stimulus processing areas such as the inferotemporal cortex.

These study series should serve as a first step of a more profound understanding of neurobiological principals of risk processing. A next step shall focus on applying mental interventions for guided intentional information processing and lead to a decision making upon risk confrontation. This may comprise the awareness for emotional and cognitive components in order to overcome for instance potential fear driven approaches bypassing the rational approach. These investigations based on models of cognitive control of emotions (Phillips et al. 2008, figure 1.3; Etkin et al. 2015) were performed in another line of research (Herwig et al. 2007, 2010) and contribute as a basis for the application in risk situations and for another step of training such mental interventions based on neurobiological findings.

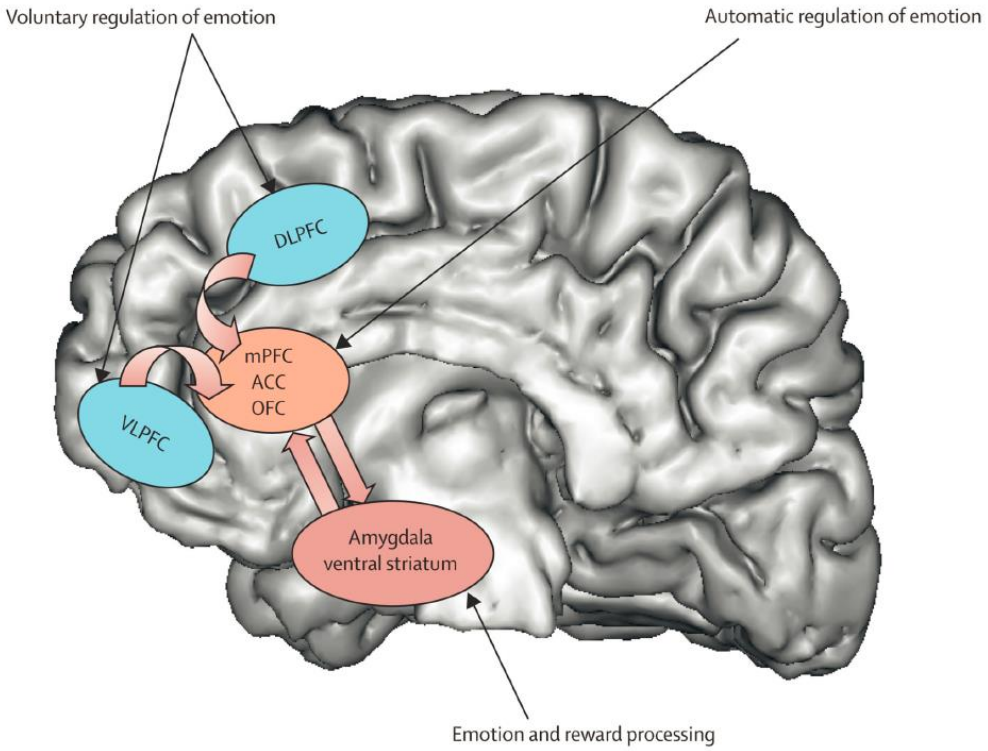

Fig. 1.3 Model of bottom-up and top-down processes in emotion regulation (figure from Phillips et al. 2008). VLPFC ventrolateral prefrontal cortex, DLPFC dorsolateral prefrontal cortex, mPFC medial prefrontal cortex, ACC anterior cingulate cortex, OFC orbitofrontal cortex. The voluntary regulation of emotion can be applied to cope with and to overcome disadvantageous consequences of automatic regulation of emotion by for instance impulse control.

This is for instance done in educational training of risk management. An additional approach from the neurobiological perspective is to train such interventions be means of neurofeedback of the targeted neural activation (Sitaram et al. 2017). In an earlier feasibility study, this principle was demonstrated for the cognitive control intervention 'reality check'. A training of this intervention with feedback of the amygdala activity led to enhanced down-regulation of the amygdala in the course of four training sessions (Brühl et al. 2014).

Such, in the fourth study we hypothesized a training effect of emotion regulation by a cognitive intervention, 'reality check', onto the presentation of emotional pictures by means of the down-regulation of amygdala activity, subserved by realtime feedback of the amygdala activity, improving over a series of training sessions unlike as in a control group.

Overview

We performed a series of studies to investigate neural correlates of risk and emotion processing in different domains as well as the potential of intentionally influencing information processing for emotion regulation.

The first study dealt with neurobiological correlates of certain environmental risk evaluation. The second study investigated neural activation associated with evaluating food items to be healthy or not healthy. The third study differentiated neural activity on evolutionary and modern threats. The fourth study assessed emotion regulation training by means of real-time neurofeedback.

The underlying hypotheses are outlined in the single studies. A general hypothesis was a close relationship between risk and emotion processing. The common method of all studies was the investigation with functional magnetic resonance imaging in healthy subjects. The results may serve to develop a higher level of awareness for dealing with environmental threats and as a basis for actively initiated mental interventions for a secure and sustainable coping with the environment.

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1. Scientific work

1.1. Neural correlates of evaluating hazards of high risk

Uwe Herwig[1*], Annette B. Brühl[1*], Marie-Caroline Viebke[1], Roland W. Scholz[2], Daria Knoch[3], Michael Siegrist[2]

[1]Psychiatric University Hospital Zürich, Switzerland

[2]Institute for Environmental Decisions, ETH, Zürich, Switzerland

[3]Department of Psychology, University of Basel, Switzerland

*both authors contributed equally

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Introduction

Analyzing the risk of environmental, personal and political hazards is an everyday challenge. A proper risk assessment is essential for survival by coping with respective threats and for allocating necessary resources. Many, if not most decisions are made with a certain grade of uncertainty resulting in a choice under risk. Then, risk can consist in a disadvantageous outcome for the person in case of the "wrong" decision or in happiness after the right one. Otherwise, risk may be represented by a concrete threat or incident that might occur or not. This has an emotional impact and such risk processing was associated with emotion processing (e.g. Bach et al. 2009; Mohr et al. 2010a). Thereby, particularly lay persons that are not familiar with for instance the scientific and statistical background of certain risky conditions are prone to a more affect- or emotion-based estimation when faced with the necessity of evaluating a risk or a benefit (Loewenstein et al. 2001; Slovic et al. 2002). They then rely stronger on previous experiences, trust, narratives or metaphors than on sound knowledge (Fischhoff et al. 1982; Sjöberg 1998), and they may use somatic signals associated with the emotional impact (Damasio 1996) related to a hazard as a cue for intuitively estimating the risks: "risk as feelings" (Slovic et al. 2004).

Recent studies provided a profound investigation and discussion on the relation of risk processing and emotions regarding the neurobiological backgrounds (Bach et al. 2009; Mohr et al. 2010a; Quartz 2009; Vorhold et al. 2007; Xu et al. 2009). A meta-analysis assessing risk-processing related brain regions identified a network including bilateral anterior insula, dorsomedial and posterior thalamus, dorsomedial and right dorsolateral prefrontal cortex (DM/DLPFC), and right parietal cortex to be

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Abbreviations: ACC anterior cingulate cortex, BOLD blood oxygen level dependent, DLPFC dorsolateral prefrontal cortex, DMPFC dorsomedial prefrontal cortex, FDR false discovery rate, fMRI functional magnetic resonance imaging, IAT implicit association task, MPFC medial prefrontal cortex, PCC posterior cingulate cortex, TR repetition time, VMPFC ventromedial prefrontal cortex.

involved (Mohr et al. 2010a). In this context, it was argued that lay-persons may recruit more emotion-associated brain areas as insula, amygdala and thalamic regions during risk processing (Anderson et al. 2003; Craig 2002; Critchley et al. 2004; Singer et al. 2009; Vorhold et al. 2007).

The studies in this field particularly aimed at elucidating the neurobiological basis of risk assessment by means of functional magnetic resonance imaging (fMRI) using conditions with a subject-related risk implemented in the experimental tasks. Presented stimuli or terms had to be assessed in relation to a personal risk for the subject, or decision making was investigated under risky conditions with reward or loss for the subject (overview in Mohr et al., 2010a, e.g. Bach et al., 2009; Christopoulos et al. 2009; Huettel 2006; Preuschoff et al. 2006; Quartz 2009; Smith et al. 2009; Vorhold et al, 2007; Xu et al. 2009, and others). This meant also a direct emotional impact of the risk-related stimulus to the subjects: a possibly immediate negative consequence as fear or enjoying a positive outcome. Further, related studies primarily investigated risk evaluation versus control conditions that did not contain a risk evaluation component. Such, activity associated with estimating specifically the grade of for instance a high level of risk was not investigated. Accordingly, it appears valuable to investigate a risk condition which does not focus on a direct personal risk but on general risk evaluation. Therefore, subjects can rate the risk of certain hazards for the society and not for themselves. Further, in order to focus on brain activity particularly associated with a higher degree of risk, conditions with a higher risk can be compared with those of lower risk. This appears more suitable to investigate activation associated with gradually increasing risk than comparing with a non-risk control condition.

Our aim was to use such a methodological approach in order to assess the neurobiological backgrounds of risk processing. Based on the mentioned previous reports associating risk and emotion processing circuits (Mohr et al. 2010a; Quartz 2009) we hypothesized that evaluating the degree of risk for the society of various hazards will in case of high risk recruit brain areas involved in different aspects of emotion processing despite addressing no imminent personal meaning. In this context, experience-based emotional signals or markers may be important to contribute to risk estimation. Primary areas of interest were thus insula, thalamus, lateral and medial prefrontal regions and amygdala.

We used non-imminent and non-personal risk terms representing possible hazards for the society with the instruction to assess the risk for the general (Swiss) society in order to differentiate from a personal emotional relation as far as possible (Slovic et al. 2004). For instance, a condition as "skiing" might be associated with positive emotions for the individual, but might represent a certain risk for the society (or not) due to a high accident rate. Further, we focused on the evaluative aspect and less on the decision making or choice process by analyzing the evaluation period prior rating feedback. Our approach is in line with the risk perception literature, in which people's risk perception is similar measured as in our study, in order to find out why societies are concerned about some hazards, but not other hazards (Slovic 1987). Thereby, the subjects had not to decide for a certain action but to indicate

their risk estimation of a presented term. In this context, and differing from previous studies contrasting general risk evaluation with non-risk control conditions, we analyzed brain activation specifically associated with high versus low risk. Further, we also discriminated activity in the earlier phases of risk evaluation in order to detect brain regions with a more phasic or initial contribution to the evaluation process. Finally, the results were exploratorily compared with individual emotion ratings of the same hazards.

Experimental Procedure

Subjects

Twenty healthy subjects (age 22-29 years, mean 25.1, all right handed, 11 females) were recruited to participate in this study and gave written informed consent. The study was approved by the local ethics committee. Two subjects were excluded afterwards because of movement artefacts (exceeding 3 mm in one direction), such that data of 18 subjects were analyzed. The subjects were healthy without any psychiatric or neurologic history and did not take any psychotropic medication.

Experimental design

During fMRI scanning, the subjects evaluated the general risk of different hazards such as "nuclear power", "smoking", "bicycling" etc. for the society (complete list originally in German, English translation in supplementary material). A related paradigm has earlier been used for examining the research question of why lay people perceive different hazards differently (Siegrist et al., 2005; Slovic, 1987), and was adapted for the current study comprising common potential hazards. The subjects were presented written terms for 5940 ms (equivalent to 3 repetition times, TR, for the fMRI volumes). In this period, they were instructed to judge the risk of the respective hazard for the local, i.e. Swiss, society. So, this period comprised the processes of perceiving, evaluating and judging/estimating of the hazards ("evaluating period"). Subsequently, a five-step visual analogue scale was presented for 3,960 ms (two volumes) on which the subjects indicated the individually estimated risk from very low to very high by moving a cursor using a trackball with the right hand (fig. 1), the "rating period". Altogether, 50 stimuli (terms) were presented in a randomized order. The following baseline period (13,700 ms, 7 TR) was of sufficient duration to allow the blood oxygen level-dependent signal to wear off before the next trial. The task was programmed with Presentation™, Neurobehavioral Systems, USA. The terms were presented in black letters on a white background via digital video goggles (Resonance Technologies, Northridge, CA) in a size approximately equivalent to font size 24 in the focus of a laptop screen in reading distance such that minimal eye movements were required to read the terms. After scanning, the subjects were asked to rate the subjective emotional valence of the risk terms on a nine-step visual analogue scale (very negative = 1, neutral = 5, very positive = 9).

Data acquisition

Imaging was performed with a 3.0 T GE Signa™ HD Scanner (GE Medical Systems, Milwaukee). Echoplanar imaging was performed for fMRI (repetition time TR/echo time TE 1,980 ms/32 ms, 22 sequential axial slices, whole brain, slice thickness 3.5 mm, 1 mm gap, resulting voxel size 3.125 × 3.125 × 4.5 mm, matrix 64 × 64 pixels, field of view 200 mm, flip angle 70°). 611 volumes were obtained per subject, 12 per trial. Four initial volumes were discarded to allow for T2 equilibration effects, seven volumes were added for a final baseline. High-resolution 3-D T1 weighted anatomical volumes were acquired (TR/TE 9.9/2.9 ms; matrix size 256×256; 1 mm × 1 mm × 1 mm resolution) for coregistration with the functional data.

Data analysis

fMRI data were analyzed using BrainVoyager™ QX 1.10.1 (Brain Innovation, Maastricht, The Netherlands). Preprocessing of the functional scans included motion correction, slice scan time correction, high frequency temporal filtering, and removal of linear trends. Functional images were superimposed on the 2D anatomical images and incorporated into 3D data sets. The individual 3D data sets were transformed into Talairach space resulting in a voxel size of 3 mm × 3 mm × 3 mm and then spatially smoothed with an 8 mm Gaussian kernel for subsequent group analysis. From each included subject (n=18), the individual ratings of each term were analyzed concerning risk value and divided in three groups: low risk, medium risk, high risk. Low risk was defined as ratings between 1.00 and 2.00, medium risk between 2.01 and 3.40 and high risk between 3.41 and 5.00 based on the distribution of the evaluation ratings (consider supplementary material figure S2). Individual protocols for each subject for the fMRI-analysis were built comprising the individually rated items meeting the three conditions low, medium, high risk and the respective three presentation conditions of the rating scale as predictors resulting in each six predictors for the design matrix. The periods were modelled as epochs using a two-gamma hemodynamic response function provided by BrainVoyager™ adapted to the applied period duration.

The fMRI data analysis, based on the general linear model (GLM), comprised the following steps: First, fixed effects analyses were calculated separately for each subject for the contrast comparing the individual conditions ‘high risk’ versus ‘low risk’ resulting in summary images. The summary images were subjected to second level group analyses. Thus, those trials in which the terms were rated with ‘high’ and ‘low’ risk were considered, irrespective of the word contents. For analyzing the whole evaluation period, three-dimensional statistical parametric maps were calculated for the groups using a random effects analysis. The main analysis focused on the contrast “high risk > low risk”. The voxel-wise threshold for reporting results in the random effects analysis was set at $p<0.001$. To correct for multiple comparisons, a Monte Carlo simulation was used (Goebel et al., 2006) for estimating cluster-level false-positive rates on these maps, yielding after 10.000 iterations a minimum cluster size threshold of 4 voxels of 3x3x3 mm (108 mm3), corresponding to a corrected cluster level $p < 0.04$.

As the evaluating period comprised early perceptual and rapid judgemental as well as later explicitly estimative and perhaps already preparatory processes we were further interested in brain activity particularly in the earlier periods of risk evaluation with an exploratory approach. When of course overlapping with the analysis covering the whole period, this analysis revealed regions particularly active in the initial phase of evaluation, of which the associated activation may be mitigated when analyzing the whole period. This appeared important for us, as risk evaluation may be regarded as a chain of process comprising initial perception of the stimulus, quick intuitive/emotional estimation, rational consideration and a final decision. Therefore, we applied a deconvolution analysis (Dale and Buckner, 1997; Pierce and Redcay, 2008) to achieve a better temporal resolution: We defined the three volumes acquired during the assessment period as single time points using no specific hemodynamic response function. We analyzed the brain activity within the period of the first volume separately (approximately 2 seconds), and also for the first two volumes (near 4 seconds) of the three volume period. For these analyses, we used a statistical threshold of $p < 0.001$, corresponding to a correction for multiple comparisons according to the FDR corrected $p < 0.05$, together with a cluster threshold of 108 mm3, and also analyzed exploratorily with a threshold of $p < 0.005$ (uncorrected). By using this approach, those areas were identified where the activation significantly differed between the conditions 'high risk' and 'low risk' during the period of the first volume and during the first two volumes together.

Based on the emotion ratings, we performed an analysis of brain activity during the presentation/ evaluation period of those terms rated individually to be associated with negative affect compared with those rated positive or neutral. Analogue to the risk analysis, the threshold was set to $p < 0.001$ in a random effects analysis. Exploratorily, we assessed activity also at $p < 0.01$. Finally, the emotion ratings were compared by using Pearson's correlation with the risk ratings during the experiment in the scanner.

Results

Behavioural data

Twenty subjects were scanned of which 18 subjects were included into the analysis. Altogether, 890 trials were presented and rated (out of 900 trials = 50 trials per subject, 10 trials were not presented in one subject due to technical reasons). The subjects attributed low risk to on average 16.9 terms (SD 7.2), medium risk to 18.8 terms (SD 6.8) and high risk to 13.7 terms (SD 7.1), resulting in overall n = 305 trials with low risk, medium risk in n = 338 trials and high risk in n = 247 trials. The correlation of the individual risk and emotion ratings revealed in 8 of 50 terms a significant correlation. In 42 terms we found no correlation between individual rating of emotional valence and rating of the risk for the society. The mean rating data for risk and emotional valence are presented in the supplementary table S1.

fMRI results

Comparing the conditions 'high risk'- and 'low risk'-evaluation for the whole presentation period of 5940 milliseconds with a random effects analysis, we found a stronger activation in the 'high risk' condition in left anterior insula, medial thalamus, left head of the caudate nucleus, the posterior cingulate cortex (PCC) and the precuneus (fig. 2, table 1).

In the deconvolution analysis, the early phase of the evaluating period during the first volume revealed activation in bilateral medial thalamus and in posterior higher perception processing regions such as bilateral temporo-occipital junction and precuneus on an exploratory significance level of $p < 0.005$. When considering the first two volumes together, we found additional activation on a level $p < 0.001$ in the anterior cingulate cortex (ACC), right anterior insula, caudate nuclei, medial prefrontal cortex (MPFC) and right dorsolateral prefrontal cortex (DLPFC; fig. 3, table 2). Results of the medium risk terms were assessed exploratorily and are provided descriptively with the time courses in the figures, showing a signal change ranging between the activations associated with low and high risk evaluation.

When comparing the activations associated with those terms individually rated to be of negative versus positive, or negative versus neutral emotional valence, we observed no stronger activation related to the negative valence on the level $p < 0.001$ (random effects). Particularly, we found no activity related to negative affect in those regions identified to be associated with high risk also on an exploratory level of $p < 0.01$ (random effects).

Discussion

Our aim was to investigate neural correlates associated with estimating a high risk of environmental and technological hazards for the society. We found distinct brain regions involved, comprising prefrontal, insular, and posterior cortical regions, as well as medial thalamus and caudate head. These results are discussed in the context of emotional and intuitive processing.

Anatomical and functional features of the brain regions involved in risk-evaluation

Estimating distinct hazards to be of high risk was associated with medial thalamic and anterior insular activation. Anatomically, medial thalamic regions receive input from viscerosensitive and pain mediating brainstem areas such as the parabrachial nucleus, the subnucleus reticularis, and the periaqueductal gray (Craig 2002; Vogt 2005). They are considered to form a relay within the viscerosensitive pathway towards particularly insular regions, ACC and amygdala (Augustine 1996; Craig 2002; Vogt 2005). The insula is involved in the processing of multimodal visceral, sensory and emotional stimuli (Calder 2003; Craig 2002; Critchley et al. 2004; Damasio et al. 2000; Paulus and Stein 2006; Singer et al. 2009). Insular regions have a wide range of reciprocal connections to prefrontal areas, ACC, medial thalamus, amygdala, hypothalamus, and brainstem regions as the parabrachial nucleus for relaying visceral afferents (Augustine 1996). It was proposed that the

interoceptive sensation of bodily signals depends on input from the viscera represented in the anterior insula (Critchley et al. 2004; Singer et al. 2009). In the context of risk processing, thalamic and insular contributions were reported during intertemporal choices involving losses which were associated with accompanying negative emotions (Xu et al. 2009). Thalamus and insula were also found to be involved in risky decisions and in anticipating risk (Huettel 2006; Mohr et al. 2010a; Mohr et al. 2010b). We here propose medial thalamus and anterior insula to be involved in the mediation of bodily interoceptive signals for evaluation purposes in response to the faced hazard.

We also found left caudate head activation associated with high risk, particularly in the initial phase of the evaluation period. The caudate head shares prominent connectivity with the DLPFC through a series of parallel loops that project from the cortex to the input and output nuclei of the basal ganglia, then to the ventral-anterior and dorso-medial nuclei of the thalamus, and then back to the cortex (Alexander et al. 1986; Middleton and Strick 2002). The caudate, particularly its head, has been proposed to be sensitive to implicit executive processing (Melrose et al. 2007; Seger and Cincotta 2005), and being involved in intuition and implicit learning (Lieberman 2000). Functional imaging studies link activity in the head of the caudate with information integration (Seger and Cincotta 2002) and with executive functions related to probabilistic classification (Poldrack et al. 1999). A recent study reported the caudate nucleus to be involved in a task assessing risk-averse attitudes (Engelmann and Tamir 2009). Taken together, the caudate in the context of risk estimation may function as a relay between cortical evaluation and thalamic signalling contributing to classification of the presented terms and to selection of implicit behavioural coping strategies.

The ACC, also activated during the 'high-risk' condition, is involved in conflict monitoring with potential affective consequences comparing the actual state with a desired state (Carter et al. 2000; Vogt 2005). Being confronted with a high risk condition means a discrepancy to the desired state, resulting in a conflict signal. Cingulate regions are known to mediate integration and evaluation of emotional, motivational and cognitive information, and to modulate attention (Bishop et al. 2004; Vogt 2005) with direct connections to amygdala, thalamus, prefrontal and insular areas and to the posterior parietal lobe (Goldman-Rakic 1988). Cingulate activity in risk tasks was associated with a higher probability of a risky choice (Christopoulos et al. 2009) and was increased when risky choices involved immediate losses (Xu et al. 2009). Activation within the PCC was suggested to signal the subjective preferences that guide visual orienting within a gambling task comprising risky choices (McCoy and Platt 2005). ACC and PCC were reported to be involved throughout all phases of risky decision making in a task concerning financial aspects (Engelmann and Tamir 2009; Shackman et al. 2011).

We also found activation in medial and dorsolateral PFC when estimating high risk. This indicates an association with internal control and executive functions (Miller and Cohen 2001; Wood and Grafman 2003) which are regularly present in studies assessing cognitive and emotional functions (Pessoa 2008). The contribution of these areas lead to argue that components of an analytical system

are also involved in risk processing in lay people, or that executive strategies may be primed or selected for instance in the DLPFC (Mohr et al. 2010a). This can be accounted also to the specific instruction to rate the hazards regarding the risk for the society which may favour analytic processes apart emotional or intuitive components.

Posterior cortical activations associated with high risk occurred in temporo-occipital cortex regions and in the precuneus. The temporo-occipital junctional cortex, covering sensory associative cortices, is involved in multisensory integration of information (Beauchamp 2005), which is increased by attention-requiring processes and efforts of performance (Mesulam 1998), and in theory of mind (Lee and Siegle 2009). The activation in the current study, found already in the very early evaluation period, may indicate an attentional bias towards risk-related terms, which also has been shown in the context of anxiety (Lee and Telch 2008). The precuneus was reported to be involved in episodic memory retrieval and self-related processing (Cavanna and Trimble 2006), which are also relevant during risk estimation.

Regarding the explorative deconvolution analysis and descriptively the time courses, we found anterior insula, medial thalamus and anterior cingulate to be active in the earlier periods of risk evaluation. This implies a role of a quicker and more phasic signalling in the context of detecting or estimating a high risk.

Taking together the brain activations and their functional implications, one might suggest pathways of risk processing. These include temporo-occipital areas for initial stimulus analysis with respect to the impact for the subject and others. They further comprise an intuitive estimation involving viscerosensitive areas as medial thalamus and insula, with a supposed bottom-up link towards prefrontal areas via the caudate for possibly selecting implicit strategies. This finally leads to evaluation and decision making involving prefrontal areas, based on a nominal value comparison regarding the impact for the person involving cingulate regions.

Intuitive risk estimation and „gut"-feelings

Risk evaluation by lay persons has been considered to be based on emotional signals, expressed as "risk as feelings" (Loewenstein et al. 2001; Slovic et al. 2004), and to involve an affect-based experiential rather than an analytical system (Slovic et al. 2004; Vorhold et al. 2007). For instance, implicit measures may reveal negative attitudes towards for instance nuclear power that were not detected by explicit measures (Siegrist et al. 2006). The experiential system may be more important than the analytic system when lay people assess technological risks compared to technical experts.

Our data enhance and differentiate this view by supporting the contribution of a viscerosensitive component to the estimation of high risk. Earlier reports addressing risk evaluation emphasized the emotional components as reflected by for instance amygdala, ventromedial prefrontal cortex (VMPFC), and insular activation (Fukui et al. 2005; Huettel 2006; Mohr et al. 2010a; Quartz 2009;

Vorhold et al. 2007; Weller et al. 2007; Xu et al. 2009). These regions may be involved in risk evaluation in general, independent of the degree of risk. This may explain on the one hand that these regions were not found to be differentially activated here when contrasting high versus low risk, and on the other hand the finding of areas specifically associated with high risk that were not identified in previous studies.

A discriminative view of emotional and evaluative aspects of risk assessment is supported by our finding that the majority of our terms, 42 out of 50, had no correlation between risk estimation and emotional valence. Further, analyzing the functional data based on the individually rated emotional valence of the potential hazards did not show any brain regions to be activated. This has, of course, to be regarded as exploratorily, also because the emotional arousal contributing to emotion related brain activation was not directly assessed, but it at least implies that risk evaluation and emotional evaluation may not be coupled tightly.

Within this context, we revealed a contribution of areas representing associations with implicit and viscerosensitive functions as caudate, medial thalamus and insula. This lends support to the assumption of an evaluation concerning the risk of hazards by lay-persons based on intuitive processes and bodily signals. These are linked to emotions and may support affective processing, however, they may form an own functional entity.

Regarding proposed systems for decision making in the context of risk analysis, the analytical and the experiential system (Slovic et al. 2004), viscerosensitive signals are suggested to serve the experiential one. This is used when lay persons have to base their decision more on experiences, which are biased more by emotional influences, than on logical and analytical considerations or on scientific facts (Finucane et al. 2000). From a phylogenetic perspective, an evaluation system based on experiences and non-analytical estimations makes sense and is important in beings without highest rational capacities, and for quick response in case of danger. In human, these evaluation systems are accordingly used in conditions without sufficient knowledge for analytical approaches, thus when intuition is required (Lieberman 2000; Volz and von Cramon 2006). In such evaluation and decision contexts, we often rely on signals that are commonly termed as “gut”-feelings.

Conclusion

We emphasize a contribution of particularly insular, thalamic and caudate regions to be involved in signalling high risk, which here was not associated with the emotional valence of the risk items. These areas have earlier been reported to be associated with, beside emotional, viscerosensitive and implicit processing. This implies assumptions of an intuitive contribution, or a “gut-feeling”, not necessarily dependent of the subjective emotional valence, when estimating a high risk of hazards for the society. In risk communication, this affective foundation, based on “gut”-feelings, of lay people’s assessment of hazards may be taken in to account.

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Figures

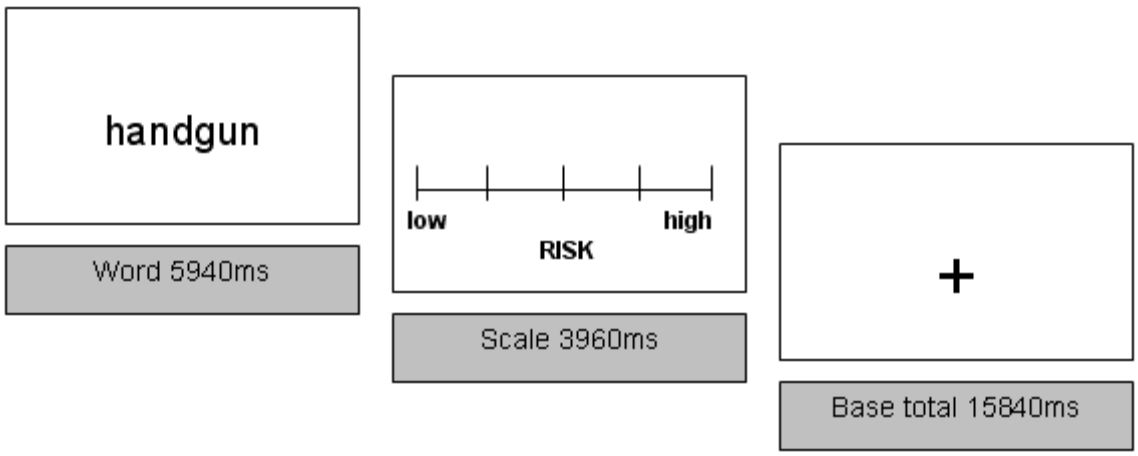

Fig. 1 Experimental task. Trials started with a term presentation of near 6 seconds with the task to evaluate the risk of the term for the society. This was followed by a feedback period of near 4 seconds. A baseline condition of near 16 seconds was implemented between the trials.

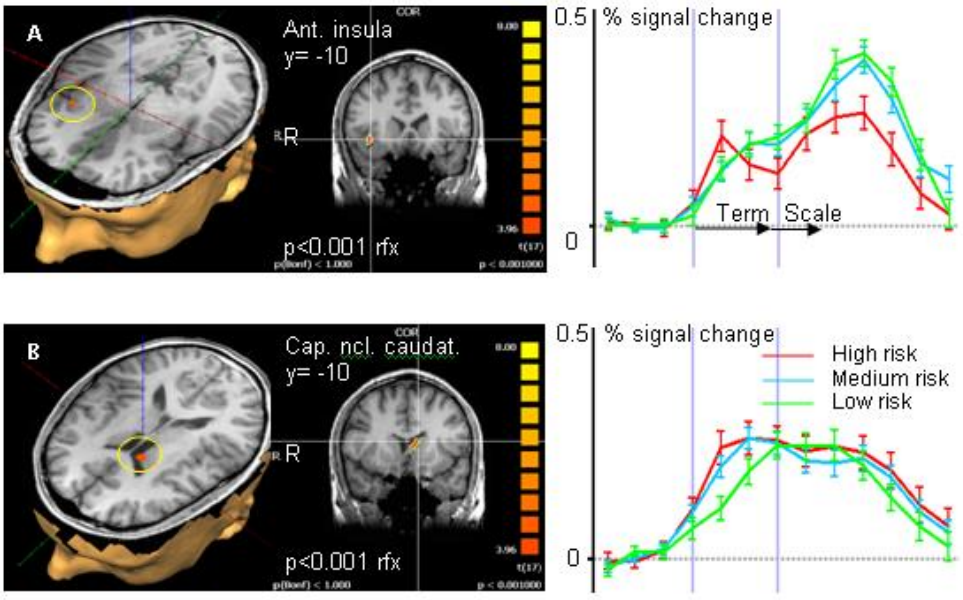

Fig. 2 Brain activation with colour coded maps and time courses according to a random effects analysis ($p<0.001$) of the whole evaluation period comparing high risk against low risk. A. insula, B. head of caudate.

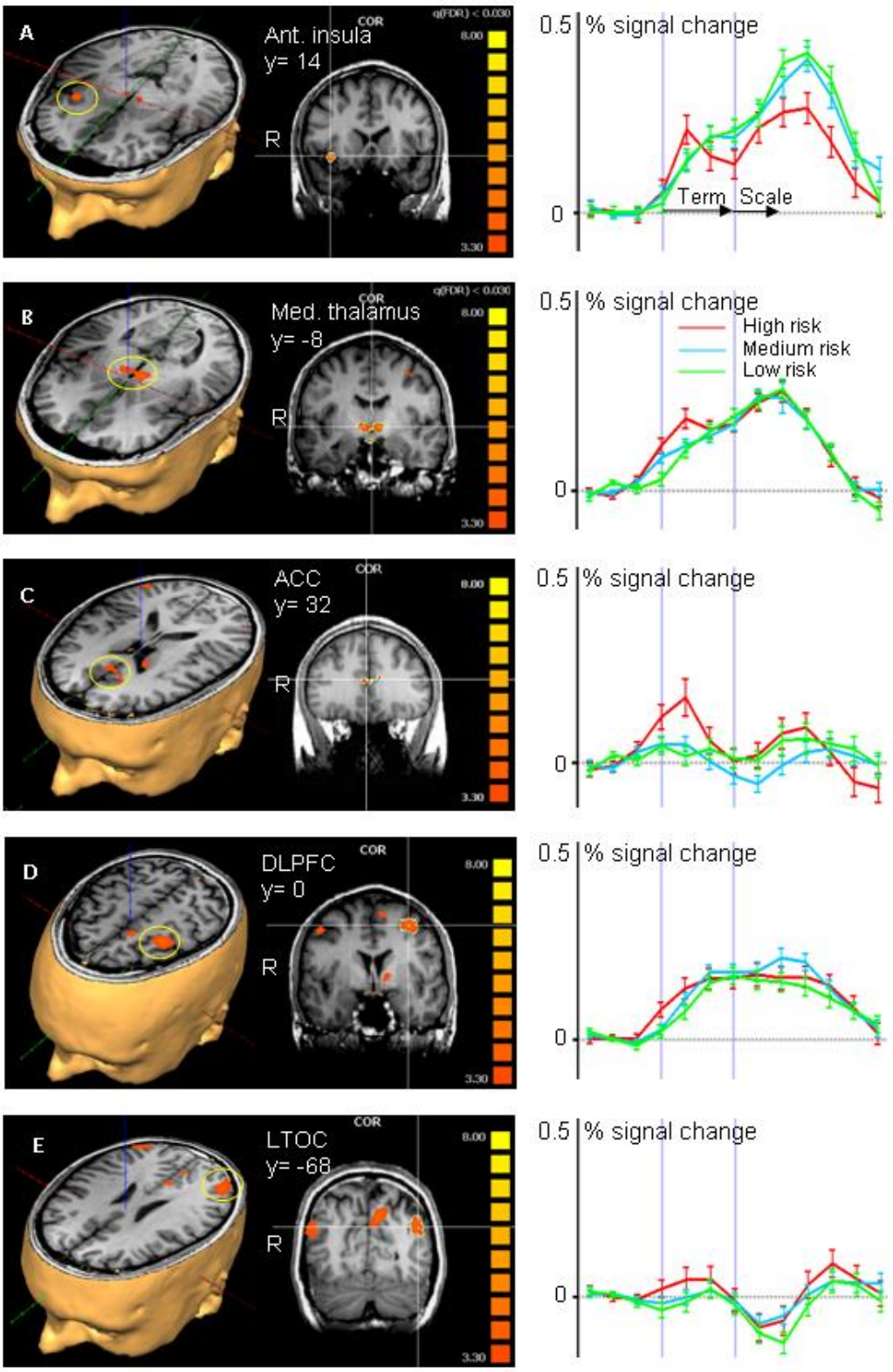

Fig. 3 Brain activation including time courses according to the deconvolution analyses ($p<0.001$) of the first four seconds of risk evaluation high vs. low risk, here A. anterior insula, B. medial thalamus regions, C. anterior cingulate cortex (ACC), D. dorsolateral prefrontal cortex (DLPFC), and E. lateral temporo-occipital cortex (LTOC).

Tables

				Cluster size	
	Peak X	Peak Y	Peak Z	mm3	t-max
Ant. insula L (fig. 3A)	36	17	-5	116	4.6
Med. Thalamus	3	-7	-2	172	4.7
Posterior cingulate cortex L	-6	-34	28	403	5.0
Caudate L (fig. 3B)	-6	11	10	264	4.5
Precuneus	-6	-73	31	122	4.7

Table 1 Contrast 'high risk versus low risk' during the complete period of risk estimation (random effects analysis $p < 0.001$). Abbreviations: ant anterior, L left, med medial.

				Cluster size	
a.) first volume	Peak X	Peak Y	Peak Z	mm3	t-max
Med. thalamus R	12	-7	-2	157	3.0
Med. thalamus L	-9	-10	1	227	3.2
Temporo-occipital cortex R	45	-67	16	928	3.3
Temporo-occipital cortex L	-42	-70	19	1503	3.6
Precuneus L	-9	-70	34	415	3.4
Inferior temporal gyrus L	-54	-40	-12	256	3.5
b.) first two volumes					
Ant. insula R (fig. 2A)	36	14	-8	355	4.3
Med./ant. thalamus blt (fig. 2B)	-6	-7	1	1631	4.2
Head of caudate nucleus L	-12	11	13	244	3.9
Precallosal cingulate cortex (fig. 2C)	6	32	16	268	3.6
Ant. cingulate cortex L	-9	23	28	142	3.7
Dorsolateral PFC L (fig. 2D)	-33	-1	49	1712	4.2
Dorsolateral PFC R	45	-4	43	287	3.9
Med. PFC L	-6	2	58	685	3.8
Posterior cingulate cortex	0	-40	28	546	3.6
Temporo-occipital cortex L (fig. 2E)	-39	-70	22	2235	4.2
Temporo-occipital cortex R	45	-70	25	981	4.1
Precuneus L	-9	-70	34	1527	4.7
Inferior temporal gyrus L	-51	-37	-15	306	4.3

Table 2 Activated regions during a.) the first ($p < 0.005$) and b.) the first two volumes ($p < 0.001$) of the presentation and evaluation period, comparing 'high risk versus low risk' by applying a deconvolution analysis. Abbreviations: R right, L left, blt bilateral, Med medial, inf inferior, ant anterior, cap caput, ncl nucleus, caud caudatus, ACC anterior cingulate cortex, PFC prefrontal cortex.

Supporting information

List of terms in English translation (alphabetic order)

English term	German term	mean risk rating (SD) Scale 1-5	mean emotional valence (SD) Scale 1-9	Correlation (Pearson's r) /significance (p)
alcoholic drinks	Alkoholische Getränke	3.40 (0.81)	5.20 (1.47)	-.181 /.445
artificial sweetener	Künstliche Süssstoffe	1.83 (0.71)	4.89 (1.33)	-.416 /.068
asbestos	Asbest	3.13 (1.05)	2.92 (1.54)	-.256 /.276
aspirin	Aspirin	1.65 (0.65)	5.41 (1.62)	-.263 /.263
atomic bomb	Atombombe	2.95 (1.21)	1.22 (0.68)	-.350 /.130
bird flu	Vogelgrippe	2.62 (0.86)	3.06 (1.01)	**-.684 .001**
BSE	BSE	2.48 (0.87)	3.08 (0.95)	-.342 /.140
caffeine	Koffein	1.67 (0.55)	5.61 (1.44)	-.303 /.195
cars	Autos	2.89 (0.97)	5.65 (1.41)	.041 /.862
cloning	Klonen	2.67 (0.88)	4.02 (1.65)	-.427 /.060
commercial aviation	Kommerzielle Luftfahrt	2.32 (0.89)	6.15 (1.31)	**-.690 /.001**
cycling	Velo fahren	1.97 (0.82)	7.50 (1.16)	-.367 /.119
electric power line	Starkstromleitung	2.33 (0.87)	4.61 (1.14)	**-.585 /.030**
electrosmog	Elektrosmog	2.65 (0.94)	3.13 (1.00)	-.252 /.297
final storage for nuclear waste	Endlager für radioaktive	3.11	2.61	**-.511**

	Abfälle	(0.93)	(1.20)	**/.021**
flood	Hochwasser	3.28	2.97	**-.587**
		(1.02)	(1.38)	**/.007**
food colouring	Lebensmittelfarbstoffe	1.89	4.74	-.256
		(0.68)	(1.07)	/.275
food irradiation	Lebensmittelbestrahlung	2.80	3.38	-.074
		(0.69)	(1.15)	/.755
food preservative	Konservierungsmittel	1.85	4.83	-.139
		(0.55)	(1.19)	/.560
genetic engineering	Gentechnologie	2.94	4.52	-.432
		(0.84)	(1.45)	/.065
handgun	Handfeuerwaffen	3.59	2.14	-.068
		(0.77)	(0.96)	/.777
high radiation nuclear waste	Hochradioaktiver Abfall	3.35	1.80	-.089
		(1.11)	(0.75)	/.708
household aids	Haushaltsgeräte	1.94	6.35	-.124
		(0.77)	(1.56)	/.604
hydro power	Wasserkraftenergie	1.71	7.51	**-.481**
		(0.73)	(1.24)	**/.032**
low radiation nuclear waste	Schwachradioaktive	2.91	3.28	-.379
	Abfälle	(0.62)	(1.35)	/.102
microwave oven	Mikrowellenofen	1.71	5.68	-.070
		(0.41)	(1.29)	/.770
mildew/mould	Schimmelpilze	2.07	3.41	-.108
		(0.63)	(0.96)	/.649
nuclear fission	Kernspaltung	2.89	4.55	-.129
		(0.95)	(1.30)	/.587
nuclear fuel rod	Brennstäbe für	2.78	3.62	-.190
	Atomkraftwerk	(0.85)	(1.22)	/.423
nuclear fusion	Kernfusion	2.57	5.36	-.127
		(0.80)	(1.54)	/.593
nuclear power	Nuklearenergie	2.88	4.49	-.290
		(0.85)	(1.86)	/.215
nuclear power	Atomenergie	2.82	4.44	-.055
		(0.71)	(1.40)	/.823
nuclear power plant	Atomkraftwerk	2.83	4.24	-.236

		(0.69)	(1.56)	/.331
nuclear power plant cooler	AKW Kühlturm	2.33	5.30	-.492
		(0.95)	(1.56)	/.032
nuclear radiation	Radioaktive Strahlung	3.13	2.19	-.310
		(0.80)	(0.91)	/.184
nuclear reactor	Kernreaktor	2.86	4.05	-.201
		(0.96)	(1.24)	/.396
nuclear warhead	Atomsprengkopf	3.10	2.03	-.045
		(1.06)	(0.97)	/.851
nuclear weapons	Atomwaffen	3.33	1.21	-.259
		(1.00)	(0.51)	/.270
ozone	Ozon	3.69	2.56	**-.813**
		(0.71)	(1.09)	**/.000**
pesticide	Pestizide	2.74	3.13	-.069
		(0.70)	(0.84)	/.780
plutonium	Plutonium	2.94	3.47	**-.637**
		(0.88)	(1.31)	**/.003**
railway	Eisenbahn	1.84	7.17	.028
		(0.87)	(0.87)	/.911
repository for nuclear waste	Atommüllager	3.08	2.31	-.394
		(0.91)	(1.81)	/.100
skiing	Skifahren	2.25	7.59	-.200
		(0.97)	(1.16)	/.399
smoking	Rauchen	3.97	2.63	.191
		(0.77)	(1.17)	/.419
swimming	Schwimmen	1.89	7.62	**-.501**
		(0.88)	(1.19)	**/.029**
transport of radioactive waste	Transport von	3.11	2.59	-.246
	radioaktivem Abfall	(0.99)	(1.40)	/.295
uranium reprocessing	Wiederaufbereitung von	2.86	3.60	.078
	Uranium	(0.92)	(1.25)	/.744
vaccination	Impfungen	1.79	5.98	-.197
		(0.64)	(1.44)	/.406
x-rays	Röntgenstrahlen	2.72	4.02	-.003
		(0.83)	(1.42)	/.989

Table S1 In the first row the English translation of the used German terms. Given are mean and SD of the risk and emotional valence ratings. Both scales had high internal reliability (Cronbach's alpha 0.89 for the risk ratings, 0.818 for the emotional ratings). Further in the last row the correlation between the risk and the emotional rating (Pearson's r and the respective p-value two-tailed). Within the 50 correlations, there were 8 significant at the level of $p<0.05$ (uncorrected). When interpreting the ratings, one has to bear in mind that a value of 1 in the risk rating means low risk, whereas a value of 1 in the emotional category means "very negative".

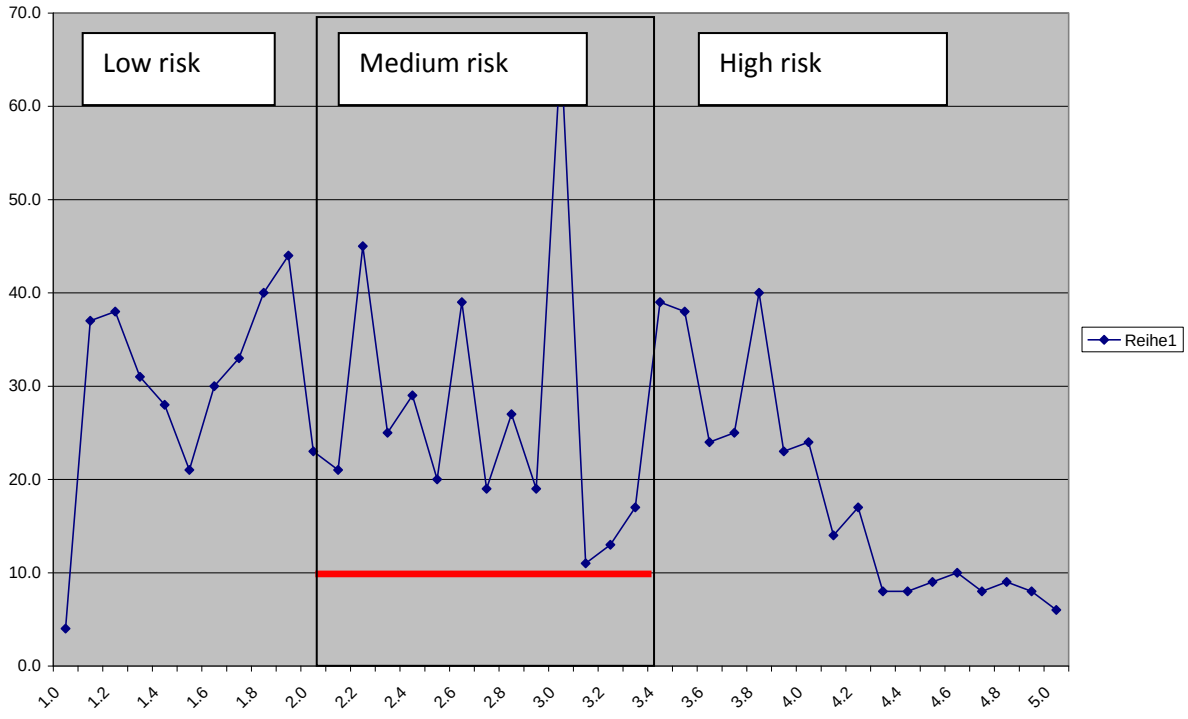

Fig. S2 Distribution of the risk ratings. For the grouping into low, medium, high according to the evaluation we considered the distribution of ratings across subjects (figure below). There we found a three-parted distribution divided by two dips at 2.0/2.1 and at 3.1-3.4, please consider figure below. Such it appeared suitable for us divide the categories of low, medium and high risk accordingly. This led to define medium risk in this middle range from 2.01 - 3.40 (red line in figure), and the rest as being low and high respectively. The mean number of items rated as high risk was 13.7 (SD: 7.2), medium risk 18.8 (6.8), low risk 16.9 (7.2).

1.2. Neural signalling of food healthiness associated with emotion processing

Uwe Herwig[1,2], Matthias Dhum2, Anna Hittmeyer[1], Sarah Opialla[1], Sigrid Scherpiet[1], Carmen Keller[2], Annette B. Brühl[1,2], Michael Siegrist[2]

[1]Clinic for Psychiatry, Psychotherapy and Psychosomatics, University Hospital of Psychiatry, Zürich, Switzerland

[2]Institute for Environmental Decisions, ETH, Zürich, Switzerland

[3]Behavioural and Clinical Neuroscience Institute and Dept. of Psychiatry, University of Cambridge, UK

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Introduction

The ability to perceive food as being advantageous or disadvantageous to one's own health, and guiding one's nutritional behaviour accordingly, promotes good health. This is particularly important considering that the burden and the economic impact of nutrition related medical conditions and unhealthy lifestyles is high (Striegel-Moore and Bulik 2007; Hoek 2008). Nutrition, or food intake, may trigger or may cause pleasant and unpleasant feelings, and can such be considered to be associated with the activation of brain regions that process emotions (Rolls 2005; Rolls 2008; Siep et al. 2008; Ziauddeen et al. 2012; Meye and Adan 2014; Morton et al. 2014). A feature of tasty, but unhealthy nutrition consists of appetitive emotions occurring with impaired impulse control and self-guiding related to respective food stimuli, despite knowledge of a possible disadvantageous health value (Glanz et al. 1998). For example, eating chocolate is accompanied by a positive emotion, whereby resisting eating an offered chocolate may result in unpleasantness and may require impulse control. This unpleasantness signal makes sense from an evolutionary point of view, as the ingestion of high caloric food is advantageous for an organism's survival. However, many people, for instance those with obesity, diabetes or other nutrition related conditions, have to control eating certain foods that they desire. Evaluating and choosing healthy food on an individual and situation based level in this way is especially important.

In everyday life, many people do not actively reflect on whether the food they eat is healthy, but more so on whether it is tasty (Glanz et al. 1998). However, consciously reflecting about the healthiness of a food item can influence eating behaviour. Identifying the brain regions involved in the evaluation of food healthiness might help to understand which cognitive strategies are utilized to promote salutary nutrition. We investigated brain activity associated with single subject's conscious food healthiness evaluation and rating and focused on brain regions known to be involved in emotion

processing and regulation. We were interested in which brain areas signal differentially when evaluatinga food item to be of higher healthiness compared to lower healthiness.

Earlier studies on the neural processing of the visual presentation of food stimuli in healthy subjects showed heightened activation in insular and orbitofrontal cortex (OFC) regions, when hungry (Porubska et al. 2006) and not hungry (Killgore et al. 2003; Simmons et al. 2005). These results are consistent with reports of gustatory representation in these areas (Kringelbach et al. 2004; Rolls 2001). Furthermore, the OFC has been shown to integrate different sensory modalities such as gustation and olfaction (Rolls 2001). Fuhrer et al. (2008) analysed brain activity during the presentation of food items compared to other stimuli. They found stronger activation of the medial prefrontal, insular, anterior cingulate and striatal regions when participants were presented with food stimuli. Siep et al. (2009) reported activity in reward-associated brain regions, the amygdala and OFC, when assessing high versus low caloric nutrition. Rolls (2008) discussed a central involvement of the orbitofrontal and anterior cingulate cortex in the multimodal representation of food particularly associated with reward. These suggestions were also supported by Frank et al. (2010). Medial and lateral prefrontal cortex areas are also involved in value based decision making (Deco et al. 2013; Dixon and Christoff 2014), which was also required in our task. Following these findings, regions of interest to be considered in our study were the medial and dorsolateral prefrontal cortex regions, anterior/subgenual cingulate gyrus, orbitofrontal cortex, anterior insula, amygdala, ventral striatum, medial thalamus and midbrain.

In previous studies that have investigated neural activation associated with food healthiness, food healthiness processing in general was related to cognitive domains such as attention (e.g. Hare et al. 2011; Grabenhorst et al. 2013), but signalling of healthiness versus non-healthiness in distinct brain areas was not addressed and the individual estimation of healthiness evaluation was not considered (e.g. Frank et al. 2010; Killgore and Yurgulun-Todd 2010). The novelty of our study relies on i) the direct comparison of brain activation associated with high and low health value in healthy subjects, and ii) on the investigation of the single subject level of food healthiness evaluation. Therefore, our analysis was not based on a general a priori categorization of the food items into healthy and unhealthy categories, but on the single subject rating of each food item concerning perceived health value. Given that the subjective valence of different foods can vary, we individually determined the grade of healthiness related to each presented food item and considered the individual results for the analysis. As it was previously shown that males and females may differ regarding their estimation of healthiness of nutrition and other food related aspects (Killgore and Yurgulun-Todd 2010; Geliebter et al. 2013), we further aimed to identify gender differences at the level of neural activation concerning food evaluation. We expected the differential evaluation of food healthiness to be associated with the activation of brain areas related to emotion processing, especially in more primordial brain regions such as the midbrain, amygdala and ventral striatum regarding an emotional connotation, and in the insula and OFC regarding viscero-sensitive interoception. A cognitive approach, however, would involve higher cortical regions such as medial prefrontal and dorsolateral prefrontal cortex regions.

Methods

Subjects

Forty-one healthy subjects (age 20-46 years, mean 24.8, SD 4.6; all right handed; 22 males, BMI mean 22.9, SD 2.4, range 19.9-28.6 with n=1 > 26; 19 females, BMI mean 21.3, SD 2.1, range 18.0-24.6; none with dietary needs, 39 with academic background, mostly students, 2 medical assistance professionals) were recruited to participate in this study and gave written informed consent. The study was approved by the local ethics committee. Further, the subjects were neither hungry throughout scanning, nor had they had a major meal within an hour prior scanning. Four subjects were excluded afterwards because of sudden movement artefacts (exceeding more than 3 mm in at least one direction) or other technical reasons, so that the data of thirty-seven subjects (age 20-46, mean 24.9, all right handed, 19 males, 18 females; Tab. 1) were analysed. The subjects were healthy (assessed with clinical interview based on ICD-10 and DSM-IV) and did not take any psychotropic medication or have any psychiatric, neurological, or other relevant medical history that would affect the results of this study. We also assessed self-ratings of depression (SDS, German version; Zung 2005) and state-trait anxiety inventory (STAI) to control for affective or anxiety symptoms (tab. 1).

Experimental design

During fMRI scanning, the subjects evaluated the healthiness of different food items presented in photographs. The photographs showed the food items on a white background, in such a way that only the food item was visible (examples in fig. 1). The food items were divided into two halves representing more or less healthy or unhealthy food, respectively. The food photographs were presented for 5940 ms (equivalent to 3 repetition times, TR, for the fMRI volumes). In this "evaluation" period, the subjects were instructed to look at the photo and to estimate the healthiness of the respective food item. Subsequently, a visual analogue scale ranging from 1 to 5 was presented for 3960 ms (two volumes) on which the subjects indicated the individually estimated nutrition value between very healthy (5) to very unhealthy (1) by moving a cursor using a trackball with the right hand (fig. 1), the "rating period". Altogether, 40 food stimuli were presented in a randomized order. The following baseline period (13700 ms, 7 TR) was of sufficient duration to allow the blood oxygen level-dependent signal to wear off before the next trial. The task was programmed with Presentation™, (Neurobehavioral Systems, USA) and presented via digital video goggles (Resonance Technologies, Northridge, CA). Photographs were sized to fill approximately two thirds of the screen diameter, so that the food item could have been identified immediately with minimal eye movements required. After scanning, the subjects were asked to rate the healthiness of the food items again and also the grade of subjective tastiness, from very tasty (5) to not tasty at all (1), on visual analogue scales.

We specifically assessed the difference in healthiness evaluation within the sample of food pictures

on a single subject level. As such, we explicitly compared the items as being of high or low healthiness as they were rated by the individual. In order to better discriminate healthy ratings from unhealthy ones, we separated both groups by a group of stimuli with intermediate healthiness (group definition below).

Data acquisition

Imaging was performed with a 3.0 T GE Signa™ HD Scanner (GE Medical Systems, Milwaukee). Echo planar imaging was performed for fMRI (repetition time TR/echo time TE 1980 ms/32 ms, 22 sequential axial slices, whole brain, slice thickness 3.5 mm, 1 mm gap, resulting voxel size 3.125 × 3.125 × 4.5 mm, matrix 64 × 64 pixels, field of view 200 mm, flip angle 70°). 528 volumes were obtained per subject, 12 per trial. Four initial volumes were discarded to allow for equilibration effects, seven volumes were added for a final baseline. High-resolution 3-D T1 weighted anatomical volumes were acquired (TR/TE 9.9/2.9 ms; matrix size 256×256; 1 mm × 1 mm × 1 mm resolution) for co-registration with the functional data.

Data analysis

FMRI data were analysed using BrainVoyager™ QX 2.0 (Brain Innovation, Maastricht, The Netherlands). Pre-processing of the functional scans included motion correction, slice scan time correction, high frequency temporal filtering, and removal of linear trends. Functional images were superimposed on the 2D anatomical images and incorporated into volume time courses. The individual volume time courses were transformed into Talairach space resulting in a voxel size of 3×3×3 mm and then spatially smoothed with an 8 mm Gaussian kernel for subsequent group analysis. From each included subject (n=37), the individual food healthiness evaluation periods of each single food item presentation were considered. We pre-defined three categories of healthiness ratings: high, medium, and low. The categories were mathematically divided considering the highest and lowest 1.5 score periods on the scale for the analysis of high and low healthiness, respectively. Thus, low healthiness was defined between 1.00 and 2.50, medium healthiness between 2.51 and 3.49, and high healthiness between 3.50 and 5.00, based on the distribution of the evaluation ratings. Individual experimental design matrices for each subject for the fMRI-analysis were built comprising the individually rated items meeting the three conditions (low, medium, high healthiness) and the respective three conditions with presentation of the rating scale as predictors, resulting in six predictors for the design matrix. The periods were modelled as epochs using a two-gamma hemodynamic response function provided by BrainVoyager™ and were adapted to the applied period duration.

The fMRI data analysis, based on the general linear model (GLM), comprised the following steps: First, fixed effects analyses were calculated separately for each subject for the contrasts comparing the individual conditions of evaluation and rating of ‘high healthiness’ versus ‘low healthiness’, resulting in summary images. The summary images were subjected to second level group analyses. Thus, those

trials in which the food photographs were rated as ‘high’ and ‘low’ healthiness were considered for contrast analysis. The ‘medium’ rated items were also modelled as a condition in the analysis protocol but not considered for the final fMRI analysis and served therefore as a “buffer” between ‘low’ and ‘high’ for better discrimination. We further differentiated between the evaluation and the rating period. The evaluation period was the primary period of interest with the pure mental act of reflecting about and estimating the healthiness of the presented food item without a motor command or other distracting activity. The rating period was the period of giving feedback concerning the healthiness estimation. Both, evaluation and rating period, were functionally and timely coupled, nevertheless, we decided on a separated analysis. To analyse the evaluation and rating periods, three-dimensional statistical parametric maps were calculated for the groups using a random effects analysis.

Because of the approach with individual data of each subject for each food item, the data were not suitable for a continuous or regression analysis, which would have been suitable for a mean value derived from all subjects for the single food items. ,The main analysis therefore focused on the contrasts “evaluation high healthiness > evaluation low healthiness” (e-hi > e-lo) and “rating high healthiness > rating low healthiness” (r-hi > r-lo). The voxel-wise threshold for reporting results in the random effects analysis was set at $p<0.005$. To correct for multiple comparisons, a Monte Carlo simulation was used (Goebel et al., 2006) for estimating cluster-level false-positive rates on these maps, yielding after 10.000 iterations a minimum cluster size threshold of 10 voxels of 3x3x3 mm (270 mm3), corresponding to a corrected cluster level $p < 0.02$.

We also assessed the brain activity associated with food healthiness evaluation and rating in predefined anatomical cubic ROIs, which are known to be related to emotion processing. For the larger cortical regions of the insula, subgenual (sg)ACC, OFC, DLPFC and DMPFC, ROIs were constructed using 4x4x4 functional voxels (edge length 12×12×12 mm, volume 1728 mm3 each). The ROIs were placed according to the Talairach Client (Lancaster et al., 2000) and prior studies: DMPFC x = 6/-6, y = 6, z = 50, covering Brodmann Area (BA) 6 and 8 in the superior frontal gyrus; and DLPFC x = 43/-43, y = 18, z = 30, covering mainly BA 9 in the middle frontal gyrus (Northoff et al., 2006, Herwig et al., 2010b, Herwig et al. 2012); anterior insula x = 33/-33, y = 16, z = -1 (Craig, 2009, Paulus and Stein, 2010); ventral striatum (10/-10, 6, -6; McClure et al., 2004, Heimer and Van Hoesen, 2006), amygdala (Costafreda 2006, edge length 6 mm, 22/-22, -6, -12), medial thalamus (0, -12, 4), midbrain (0, -23, -12), OFC (0, 52, -1), ACC (0, 38, 1), sgACC (0, 17, -9).

Finally, in order to assess the influence of gender on the food evaluation and food rating periods, we introduced this variable as a covariate in a further analysis using the same statistical approach and thresholds.

Results

Behavioural data

37 subjects were included in the analysis (demographic data including normal anxiety and depressiveness ratings in table 1). The subjects attributed high healthiness to 16.2 items (SD 3.0),

medium healthiness to 5.7 items (SD 3.1), and low healthiness to 18.0 items (SD 2.1), on average. This resulted in n = 601 trials with high healthiness, n = 212 trials with medium healthiness, and n = 667 trials with low healthiness, overall. Correlating healthiness with tastiness in the groups of pictures that were rated as healthy and in the group rated as unhealthy did not reveal any significant results: healthy/taste r = -0.06 (mean healthy food pictures 4.4, taste health group 4.1), unhealthy/taste r = -0.11 (mean health rating in the unhealthy food pictures 1.8, mean taste in that group 3.6). However, when correlating the grades of healthiness and tastiness in the whole group, we found a positive correlation (r = 0.52), meaning that healthier food items were also rated as tastier.

fMRI results

We performed whole brain analyses on the contrasts "evaluation high healthiness > evaluation low healthiness" (e-hi > e-lo) and "rating high healthiness > rating low healthiness" (r-hi > r-lo).

Regarding the evaluation period, we found higher activation in a left superior/medial prefrontal cortex region covering Brodmann areas (BA) 6, 8, 9, (premotor cortex (PMC) and DLPFC, fig. 2A) and in precuneus and lateral parietal cortex regions associated with the evaluation of food pictures subjectively rated as high in healthiness. Higher activation in primary and associative visual cortex was associated with the low healthiness food pictures (table 2, fig. 2). The ROI analysis was used in order to assess activation in emotion processing related brain areas, and revealed higher activity in the right amygdala associated with evaluation of unhealthy food stimuli compared to healthy stimuli ($p = 0.048$, $t = -2.05$). Both evaluations (healthy and unhealthy) activated the amygdala compared to baseline (right and left $p < 0.00001$, $t > 7$; fig. 3).

Regarding the rating period, right ventral striatal activity was stronger when rating food as being healthy compared to unhealthy ($p = 0.008$, $t = 2.82$). DLPFC BA 46 was bilaterally more active with the rating of unhealthy food compared to healthy (right $p = 0.020$, $t = -2.44$; left $p = 0.041$, $t = -2.12$).

Food healthiness evaluation and rating were both associated with significant neural activation in the other ROIs such as the MPFC, OFC, ACC, insula (apart for right anterior insula and evaluation), medial thalamus and midbrain, but without differences in regard to subjective healthiness (fig 4, table 3).

When assessing differences between males and females in the ROI analysis, we found higher activity in the right ventral striatum among males compared to females in the perception and evaluation of unhealthy stimuli (m>f; $p = 0.008$, $t= 2.813$). This contrast was at the borderline to significance in the medial thalamus ($p = 0.058$, $t = 1.959$). Activation in the midbrain region was higher among females associated with the rating period of healthy stimuli compared to unhealthy stimuli ($p = 0.028$, $t = 2.293$; fig. 5). We found no differences between males and females in the amygdala, insula and prefrontal regions.

Discussion

Our main interest was on neural signalling associated with evaluating higher versus lower healthiness of food stimuli on a single subject level. We found an association of *higher* healthiness evaluation with higher activity of the PMC/DLPFC BA 6/8/9 and in the precuneus in the whole brain analysis and in the ROI analysis of the rating period in the ventral striatum and the orbitofrontal cortex. Contrary to our hypothesis, we found an association of *lower* food healthiness with higher activation in the amygdala. This was also found bilaterally in more anterior and lateral regions of the DLPFC (BA 46) as well as in primary and secondary visual areas. Nearly all other areas known to be involved in a network of brain areas associated with emotion processing, such as the MPFC, OFC, ACC, insula, medial thalamus and midbrain, were activated generally when associated with the act of food healthiness evaluation and/or respective rating, but not specifically for healthy or unhealthy, and of course within the frame of our study without exclusion of other cognitive components that may be attributed to the activation. However, this at least implicates a relationship between the estimation of nutritional healthiness and emotional signalling in distinct emotion processing areas. The only gender difference was reflected by a higher activation in the midbrain of females associated with healthy stimuli.

Food health evaluation and emotion processing

The general association between nutrition and emotion has been proven by multiple studies (Rolls 2005; Rolls 2008; Siep et al. 2009; and many others). Denton et al. (2009) have shown hunger and satiation as belonging to "primordial emotions" deeply rooted in central-nervous information processing and developed early in evolution. From an evolutionary point of view, one can even suggest that the complex system of emotions may at least in part originate from the bodily signalling associated with the ingestion of food and its evaluation in early species throughout evolution (Denton et al. 2009). Getting nutritious food is necessary for survival and associated with feelings of reward. Conversely, being deprived of food is unpleasant and potentially life threatening. As such, one may argue that neural emotion processing might have evolved at least in part from nutrition related neural processing. Considering the long term consequences of healthiness and favouring them over the short term benefits of tasty but unhealthy food, however, is often difficult and requires awareness for health aspects and self-control (Hare et al. 2011; Liberman and Trope 2008).

According to our findings, regions associated with a differential healthiness signal comprise the DLPFC, MPFC, precuneus, amygdala and ventral striatum. The PMC/DLPFC region showed stronger activation in more posterior and superior regions (BA 6/8/9) associated with the rating of higher healthiness, and in more rostral regions (BA 46) associated with the rating of lower healthiness. Hare et al. (2011) reported the lateral prefrontal cortex in BA 8/9 and 46/47 showed increased activity during a condition where subjects were asked to generally consider the healthiness of a food item. Further, BA 9 of the DLPFC appeared to modulate ventromedial PFC to promote health information

(Hare et al. 2009; Hare et al. 2011). In a study assessing brain activation during the regulation of the desire for food intake by using reappraisal strategies, the DLPFC, as well as medial and inferior frontal PFC areas, were activated. This suggests that these areas have an impact in controlling food intake (Hollmann et al. 2012). The identified regions of the DLPFC, which is generally known to be involved in cognitive and executive control (e.g. Disner et al. 2009; Fuster 2000), are suggested to be involved centrally in guiding nutrition relevant evaluation and behaviour. The anterior and subgenual cingulate cortices, involved in conflict detection (Carter and van Veen 2007), were not activated specifically with evaluation of healthy or unhealthy food.

Important concomitant signals for evaluations of food may arise from the amygdala and ventral striatum. However, contrary to whatwe hypothesized, the amygdala in our study exerted a stronger signal associated with lower healthiness and the ventral striatum signalled higher healthiness. Both regions are known to be tightly coupled with emotion processing. The ventral striatum is part of the reward system (Salamone and Correa 2012) and the amygdalae are central processors of emotional signals (Costafreda 2008; Pessoa and Adolphs 2010). In the context of food processing, Grabenhorst et al. (2013) found that nutritional information biased food evaluations in the amygdala, potentially reflecting an active amygdala participation in food choice. Siep et al. (2009) also reported the amygdala as being active when attending and evaluating food. However, when simply watching food, the amygdala was reported as inactive (Siep et al. 2009; Frank et al. 2010). This underlines the role of the amygdala in evaluation and, possibly, choice or "what if" processes. It may provide bottom-up signalling of the grade of healthiness and, as our findings imply, "warn" by a higher activation when an unhealthy food item is detected. Furthermore, amygdala signalling may be associated with the retrieval of autobiographic and episodic content concerning food, biasing approach or avoidance behaviour. On the other hand, the amygdala is a central recipient of cognitive control processes (e.g. Ochsner and Gross 2003; Herwig et al. 2007) and thus is susceptible to deliberate regulation of food choice and consumption. In that context, "warning" signals can also be intentionally ignored or suppressed when deciding to eat unhealthy food.

The ventral striatum was consistently activated during evaluation and rating bilaterally, and showed right-sided higher activity during the rating of higher healthiness. This may be interpreted as a reward signalling, since the ventral striatum is centrally involved in the brain reward system (Hollmann et al. 2011; Kringelbach et al. 2012). Earlier studies reported, however, that salutary food might be regarded as less tasty than unhealthier food items (Glanz et al. 1998), because healthy food might cause less reward signalling than unhealthy food. We found a positive correlation between health and taste rating, which might result from a bias due to actively making oneself aware of the health value, which promotes a reward signal in the case of healthy foods. It has been shown that food and drug cues activate the same reward related brain regions (Tang et al. 2012). The cognitive act of intentionally reflecting on the healthiness of food prior to the concrete selection of nutrition, which is often not regularly done, might lead to preferring healthy food, as biased by a reward signal in the context of

health evaluation. This might be a simple cognitive strategy for healthier nutrition and better impulse control compared to every-day nutrition without actively reflecting on health value.

The OFC is assumed to be involved in the representation of emotional value linked to reward and decision-making, thereby guiding behaviour (Kringelbach 2005; Schoenbaum et al. 2011). In our case, the OFC was more strongly activated when rating healthy food than when rating unhealthy food. This supports an association between health evaluation and reward in our context. We expected insular regions to be differentially activated by healthiness, but despite a bilaterally prominent general activation during the rating period, no specific healthiness signal was detected. Nevertheless, the strong activation reflects its involvement in associated interoceptive awareness processes (Critchley et al. 2007; Paulus and Stein 2010). Finally, the unhealthy food items activated the areas within the primary and associative visual cortex more strongly. Whether this may be due to neural processing related to the unhealthiness or to basic visual aspects remains open.

A potential clinical application could be the utilization of cognitive regulation strategies such as reappraisal in order to control food intake when needed (Siep et al. 2012, Yokum and Stice 2013). A recent reappraisal study for instance supported applying the strategy of reflecting about the long-term benefits of not eating (Yokum and Stice 2013). Incorporating health aspects in such strategies may advance the application within psychotherapeutic control of eating behaviour.

Nutrition and self-related brain activation

Another interesting finding was the prominent activation of the precuneus, particularly its cognitive self-representation related domain, associated with health evaluation. In an earlier study, participants had estimated the risk of certain hazards presented as verbal terms. It was found that the precuneus was activated when evaluating a higher risk (Herwig et al. 2012). The precuneus is regarded evolutionarily as a newer brain region, particularly present in primates (Cavanna and Trimble 2006). The precuneus was also reported to be involved in self-imagery, representation of the mental self, and autobiographical memory (Cavanna and Trimble 2006). Furthermore, the precuneus was found to be involved in the evaluation of risks and benefits when establishing good reputations (Watanabe et al. 2014). Another study reported evidence on the role of the precuneus in the integration of both visuospatial information and self in the context of navigation within personal space (Freton et al. 2013). Cavanna (2007) summarized the function of the precuneus as a richly connected multimodal associative area that belongs to a neural network, subserving awareness and producing a conscious self-percept. Regarding the activation of the precuneus in our current study, self-relevance and a link to self-representation appears to be a relevant common denominator, with a higher signalling associated with healthier food particularly in the anatomic subdivision of the cognitive/associative central area of the precuneus (Margulies et al. 2009). This area has connections to the prefrontal cortex, BA 10, 46, 8, and also to the dorsal thalamus including the lateral pulvinar, pretectal area, and superior colliculi, thus being connected with very early visual processing that is also related to

emotion processing (Tamietto et al. 2011).

Gender differences

Several studies on food processing in the central nervous system have reported gender differences. Killgore and Yurgulun-Todd (2010) showed that women, when compared to men, had significantly greater activation to high-caloric foods within dorsolateral, ventrolateral, and ventromedial prefrontal cortex, middle/posterior cingulate, and insular brain regions. They concluded that when viewing high-calorie food images, women appear to be more responsive than men within cortical regions involved in behavioral control and self-referential cognition. Frank et al. (2010) revealed that satiation seems to influence the processing of food pictures differently in men and women in areas such as the MPFC and fusiform gyrus. On the other hand, Grabenhorst et al. (2013) did not find gender differences concerning taste preference and health-based decision variables. Geliebter et al. (2013) found obese men and women exert different brain activation towards high versus low calorie food in fed and hunger states, comprising prefrontal and subcortical areas such as the caudate. The only difference we found was stronger midbrain activation towards evaluating more healthy food items in women compared to men. One might consider a very deeply rooted healthiness signal in midbrain regions in women reflecting a primordial emotion, even though this remains speculation.

Limitations

Reflecting on limitations, we have to consider the experimental condition in which the task was restricted to evaluating healthiness without the implementation of another cognitive control condition in order to assess specificity for this aspect. However, we attempted to differentiate between high and low healthiness, with the active cognitive requirement to reflect on healthiness, so that both conditions served as a control for each other with the general basis of health estimation.

Conclusion

Differential signalling of perceived food healthiness is associated with activity in the DLPFC, MPFC, precuneus, amygdala and ventral striatum. The overlap between food processing and emotion processing is obvious. Certainly, this overlap can be explained from an evolutionary perspective and one may even suggest that emotion processing might have its roots, at least in part, in food processing. Regarding possible implications for interventions towards nutrition behaviour, one might propose an intentional active mental evaluation of the health value of food intended to be consumed. This might be combined with emotion regulation strategies aimed to reflect the accompanied appetitive emotions, such as incorporating a reality check and reappraisal towards unhealthy food. Actively reflecting on the health value of food may also enhance impulse control. The healthiness associated activation of areas involved in basic emotion processing, such as the amygdala, supports applying emotion regulation strategies in psychotherapeutic attempts to support healthy nutrition.

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Tables

	Total mean (SD, range)	**Males mean (SD, range)**	**Females mean (SD, range)**	**Males vs. females p**
Subjects (n)	37	19	18	
Age (years)	24.8 (4.6, 20-46)	24.4 (4.0, 20-36)	25.3 (5.5, 20-46)	0.58
SDS	45.6 (3.8, 36-53)	45.2 (1.9, 42-48)	46.1 (4.2, 36-53)	0.48
STAI - **State** - **Trait**	 43.6 (2.8, 38-49) 43.7 (4.3, 37-56)	 43.4 (2.9, 38-49) 43.0 (4.2, 37-52)	 43.8 (2.7, 39-48) 44.4 (4.2, 39-56)	 0.71 0.32

Tab. 1 Demographic and psychometric data of the subjects. Abbreviations: SDS Self-ratings of depression, STAI state-trait anxiety inventory.

	BA	**Peak x**	**Peak y**	**Peak z**	**t**	**P**	**mm^3**
PMC/DLPFC L	6, 8, 9	-18	8	49	3.73	0.0007	766
IFG R	11, 44, 45	42	44	-8	4.96	0.0000	6832
Posterior insula R	13	36	-37	22	3.63	0.0009	521
Superior temporal ctx	22	36	-40	1	3.46	0.0014	649
Precuneus	7	-3	-70	34	4.75	0.0000	16393
Occipital ctx	18	-30	-88	-2	-5.41	0.0000	31725
Postcentral gyrus L	3	-33	-28	52	3.55	0.0011	472
Temporo-occipital ctx L	22, 39	-39	-52	16	4.84	0.0000	8614
Temporo-parietal ctx R	39	45	-58	28	4.50	0.0001	5269

Tab. 2 Contrast evaluation period of healthy vs. non-healthy food photographs in the whole brain analysis. Abbreviations: PMC premotor cortex, DLPFC dorsolateral prefrontal cortex, IFG inferior frontal gyrus, ctx cortex, BA Brodmann Area, R right, L left, SD standard deviation.

Anatomic region **Tal. X / Y / Z**	**Eval h>uh** **p / t**	**Eval h** **p / t**	**Eval uh** **p / t**	**Rat h>uh** **p / t**	**Rat h** **p / t**	**Rat uh** **p / t**
Amygdala R 22 / -6 / -12	0.048 / -2.05	<0.0001 / 7.26	<0.0001 / 7.16	0.54 / -0.62	0.53 / -0.64	0.93 / -0.09
Amygdala L -22, -6, -12	0.14 / -1.50	<0.00001 / 6.92	>0.00001 / 6.01	0.85 / 0.19	0.63 / 0.49	0.71 / 0.37
Ventral striatum R 10 / 6 / -6	0.083 / -0.25	0.001 / 3.64	0.005 / 3.03	0.005 / 3.00	0.001 / 3.71	0.08 / -1.81
Ventral striatum L -10 / 6 / -6	0.48 / -0.75	0.0002 / 4.12	0.0003 / 4.03	0.30 / -1.05	<0.00001 / 5.74	<0.00001 / 6.12
DMPFC R 6 / 6 / 50	0.73 / -0.342	<0.00001 / 5.005	<0.00001 / 5.27	0.94 / 0.071	<0.00001 / 22.23	<0.00001 / 17.14
DMPFC L -6 / 6 / 50	0.51 / -0.66	<0.00001 / 8.77	<0.00001 / 8.59	0.56 / -0.59	<0.00001 / 19.11	<0.00001 / 17.16
DLPFC R 43 / 18 / 30	0.77 / 0.30	0.20 / 1.28	0.34 / 0.96	0.020 / -2.44	<0.00001 / 9.18	<0.00001 / 9.62
DLPFC L -43 / 18 / 30	0.34 / -0.98	<0.00001 / 9.60	<0.00001 / 10.04	0.036 / -2.17	<0.00001 / 8.58	<0.00001 / 9.48
Anterior cingulate	0.079 /	<0.00001 /	<0.00001 /	0.38 /	<0.00001 /	<0.00001 /

0 / 38 / 1	-1.81	9.26	9.32	-0.89	13.69	12.20
Subgenual cingulate 0 / 17 / -9	0.29 / -1.08	0.00015 / 4.28	0.00002 / 4.91	0.59 / 0.54	0.036 / -2.18	0.027 / -2.31
Anterior insula R 33 / 16 / -1	0.94 / 0.07	0.37 / 0.90	0.39 / 0.86	0.37 / 0.90	<0.00001 / 10.48	<0.00001 / 9.19
Anterior insula L -33 / 16 / -1	0.90 / -0.13	0.039 / 2.14	0.030 / 2.26	0.73 / 0.35	<0.00001 / 9.98	<0.00001 / 9.16
Medial thalamus 0 / -12 / 4	0.74 / 0.33	<0.00001 / 5.23	<0.00001 / 4.40	0.79 / 0.27	<0.00001 / 14.57	<0.00001 / 11.28
Midbrain 0 / 23 / -12	0.19 / -1.32	<0.00001 / 8.06	<0.00001 / 7.95	0.71 / -0.37	<0.00001 / 11.17	<0.00001 / 11.9
Orbitofrontal cortex 3 / 49 / -12	0.80 / 0.25	0.00015 / 4.23	0.0036 / 3.12	0.0056 / 2.97	<0.00001 / -6.00	<0.00001 / -8.03

Tab. 3 Results ofthe comparisons of the conditions healthy (h) and unhealthy (uh) in different regions of interest providing statistical p- and t-values for each, the evaluation (Eval) and the rating (Rat) periods. The x, y, z coordinates correspond to the centres of the named regions.

Figures

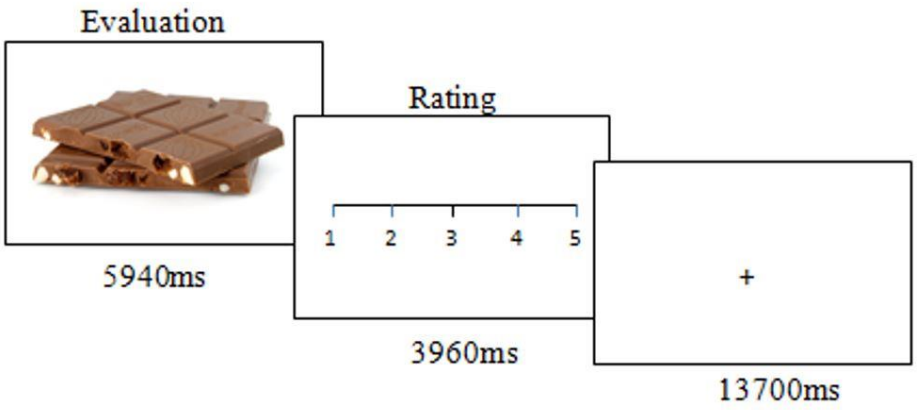

Fig. 1 Experimental task. The trials started with displaying a food photograph for nearly 6 seconds while the participants evaluated the healthiness of the food item. This was followed by a rating period of nearly 4 seconds. A baseline condition of nearly 16 seconds was implemented between trials.

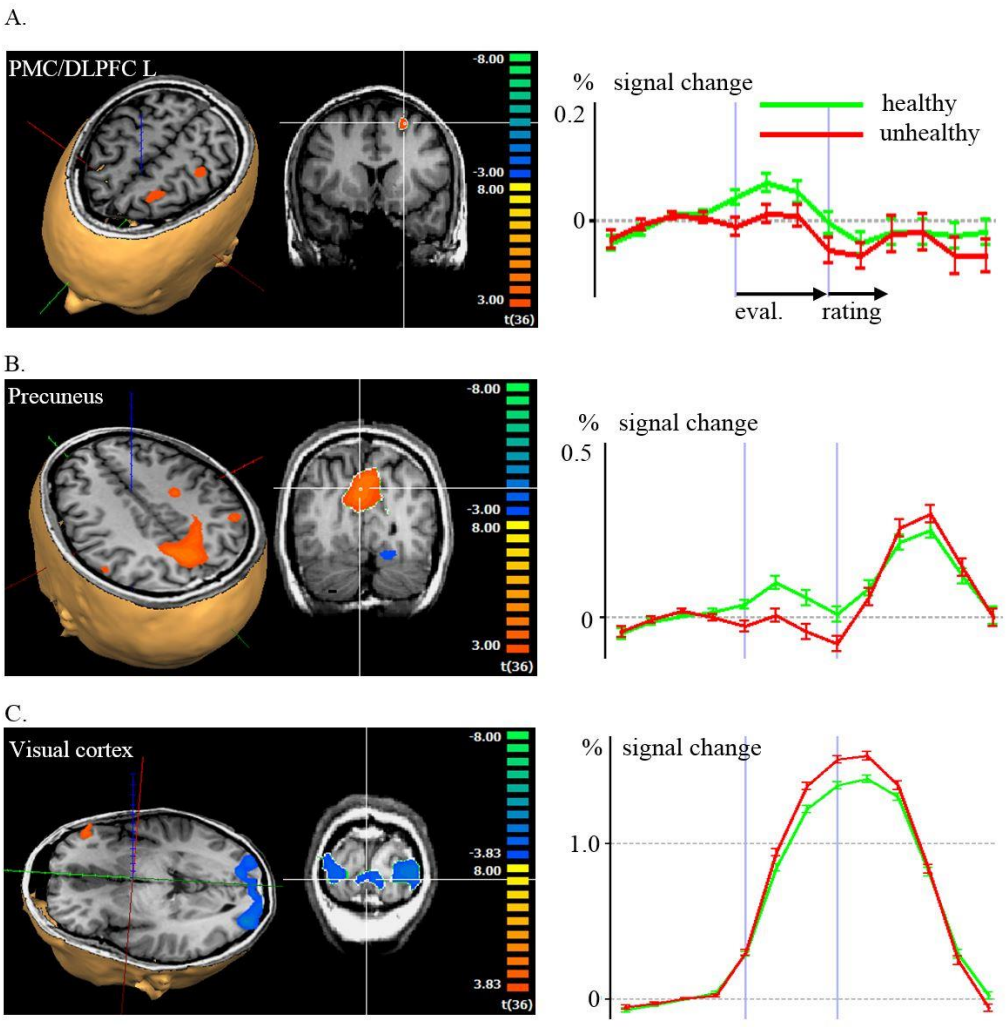

Fig. 2 Brain activation with colour coded maps and time courses with signal changes according to a

random effects analysis ($p<0.001$) of the evaluation (eval.) and rating period, comparing high healthiness against low healthiness: A. left dorsolateral prefrontal cortex (DLPFC), B. precuneus, C. visual cortex. In the 3D visualisations, red, blue and green axes indicate the coordinate system. In the 2D coronal slice, the region with statistical significant activation of which the time course is derived is marked with the white crosshair. In the time course diagrams on the left side, the first vertical violet bars after the y-axis correspond to the activations during the first volume of the evaluation period. The second vertical violet bars correspond to the activations during the first volume of the rating period.

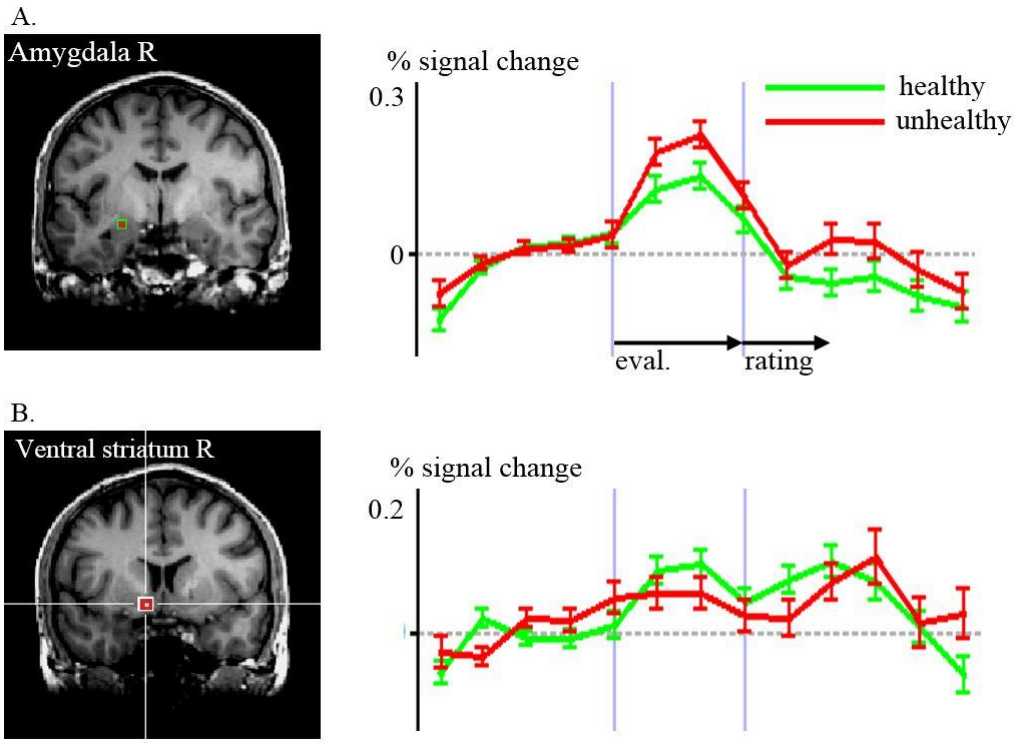

Fig. 3 Time courses of signal changes according to the region of interest analyses of healthy versus unhealthy food in A. amygdala, B. ventral striatum. The regions are indicated by the red squares in the structural MR images Differences in the time courses were significant for the evaluation (eval.) period in the amygdala ($p=0.048$) and for the rating period in the ventral striatum ($p=0.005$).

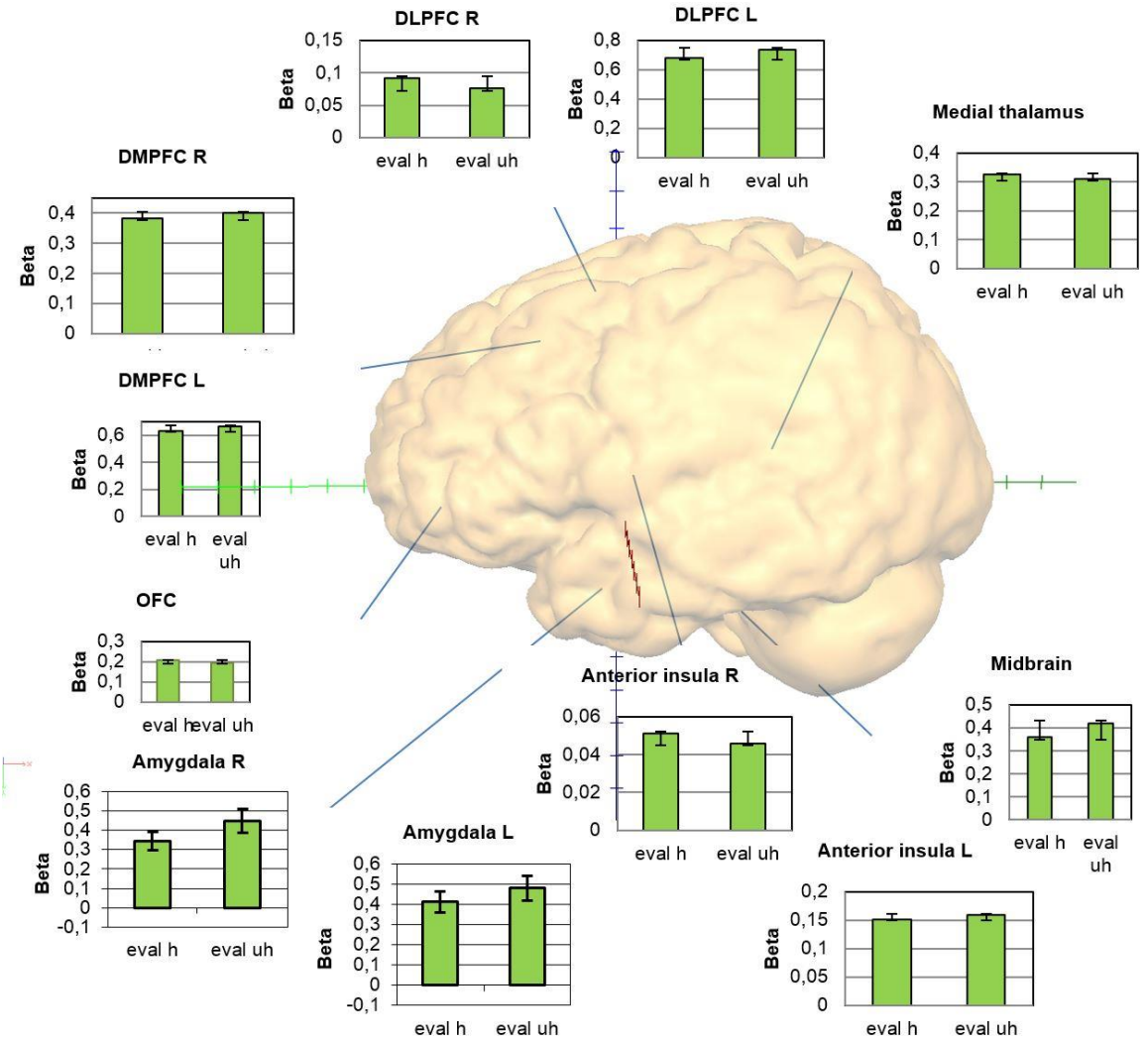

Fig. 4 Overview of the activations within the regions of interest providing graphs with the beta weights for the conditions evaluation healthy and unhealthy.

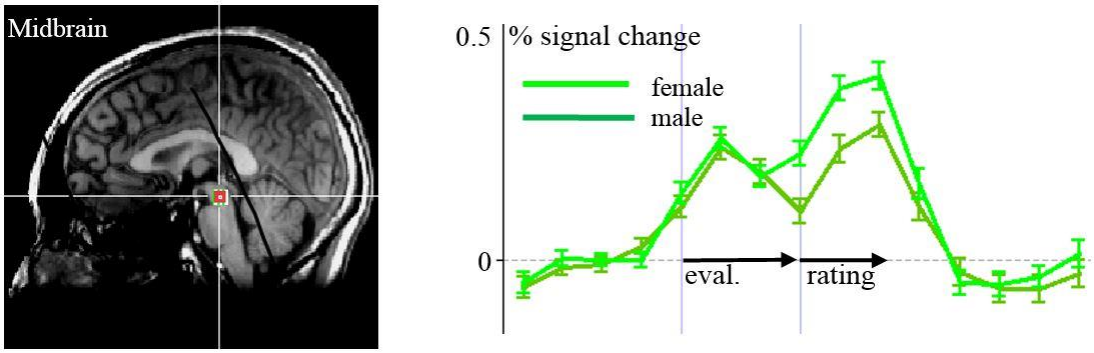

Fig. 5 ROI-Analyses of females vs. males: activation in the midbrain to healthy food items is stronger in females than in males.

1.3. Evolutionary and modern image content differentially influence the processing of emotional pictures

Matthias Dhum[1,x], Uwe Herwig[1,2,3,x], Sarah Opialla[2], Michael Siegrist[1], Annette B. Brühl[2]

[1]Institute of Environmental Decisions, Consumer Behavior, ETH, Zurich, Switzerland

[2]Department of Psychiatry, Psychotherapy and Psychosomatics, University Hospital of Psychiatry, University of Zurich, Zürich, Switzerland

[3]Department of Psychiatry and Psychotherapy III, University of Ulm, Ulm, Germany

[x]Both authors contributed equally, please also consider Author Contributions

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Introduction

Identifying threatening situations is a vital feature of human perception that has evolved over the history of animals. A failure in this aptitude could have had fatal consequences for our ancestors. The vital relevance of a working adaptive behavior is assumed to have led to the evolution of a dedicated fear module that, in turn, governs this behavior (Öhman and Mineka, 2001; Sander et al., 2003). Öhman and Mineka further argue that this module has evolved to recognize all natural threats facing our ancestors, such as predators and poisonous animals (Öhman and Mineka, 2001). The concept of the fear module is based on the theory of preparedness, which posits that the successful perception and identification of environmental threats lead to a reproductive advantage for the individual (Seligman, 1971).

In modern times, however, the environmental threats prevalent until a few centuries ago are no longer the main threats to most people, particularly in modern urban societies. Instead of the natural and evolutionary established threats we now increasingly face threats that are qualitatively different, more technical, and in some cases less tangible. This might require an adaptation of our perception, evaluation and reaction to these threats: We no longer have to fear for instance snakes, spiders and predators, but should rather be cautious in motor traffic, when facing guns as well as when handling tools like knives. These stimuli are in this study referred to as modern threats. The question is how the neural processes have adapted in response to these 'newer' threats. In our study we therefore investigated the neural differences when comparing the perception of evolutionary threats to modern threats. In this line of investigation, we define the term "threat" as the anticipation of a spatially and temporally proximate source of potential harm for the individual (Baldwin, 1971; Davis et al., 2009). The concept of threat involves the identification of emotional significance, the generation of an affective state, and a subsequent behavior, they both engage overlapping neural structures and

functions (Phillips et al., 2003; Mohr et al., 2010; Herwig et al., 2011). Earlier studies addressed the question of differences in the central nervous processing of evolutionary versus modern threats (Blanchette, 2006; Fox et al., 2007; Brown et al., 2010; Sakaki et al., 2012). Regarding the threat-superiority effect, modern threats were reported to be detected in some instance better than evolutionary ones (Blanchette, 2006), whenever such a difference was not observed in an event-related potential study (Brown et al., 2010) or regarding reaction time (Fox et al., 2007). Sakaki and collegues reported differences regarding involved brain areas when comparing evolutionary and social stimuli (Sakaki et al., 2012) with more activation in dorsomedial prefrontal areas in the social context.

The neural underpinnings of the perception of threats in general and associated negative emotions have been studied extensively with a range of methods and stimuli (LeDoux, 2000; Phan et al., 2002; Wager et al., 2003; Pessoa, 2008; Pessoa and Adolphs, 2010). The current model posits that a network of cortical and subcortical regions, including the amygdala, orbitofrontal cortex, anterior insula, anterior cingulate cortex, and inferotemporal visual cortex, play a central role in the perception and identification of threatening stimuli (Sabatinelli et al., 2005; Pessoa, 2008). While the amygdala was previously thought to be involved primarily in the perception of threatening (or more general, emotionally negative) stimuli, the concept of this subcortical region has progressed to a more general function of significance detection and processing (Sander et al., 2003; Williams, 2006; Pessoa and Adolphs, 2010). According to this concept, the amygdala should be activated when encountering any stimuli that convey a biological significance for the individual, which can be of either positive or negative valence (Sergerie et al., 2008). Neuroimaging studies support this assumption by showing that amygdala activity varies according to the level of arousal evoked by a stimulus (Kensinger and Corkin, 2004; Sabatinelli et al., 2005; Kensinger and Schacter, 2006; Kryklywy et al., 2013). However, valence, which is the other main dimension in the Circumplex model of affect (Russell, 1980), seems to have a smaller effect on amygdala activity (Phan et al., 2002; Wager et al., 2003; Sergerie et al., 2008).

To investigate the influence of content on the neural circuits involved in processing threatening stimuli, we chose pictures showing a different phylogenetic origin by selecting those with a strong evolutionary history vs. modern pictures. As a reference, we included two neutral categories, again comprising evolutionary prepared vs. modern pictures. Thus, our study included four experimental conditions: evolutionary-threatening, modern-threatening, evolutionary-neutral, and modern-neutral. The pictures included in our study displayed threatening stimuli related to the basic emotion of fear. In contrast, pictures showing disgust and sadness were not covered in our study.

On a neurophysiological level we propose that the affective pictures will engage a network of brain regions comprising amygdala, orbitofrontal cortex, anterior insula, anterior cingulate cortex, inferotemporal visual cortex as well as medial thalamus and midbrain (Sabatinelli et al., 2005). We consider two complementary lines of reasoning which serve as a theoretical frame in our study. Firstly, literature (Sander et al., 2003; Wager et al., 2003; Sergerie et al., 2008) suggests that the neural

activity in emotion processing circuits reflects the affective rating of the IAPS pictures – especially the arousal dimension, and to a lesser extent the valence dimension. Secondly, the theory of the evolved fear module (Öhman and Mineka, 2001) suggests differences between evolutionary and modern stimuli in the activation of emotion processing circuits. The evolution of the fear module in response to threats such as snakes and spiders implies that evolutionary threatening stimuli might be associated with a stronger activation particularly in evolutionary older regions as amygdala, thalamus and midbrain than the modern stimuli, which are supposed to evoke stronger activation in cortical stimulus processing areas as inferotemporal cortex.

Materials and Methods

Subjects

We recruited healthy subjects through a mailing list and pin board postings. Exclusion criteria were any history of major medical conditions, head trauma, neurological and psychiatric disorder (both individually and in the family), current substance abuse and medication; further contraindications against MRI such as claustrophobia, pregnancy, pace maker or ferromagnetic implants. These criteria were assessed in a semi-structured clinical interview. Subjects received CHF 50 compensation.

In total, 44 subjects (22 females) were scanned for the study. Three subjects were excluded from the final analysis (two subjects due to performance in the behavioral task suggesting a lack of attention or cooperation or otherwise misunderstanding of the instructions [reaction times in 35% of the trials > 1.5 seconds or button presses outside the required time frame] and one subject due to potential clinical conditions which the subject revealed only after inclusion). Thus, the final sample comprised 41 subjects (21 females) with an average age of 25.0 years (SD = 5.3 years).

All subjects had normal or corrected-to-normal vision and were right-handed according to the Annett handedness questionnaire (Annett, 1967). All subjects were within the normal range of anxiety according to the State-Trait Anxiety Inventory X1 and X2 (Spielberger et al., 1970; Laux et al., 1981). No subject reported phobic symptoms related to the stimulus material (e.g., arachnophobia).

The study was approved by the Ethics Committee of the Canton of Zurich (Kantonale Ethikkommission Zürich, http://www.kek.zh.ch). All subjects gave their written informed consent. The study was conducted in accordance to the Declaration of Helsinki (World Medical Association, 2008).

Stimulus material

For each of the four experimental conditions we selected 16 representative pictures from the IAPS database (Lang et al., 2008). First, the pictures were assigned to the respective condition based on a content analysis. This was done independently by two of the authors (MD, ABB) and discussed with the co-authors in case of divergent assignments. Second, the assignment was based on the ratings provided by the IAPS technical report (Lang et al., 2008). Each picture condition was constructed with

the aim to not contain outliers in the valence and arousal ratings. Thus, pictures were selected for a condition if their rating was homogeneous within the condition and distinct to the other conditions. This manual selection process was validated in a pre-test with an independent larger sample (N = 201) by running a confirmatory factor analysis across all pictures (unpublished data). The evolutionary-threatening condition included pictures of predatory animals (e.g., snakes, spiders, dogs, bears, sharks) whereas the modern-threatening condition displayed pictures of guns, knives, and accidents involving cars, ships and airplanes. Evolutionary-neutral pictures comprised landscapes, forests, and flowers, while the modern-neutral pictures showed inanimate objects such as cars, trains, ships, bridges, suitcases, and drawers. Picture numbers are provided in the Supporting Information Table S1.

Experimental Procedure

In the scanner, pictures were displayed covering the full screen of digital video goggles (Resonance Technologies, Northridge, CA) using Presentation software (www.neurobs.com, version 15.1). We presented blocks of eight consecutive pictures from the same experimental condition (Fig. 1). Each picture was shown for 1980 ms. Thus, each block lasted 15840 ms in total. Before the first block and between the blocks, a black screen with a white fixation cross was shown for 15840 ms to allow the Blood-Oxygen Level-Dependent (BOLD) signal to return to a baseline (Ogawa et al., 1990).

The pictures for each block were randomly taken from the 16 pictures selected for the respective experimental condition. The block order was pseudo-randomized across an experimental run to control for serial position effects. One experimental run included four blocks of each experimental condition. Thus, each of the 16 pictures of every experimental condition was shown twice in an experimental run. The whole experiment consisted of three experimental runs, each lasting approximately 11 minutes.

The subjects were instructed to press the button of a response box with their right index finger at the onset of the first picture of a new block. The recorded reaction times served as a control for general attention and wakefulness of the subjects. Further, fast reaction times are generally associated with higher fear relevance of the stimulus (Fox et al., 2007). After the scanning session, subjects rated the pictures on the valence (scaled from 1 = negative to 9 = positive) and arousal (scaled from 1 = low to 9 = high) scales using a digital version of the original IAPS self-assessment manikin (Mogg et al., 1994).

Similar to previous studies (Anders et al., 2008), we deliberately decided against an online rating during the fMRI task since it has been shown that emotional rating instructions may influence neural activity already during the perception of a stimulus (Taylor et al., 2003). Moreover, the post-scan evaluation of stimuli has been demonstrated to correspond well with the emotional experience during the scan (Hariri et al., 2000; Phan et al., 2004).

Behavioral data

We removed outliers (reaction time < 100 ms or > 1500 ms) from the data gathered during the scan. A repeated-measures ANOVA was performed to check for differences in reaction times to the different experimental conditions. In case of significant Mauchly's tests of sphericity, Greenhouse-Geisser correction was applied. Bonferroni-corrected post-hoc tests were performed to reveal differences between single conditions. Similarly, repeated-measures ANOVAs and subsequent post-hoc tests were performed to test for differences in valence and arousal ratings between experimental conditions. Statistical analysis was performed with SPSS (Version 19.0.0.1, SPSS Inc., Chicago, IL, USA) and Matlab (Version R2014a; The MathWorks Inc., Natick, MA, USA).

Image acquisition

Imaging was performed using a 3.0 T GE Signa HD Scanner (GE Medical Systems, Milwaukee, WI, USA; 8-channel head coil). Functional magnetic resonance imaging (fMRI) was conducted using echo-planar imaging (EPI) with the following configuration: 28 interleaved axial slices, 3.5 mm slice thickness, 0.5 mm gap, matrix 64 × 64, 240 mm field of view, resulting voxel size 3.75 × 3.75 × 4.0 mm, repetition time (TR) 1980 ms, echo time (TE) 32 ms, flip angle 70°. The slice angle was optimized to reduce susceptibility artifacts in the amygdala and frontal regions. Per run a total of 328 volumes were acquired, 16 for each of the 20 experimental blocks. The first four volumes of each run were discarded to allow for T1 equilibration. In addition, 3-D T1-weighted anatomical volumes (172 axial slices, TR 9.9 ms, TE 2.9 ms, matrix size 256 × 256, voxel size 1 × 1 × 1 mm) were acquired for co-registration with the functional data. Furthermore, T2-weighted images in parallel to the EPI sequence were acquired to exclude possible T2-sensitive brain abnormalities.

Image analysis and statistics

Imaging data was analyzed using BrainVoyager QX 2.8.4 (Brain Innovation, Maastricht, The Netherlands; Goebel et al., 2006). Pre-processing of the functional data included slice scan time correction, 3-D motion correction with intra-session alignment, and temporal high-pass filtering with removal of linear trends. Functional data was co-registered to the individual anatomical 3-D datasets. Anatomical datasets were corrected for intensity inhomogeneity and transformed into Talairach coordinate space (Talairach and Tournoux, 1988). Volume time courses with a 3 × 3 × 3 mm^3 voxel size were created from the functional datasets. For the subsequent group analysis, the volume time courses were spatially smoothed with a 6.0 mm full-width at half-maximum Gaussian kernel.

The experimental conditions were used as HRF-convolved box-car function predictors in the General Linear Model (GLM) design matrix. In addition, the individual 3-D motion correction parameters were z-transformed, high-pass filtered (10 cycles) and linear detrended using the BVA Predictor Tool (Version 1.52, J.M. Born, Maastricht, The Netherlands), and added as predictors of no interest to the design matrix to account for BOLD artifacts caused by task-correlated motion (Morgan

et al., 2007). From the individual GLM matrices, we calculated a Random Effects General Linear Model as a first step in the group analysis. Voxel time courses from the single runs were percent-transformed. Serial correlations were detected and removed using the AR(2) model approach. We automatized most pre-processing steps using BrainVoyager scripts or WinAutomation software (Version 4.02, Softomotive Ltd, Athens, Greece).

Our aim was to analyze the differential activation of those brain regions centrally involved in the processing of negative emotional stimuli. In a first step, we identified brain regions activated by all threatening stimuli compared to neutral stimuli. Therefore, we calculated a repeated measures 2x2 ANOVA with the factors threat (levels: threatening, neutral) and origin (levels: evolutionary, modern). The voxel-wise threshold for statistical maps correspond to $p < .001$ uncorrected. To correct for multiple comparisons, a Monte Carlo simulation with 1000 iterations was used for estimating cluster-level false-positive rates on these maps (statistics implemented in BrainVoyager). This resulted in a minimum cluster size of 34 voxels at 3x3x3 mm (904 mm3), corresponding to $p < .05$ corrected cluster-wise.

In a second step, we analyzed the differential effect of the factor origin in the threatening stimuli. Therefore, we created individual maps for the two contrasts (evolutionary-threatening > evolutionary-neutral) and (modern-threatening > modern-neutral). These individual maps were subsequently used as input for a paired t-Test where we contrasted the maps "evolutionary-threatening > evolutionary-neutral" and "modern-threatening > modern-neutral" against each other. The voxel-level threshold for statistical maps correspond to $p< .001$. To correct for multiple comparisons, a Monte Carlo simulation with 1000 iterations was used for estimating cluster-level false-positive rates on these maps. This led to a minimum cluster size of 38 voxels at 3x3x3 mm (1007 mm3), corresponding to $p < .05$ corrected cluster-wise. Further, we extracted t values from the resulting clusters to quantify the effect of origin. In selected regions, we additionally computed for each condition the mean time course by averaging all peri-stimulus BOLD time course segments belonging to the same condition using the respective tool in Brainvoyager. Anatomical regions were identified using the Talairach Client (Lancaster et al., 2000).

Results

Behavioral results

We performed a repeated-measures ANOVA to test for differences in reaction times (RT) to the first picture of a block. Mauchly's test indicated that the assumption of sphericity had been met ($\chi 2(5) = 7.00$, $p > .05$). The results showed no significant effect of experimental condition on RT ($F(3, 120) = 1.58$, $p > .05$). Mean RT ranged from 542.49 ms to 561.56 ms (Table 1).

The general pattern of the post-scan rating of valence and arousal of the IAPS pictures did not deviate from the original ratings provided in the IAPS technical report (Lang et al., 2008) and from our own data in an independent sample (unpublished data). A confirmatory factor analysis in this

independent sample on the valence and arousal ratings supported the assignment of the pictures to the four conditions, thus adding to the validity of the experimental design.

To test for differences in the valence rating between experimental conditions, we conducted a repeated-measures ANOVA. Mauchly's test was significant ($\chi2(5) = 28.75$, $p < .05$), indicating a violation of the sphericity assumption. Greenhouse-Geisser corrected values showed significant differences between experimental conditions ($F(2.11, 84.40) = 222.76$, $p < .05$). Bonferroni-corrected post-hoc tests revealed significant differences for all pairwise comparisons between all conditions at $p < .05$ (Table 1).

Differences in the arousal rating between experimental conditions were assessed with a repeated-measures ANOVA. Mauchly's test was significant ($\chi2(5) = 26.14$, $p < .05$), indicating a violation of the sphericity assumption. Greenhouse-Geisser corrected values showed significant differences between experimental conditions ($F(2.02, 80.79) = 119.36$, $p < .05$). Bonferroni-corrected post-hoc tests indicated that each threat condition was rated significantly different to both neutral conditions at $p < .05$ (Table 1).

To summarize, evolutionary-neutral pictures were rated significantly more positive in valence compared to all other conditions and within the positive spectrum of the IAPS set. Subjects rated both threat conditions significantly higher in arousal and more negative in valence than the two neutral conditions. While the two threatening conditions did not differ in arousal ($p > .5$), modern-threatening pictures were rated significantly more negative in valence than evolutionary-threatening pictures. Across all presented pictures, the subjects' ratings varied significantly more on the arousal scale than on the valence scale ($N = 64$ pictures; average SD across pictures: valence = 1.39, arousal = 1.77, Wilcoxon $Z = -5.5$, $p < .001$).

fMRI results

The main effect of threat in the 2x2 repeated measures ANOVA (factors threat and origin) revealed a network of cortical and subcortical regions (Figure 1) including the left middle frontal gyrus, right inferior frontal gyrus, right posterior cingulate gyrus, right cuneus, large portions of the bilateral occipital lobe including extrastriate and inferotemporal regions, and bilateral amygdala (see Table 2 and Figure 2A).

To identify regions showing a differential activation to the evolutionary vs. modern origin within the threatening stimuli, we applied the combined contrast "evolutionary-threatening > evolutionary-neutral" > "modern-threatening > modern-neutral". This analysis revealed a network of regions including the left inferior frontal gyrus, right middle frontal gyrus, right parietal lobe (sub-gyral), right precuneus, left thalamus, bilateral fusiform gyrus, bilateral superior parietal lobule, bilateral amygdala (see Table 3 and Figure 2B).

The opposite contrast revealed that only in the bilateral posterior cingulate and the bilateral parahippocampal gyrus the activity was higher for modern-threatening stimuli than for evolutionary-

threatening pictures (see Table 3). For the amygdala, the fusiform gyrus, and the parahippocampal gyrus, we created event-related averages of all conditions to characterize the BOLD response of each experimental conditions (see Figure 3).

Discussion

Functional implications

We systematically investigated the effect of image content in threatening stimuli on the activation of neural networks involved in emotion processing. By contrasting threatening with neutral pictures, we revealed a network of regions typically found in emotion processing (Pessoa and Adolphs 2010), thus supporting the validity of our threatening stimuli. Evolutionary-threatening pictures evoked significantly stronger activations than modern-threatening pictures in most regions of the network for processing threatening stimuli. Surprisingly, however, this finding is in contrast to the behavioral part of the experiment, the post-scan rating of the IAPS pictures. Subjects rated modern-threatening stimuli as significantly more negative in valence than evolutionary-threatening pictures, indicating a higher level of perceived threat or fear for stimuli such as guns, knives, and car accidents. At the same time, the two threatening conditions did not differ in the arousal rating, thus implying no relevant association of subjective arousal with the difference of neural activity between the threatening conditions. According to the prevalent opinion in the literature, our behavioral findings would have suggested that modern-threatening pictures evoke a stronger BOLD response than the evolutionary-threatening pictures in regions involved in emotion processing, or the fear module, respectively (cf. Sabatinelli et al. 2005; Kensinger and Schacter 2006). However, since the opposite was the case in our study, we argue that the evolutionary preparedness of the evolutionary-threatening stimuli is the actual driver of the neural activity.

The network of brain regions that activated stronger for evolutionary-threatening stimuli than for modern-threatening stimuli comprised bilateral amygdala, the left inferior frontal gyrus, right middle frontal gyrus, right parietal lobe (sub-gyral), right precuneus, left thalamus, bilateral fusiform gyrus and bilateral superior parietal lobule (Sabatinelli et al., 2005). The finding in the amygdala as central emotion processing region supports the close relationship to emotion processing (Fig. 3A), but also early region in the visual stream as fusiforme gyrus, known for face processing (Sabatinelli et al., 2005), are involved (Fig 3B). We found the opposite effect (higher activity for modern-threatening than for evolutionary-threatening stimuli) in the bilateral posterior cingulate and the bilateral parahippocampal gyrus (Fig. 3C). A possible explanation of this reversal could be the connection of the posterior cingulate gyrus with the hippocampus (FeldmanHall et al., 2016). Modern stimuli might engage more processes of memory, self-reflection, and appraisal, which could be mediated by the posterior cingulate cortex. The parahippocampal gyrus has been found to encode complex visual scenes and the local environment (Epstein and Kanwisher, 1998). This could explain the low activation in this area for evolutionary-threatening stimuli, where the focus is on the animal itself and

not so much on its surroundings (cf. figure 3). In the other experimental conditions, however, about half of the pictures show wide-angled shots of natural landscapes or built environments, thus possibly activating the parahippocampal place area (Aguirre et al., 1996; Epstein and Kanwisher, 1998; Ishai et al., 2004).

The results of our study support the hypothesis of the amygdala and its connected regions as an evolved module for the detection of threat. This detection takes place automatically, without the need of cognitive processing of the stimuli (Lundqvist and Ohman 2005). Furthermore, our results stress the perceived biological significance of evolutionary prepared stimuli, even if they do not pose such an actual threat to the individual anymore. A recent study found neurobiological evidence for a rapid snake detection mechanism in the pulvinar, which could represent a part of the evolved module (Stuber et al., 2011).

Our findings suggest that the assumption of amygdala activity explained by arousal ratings may not be fully comprehensive. Also, at least in our study, the valence ratings do not seem to reflect the activation of the emotion network. The study by Anders and colleagues (2008) showed effects of valence in line with the majority of the literature (i.e., less neutral valence ratings correlating with higher amygdala activity). However, the most negative rated pictures (modern-threatening) interestingly did not show the highest amygdala activity. When investigating amygdala activity to threatening stimuli, the explicit arousal and valence ratings might not be the strongest indicators to predict the neural activity. Even when made quickly and intuitively, these ratings might comprise elaborate cognitive evaluations and might thus not be strongly indicative of the amygdala's role of automatic significance detection.

Also, alternative explanations of the difference in activation between the evolutionary-threatening and the modern-threatening condition can be taken into account. Firstly, a complimentary explanation for the diminished BOLD response towards the modern-threatening stimuli could lie in a cognitively more demanding evaluation after the perception. Since an evolved module for these modern pictures can hardly exist, the evaluation of threat might require higher-level cortical processing, which in turn reduces amygdala activity (Hariri et al., 2000; Hariri et al., 2003). This demanding evaluative process might set in involuntarily even in the absence of an additional experimental task.

Secondly, the conditions could potentially differ in perceived threat, when assuming that threat could not be defined by valence and arousal ratings. Some of the pictures display an immediate threat (e.g., snarling snakes and pointing guns), whereas other pictures only show a distal or already occurred threat (e.g., resting spiders or car accidents). It might be possible to quantify the threat potential of either experimental category in terms of probabilities, for instance by pooling lethality rates of each stimulus displayed. However, we assume that the individual rating of valence and arousal represents an appropriate and valid proxy to the subjective feeling of threat. Moreover, by averaging across a broad range of image content, we reduce the influence of outliers in terms of perceived threat. Further, we argue that this averaging, together with the randomized presentation of the stimuli, reduced

possible effects of image features (e.g., eyes, color, and spatial frequency) that differed between the two threatening conditions.

Methodological implications

As we have demonstrated in this study, the content of the pictures shown to the subjects has a pronounced effect on the neural response, even if the pictures are believed to be in the same emotion category (i.e., threatening pictures). As a consequence, we suggest that greater care should be taken when selecting stimuli for studies on emotion processing. In addition to the selection based on normative ratings, we recommend to characterize images also on qualitative dimensions, with the evolutionary-to-modern dimension being only one of several. For instance, Kensinger and Schacter (2006) had the subjects rate picture or word stimuli on the dimensions animacy (animate vs. inanimate) and commonality (common vs. uncommon). However, the authors report that the emotion processing did not differ depending on the task, whereupon the authors collapsed the data of both tasks. For a study containing a matching task and a labeling task, Hariri and colleagues (2003) used sets of threatening IAPS pictures which were virtually identical to our selection. Instead of evolutionary vs. modern, they denoted the stimuli being of natural vs. artificial origin. In the subsequent analysis, however, the authors collapse the fMRI data across these two different categories, since the focus of the study was the difference in task but not the differentiation of the two origins. In this case, pooling the data might cause undesired variance, assuming that the two categories engage the network of emotion processing differently.

From a more technical perspective, even measurable image parameters such as color, contrast, or spatial frequency do not directly account for the aspect of image content. Interestingly, also the reaction time did not differ between experimental conditions, giving no indication of a prioritized perception of evolutionary or modern threats (and thus, not reflecting our fMRI or behavioral results). Empirical evidence of this effect would suggest faster perception of threatening vs. neutral stimuli (Öhman et al., 2001) and no differences between evolutionary and modern threats (Fox et al., 2007; Pool et al., 2016).

In conclusion, exerting a more elaborate process of stimulus classification and selection will consequently lead to better experimental designs and thus more valid results. Effects that might have been confounded by the selection of overly heterogeneous stimuli could thus be revealed. We point out that researchers should be more aware of the possible effect of image content when selecting pictures as well as reporting results of studies using IAPS and comparable databases. We suggest that future studies utilizing affective picture stimuli should firstly replicate our findings of marked differences between evolutionary and modern stimuli, and secondly characterize the image content on more dimensions than only valence and arousal. The IAPS database was originally conceived on a theoretical foundation representing the basic emotion dimensions valence and arousal (Russell, 1980) and the dominance dimension. However, when applied in studies investigating neural processes, these

dimensions might fall short of representing the complexity of the brain mechanisms adequately. Thus, adding further dimensions that are relevant and tailored to neurophysiological research might greatly improve the IAPS database and future studies. In addition, this study only investigated still pictures. However, real life visual perception is much more adapted to the perception of moving and animated scenes. Future studies might therefore also use short video clips to investigate effects of content on the processing of scenes.

Limitations

The content selected for our experimental conditions could be criticized in different aspects. While both modern stimuli conditions show similar scenes from a wide-angle perspective, the modern-threatening condition also comprises close-up views of weapons. Also, the evolutionary-threatening pictures present menacing animals in their natural surroundings, whereas the evolutionary-neutral pictures do not contain any non-threatening animals. We are aware of this difference but argue that the inclusion of animals in both categories would have changed the focus to the comparison of animate vs. inanimate stimuli.

Similar to previous studies (Anders et al., 2008) and the original IAPS sample (Lang et al., 2008), we found that ratings varied significantly more on the arousal scale than on the valence scale. Anders and colleagues (2008) concluded that arousal ratings were thus less directly related to the actual stimulus than valence ratings. Moreover, the validity of the valence and arousal ratings should be cross-checked by other measures (e.g., verbal descriptions, thinking-aloud, etc.). The results are further to regard with the limitation that we did not match for physical properties of the pictures as luminance, color or complexity, as this would lead to very low samples of pictures with identical properties not suitable any more for statistically sufficient stimuli samples.

Furthermore, this study relies on subjective ratings of arousal combined with fMRI measures of brain activity. Psychophysiological measures such as heart rate, heart rate variability and skin conductance might increase the specificity of the findings.

Finally, we acknowledge that our findings and implications are not readily transferable to other basic emotions. While the effect of the evolutionary origin might be valid for threatening stimuli evoking fear or anxiety, it might not hold true for emotions such as happiness, disgust, sadness, or surprise.

Conclusions

We provide evidence that neural activity in the fear module is not only driven by arousal or valence, but presumably also by the evolutionary content of the stimulus. Methodologically, we thus suggest that a more elaborate classification of stimulus content will improve the validity of experimental designs.

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Tables

	Reaction time [ms]	**Valence**	**Arousal**
Condition	***M (SD)***	***M (SD)***	***M (SD)***
Evolutionary-threatening	546.30 (175.31) [a]	3.93 (0.21) [a]	4.97 (0.25) [a]
Modern-threatening	542.49 (154.88) [a]	2.78 (0.17) [b]	5.07 (0.23) [a]
Evolutionary-neutral	556.74 (167.37) [a]	7.47 (0.11) [c]	2.21 (0.19) [b]
Modern-neutral	561.56 (165.70) [a]	5.35 (0.12) [d]	1.95 (0.15) [b]

Table 1. Reaction times to the first picture of a block during the scan session, and means and standard deviations of the normative ratings of the IAPS pictures. Valence scale from 1 = negative to 9 = positive and arousal scale from 1 = low to 9 = high. Non-identical superscripts indicate conditions that are significantly different at $p < .05$. Reaction times to the first picture of a block were recorded during the fMRI scan, valence and arousal ratings after the scan.

	Talairach coordinates				
Region (BA)	***x***	***y***	***z***	**Cluster size (mm³)**	F_{peak}
L middle frontal gyrus (9)	-40	13	24	197	14.31
R inferior frontal gyrus (46)	35	31	12	423	17.62
R posterior cingulate gyrus (31)	8	-38	30	893	16.37
R posterior cingulate gyrus (29)	9	-50	12	1384	42.74
R cuneus (17)	8	-80	9	4742	54.31
L occipital lobe, extending into the inferior temporal lobe (18, 19, 37)	-43	-80	-9	54405	99.61
R occipital lobe, extending into the inferior temporal lobe (18, 19, 37)	25	-32	-15	47724	75.84
L amygdala	-25	1	-15	1069	20.67
R amygdala	20	-2	-12	1145	21.37

Table 2. Anatomical regions activating stronger for threatening stimuli than for neutral stimuli. Statistical threshold: $p < .05$ (cluster level corrected for multiple comparisons using a Monte Carlo simulation with 1000 iterations to estimate cluster-level false-positive rates). Abbreviations: L left hemisphere, R right hemisphere, BA Brodmann Area, Talairach coordinates of peak voxel, F values of peak voxel for the ANOVA.

	Talairach coordinates				
Region (BA)	***x***	***y***	***z***	**Cluster size (mm^3)**	***t***
L inferior frontal gyrus (9)	-39	14	25	4022	5.67
R middle frontal gyrus (9)	36	11	25	623	5.21
L fusiform gyrus (19)	-39	-82	-11	35262	13.36
R fusiform gyrus (19)	39	-58	-14	31408	12.46
L superior parietal lobule (7)	-24	-67	37	2195	5.16
R superior parietal lobule (7)	21	-58	67	673	5.90
R parietal lobe (sub-gyral) (7)	24	-52	52	400	4.93
R precuneus (7)	27	-73	43	1827	5.51
L amygdala	-30	-4	-14	1197	5.74
R amygdala	24	-1	-11	2815	6.42
L thalamus	-21	-28	1	434	6.19
L posterior cingulate (30)	-21	-55	13	2244	-6.20
R posterior cingulate (30)	18	-31	-13	1641	-7.15
L parahippocampal gyrus (36)	-21	-40	-8	3359	-8.79
R parahippocampal gyrus (35)	21	-34	-11	1775	-8.04

Table 3. Anatomical regions showing the differential effect of origin in threatening pictures. Two-sample t-Test, contrast (Evolutionary-threatening > evolutionary-neutral) > (Modern-threatening > modern-neutral). All t-Tests significant at $p < .001$. Abbreviations: L left hemisphere, R right hemisphere, BA Brodmann Area, Talairach coordinates of peak voxel.

Figures

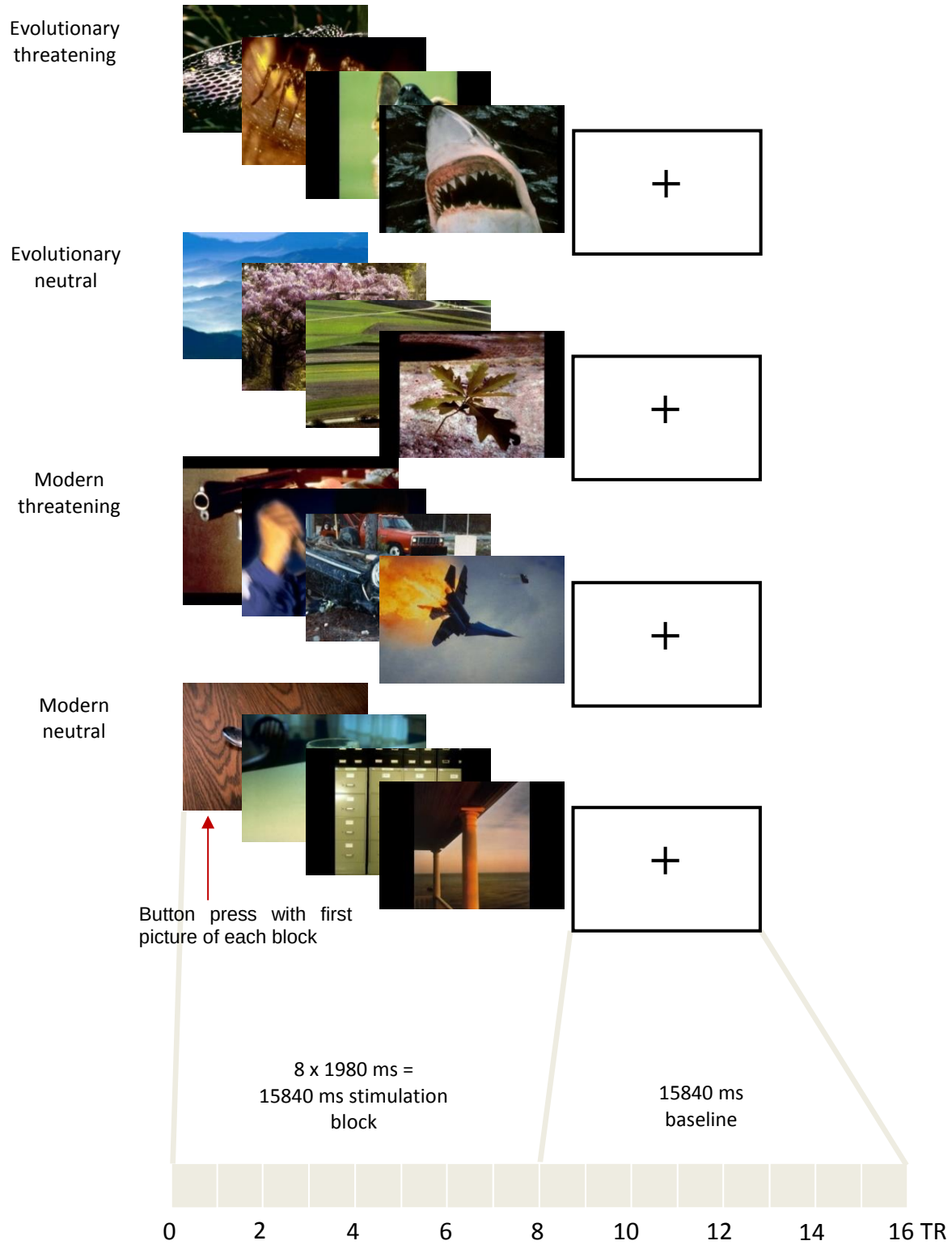
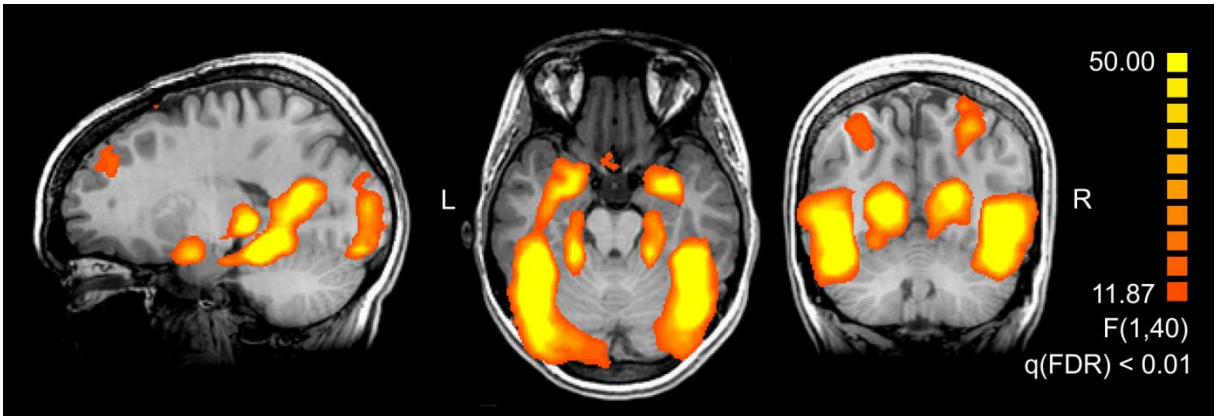
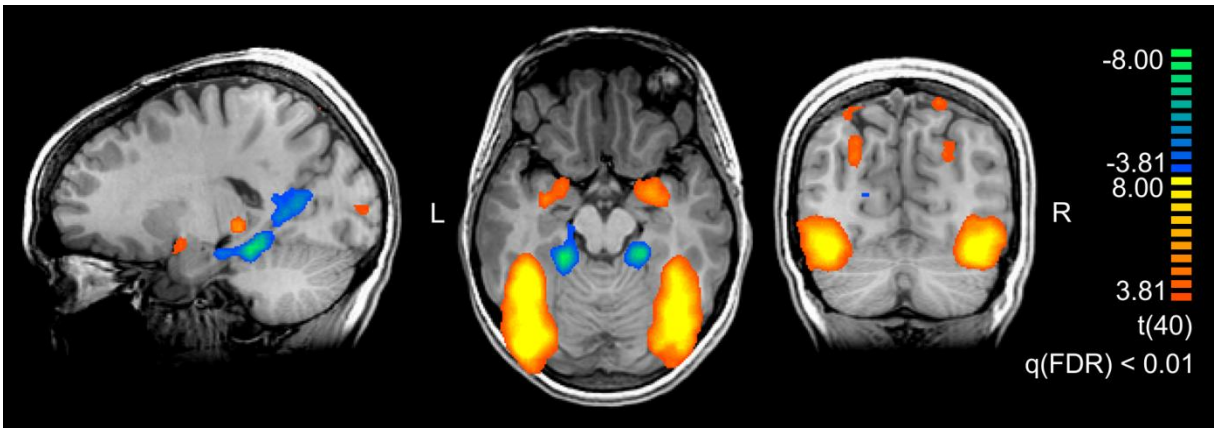


Figure 1. Experimental task. For representational reasons, only four pictures for each category are shown. In the experiment, each block consisted of eight pictures. In order to make the pictures less identifiable in the sense of the IAPS providers, in the figure black boxes are pasted over the front picture, which of course was not the case in the experiment.



A.



B.

Figure 2. A. Brain areas activating stronger for threatening stimuli than for neutral stimuli. The map shows the main effect of threat, derived from a repeated measures 2x2 ANOVA with factors threat (levels: threatening, neutral) and origin (levels: evolutionary, modern). The thresholds in the figures are chosen for representational purposes, q(FDR) < .01. Talairach coordinates of slices x: 18, y: -56, z: -17.

B. Brain areas showing the differential effect of origin in threatening pictures. Contrast: (Evolutionary-threatening > evolutionary-neutral) > (Modern-threatening > modern-neutral), q(FDR) < .01. Talairach coordinates of slices x: -18, y: -66, z: -12.

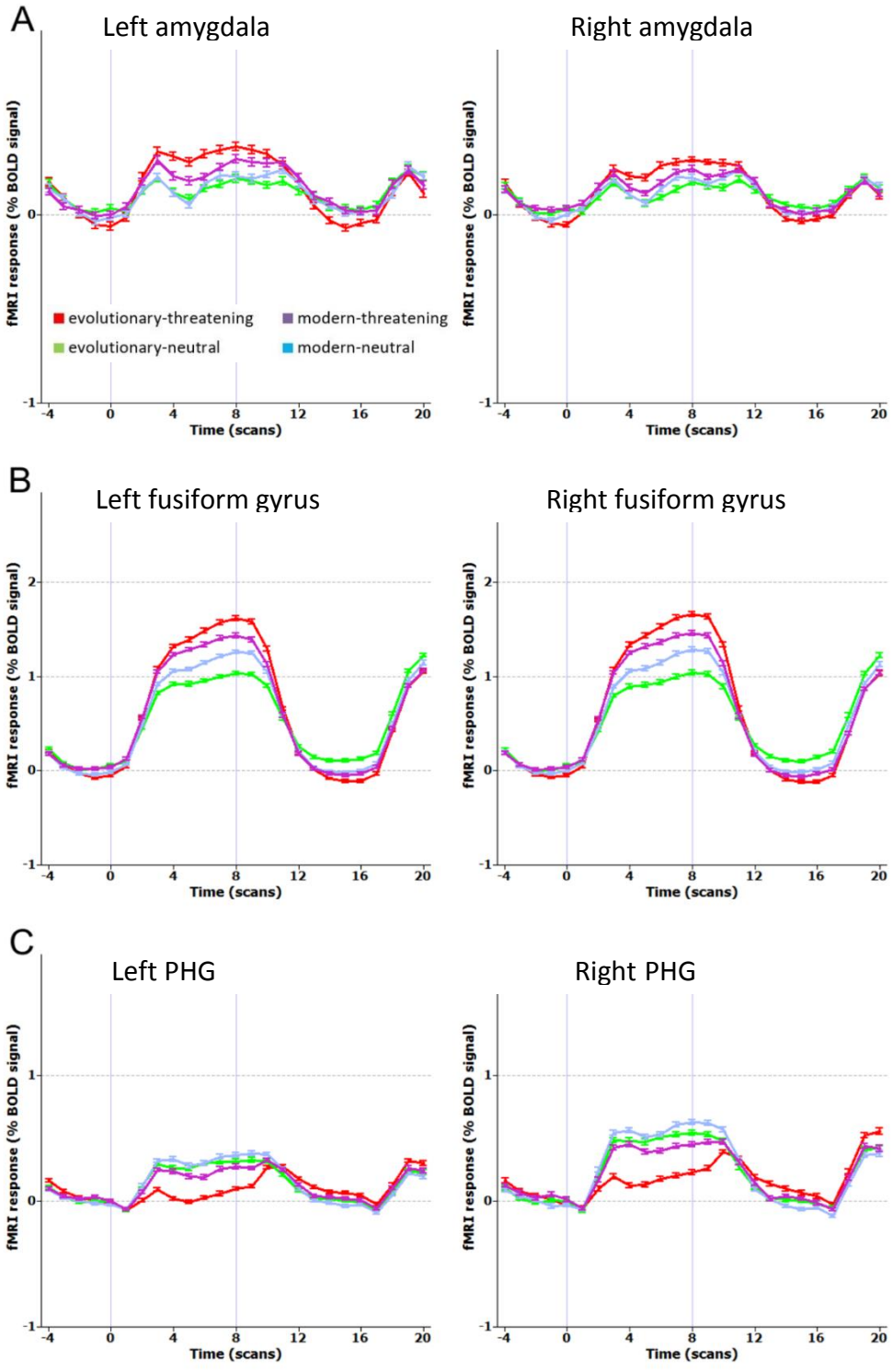


Figure 3. Mean time course for selected regions. A: Left and right amygdala, B: Left and right fusiform gyrus (BA 19), C: Left and right parahippocampal gyrus PHG, (BA 36 left, BA 35 right). Contrast: (Evolutionary-threatening > evolutionary-neutral) > (Modern-threatening > modern-neutral).

Supplementary Material

1026	6230
1030	6244
1033	6300
1040	7003
1050	7017
1051	7033
1052	7036
1070	7037
5726	7039
5750	7135
5760	7140
5780	9611
5800	9901
5811	9911
5814	9920
5825	
6190	

Table S1 Number of IAPS pictures used for the experiment

	MNI coordinates				
Region (BA)	***x***	***y***	***z***	**Cluster size (mm³)**	F_{peak}
L middle frontal gyrus (9)	-42	16	-1	197	14.31
R inferior frontal gyrus (46)	36	30	30	423	17.62
R posterior cingulate gyrus (31)	8	-39	10	893	16.37
R posterior cingulate gyrus (29)	9	-53	31	1384	42.74
R cuneus (17)	8	-83	10	4742	54.31
L occipital lobe, extending into the inferior temporal lobe (18, 19, 37)	-43	-82	5	54405	99.61
R occipital lobe, extending into the inferior temporal lobe (18, 19, 37)	26	-31	-16	47724	75.84
L amygdala	-26	4	3	1069	20.67
R amygdala	21	1	-22	1145	21.37

Table S2 MNI coordinates of table 2; converted with:
http://sprout022.sprout.yale.edu/mni2tal/mni2tal.html

1.4. Training emotion regulation through real-time fMRI neurofeedback of amygdala activity

Herwig U[1,2,3]; Co-workers and foreseen co-authors: Lutz J[1,4], Scherpiet S[1], Opialla S[1], Scheiblich A[1], Scheerer H[1], Steiger VR[1,4], Kohlberg J[1,4], Sulzer J[5], Weidt S[6], Stämpfli P[1], Rufer M[6], Seifritz E[1], Jäncke L[4], Brühl AB[1]

[1]Department for Psychiatry, Psychotherapy, Psychosomatics, Psychiatric Hospital, University of Zürich, Zürich, Switzerland

[2]Institute of Environmental Decisions, Consumer Behavior, ETH, Zurich, Switzerland

[3]Department of Psychiatry and Psychotherapy III, University of Ulm, Ulm, Germany

[4]Department of Psychology, Division Neuropsychology, University of Zürich, Zürich, Switzerland

[5]Department of Robotics, Biomechanics and Neuroscience, The University of Texas, Austin, USA

[6]Department of Psychiatry and Psychotherapy, University Hospital Zurich, University of Zurich, Switzerland

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Introduction

Gaining control over and guiding one's own mental, and as such central nervous processes is an evolutionary highly developed capability. Strategies to direct voluntarily cognitive and emotional processes are old in human intellectual history. Marc Aurel stated nearly 2000 years ago that the own appraisal of external events is what disturbs and that we have the power to change it ("*If thou are pained by any external thing, it is not this that disturbs thee, but thy own judgment about it. And it is in thy power to wipe out this judgment now."* Meditationes VIII. 47, transl. Long 1862). However, we regularly experience anxiety, sadness, anger, or even rage, and we are often not capable to simply "wipe" this out. We try to cope with and regulate emotions and accompanying cognitions by applying individual strategies; but strengthening emotion regulation is often desired. In mental disorders, such as anxiety disorders, depression, and emotional instability, cognitive control and emotion regulation are typically impaired, representing a characteristic of the disorder, and improving emotion regulation skills is a primary aim of psychotherapeutic interventions (Anthes 2014). A well-established neurobiological model of emotion regulation locates the cognitive control of emotions in prefrontal cortex areas which down-regulate emotion-generative areas, such as the amygdala (Buhle et al. 2014; Diekhof et al. 2011; Disner et al. 2011; Ochsner, Gross 2005). A basic cognitive strategy which is also an element of cognitive-behavioral psychotherapy is the *reappraisal* of an emotionally laden situation (Gross, John 2003). Faced with such a situation one may reinterpret the emotional meaning for oneself and adopt a describing view of the situation in the sense of a *reality check*, thereby intentionally activating cognitive resources (Herwig et al. 2007). However, clinical experience shows that such strategies may not be as successful as desired, and particularly in mood and anxiety disorders or in

emotional instability they are trained in psychotherapy with sometimes limited success. Accordingly, developing tools to improve training of emotion regulation strategies is desirable. A problem in this context is the difficulty to measure the success or the effect of an applied strategy which may lead to inefficient development of regulation skills. Providing such a measure to a training subject may enhance learning and help developing successful emotion regulation. A potential measure at the neurobiological level is the extent of amygdala down-regulation, associated with the application of a regulation strategy. Therefore, real-time functional magnetic imaging neurofeedback (rt-fMRI) may offer an opportunity (Brühl et al. 2014; Paret et al. 2014).

Rt-fMRI provides the possibility to guide mental activity based on a few seconds delayed feedback of the activity in the brain region to be regulated. The feasibility of rt-fMRI to selectively modulate regional brain activity has been well demonstrated (e.g. Sulzer et al. 2013a). It has also been shown in clinical context, for instance, in depression with an up-regulation of reward related brain areas or amygdala by means of a task inducing positive emotions (Linden 2014; Linden et al. 2012; Young et al. 2014). Increasing evidence points to the possibility to influence emotion regulation by amygdala-down-regulation (Buhle et al. 2013) ,which would represent a direct influence on the main neural substrate of emotion regulation (Gross, John 2003). Sarkheil et al. (Sarkheil et al. 2015) recently demonstrated that feedback-guided up-regulation of the lateral prefrontal cortex consecutively improved down-regulation of amygdala activity. Paret et al. (2014) reported amygdala-down-regulation in a single session feedback-trial. Such, the feasibility of amygdala down-regulation through rt-fMRI has been demonstrated. For a practical application, however, for instance as a potential treatment tool in psychotherapy it should be demonstrated that the emotion regulation is trainable and improves over a series of training session (Brühl et al. 2014).

Accordingly, we trained a group of subjects over four sessions to improve their emotion regulation using a cognitive strategy based on feedback of the amygdala activity as measured by fMRI, the 'feedback' group. Another group was asked to train emotion regulation in the same setting but without obtaining feedback, the 'control' group. The amygdala was individually localized prior each session. The subjects were presented series of emotional pictures of negative valence and were asked to perform two tasks: First, they should just look at the pictures, the *'view'*-condition, and second, they should regulate their emotions by performing cognitive reappraisal based on a reality check, '*regulate'*. We hypothesized that four weekly feedback-supported cognitive emotion regulation training sessions would improve down-regulation of amygdala activity from the first to the last session, and that this effect is stronger when compared to training without feedback. The primary outcome measure thus was the difference of beta weights of the fMRI signal between the emotion regulation condition ('*regulate'*) and the viewing condition ('*view'*) in training session 1 versus training session 4, with the hypothesis that the difference would be larger in session 4 compared to 1 in the feedback group and that this difference was bigger/larger than in the control group. Additionally, we tested the effect within a control region in the sensorymotor cortex supposed to be

not affected by emotion regulation. The subjects also performed emotion regulation tasks in another domain, regarding short emotional clips, in order to assess transfer effects of training success.

Methods

Participants

Out of initially 24 subjects assigned to the regulation group, 15 subjects (age mean 26.67 years, SD 4.8, range 21-38, 8 male, 7 female) completed the four training sessions and had technically suitable data (sufficient amygdala activation onto emotional stimulation in session 1, no movement artefacts of more than 3 mm in any direction). Out of 16 subjects assigned to the control group this was the case for 11 subjects (age mean 27.3 years, SD 7.3, range 19-42, 5 male, 6 female). We realized a high drop-out rate of 37.5% in the regulation group and 31.3% in the control group due to artifacts, technical problems and non-completion of the session series by the subjects. The resulting 26 subjects with a total of 104 fMRI scanning sessions provide the basis for our analysis. The subjects were recruited via personal contact and email-lists.

All participants were healthy, as assessed with semi-structured interviews and checklists (abbreviated version of the Mini Neuropsychiatric Interview (MINI, Sheehan et al. 1998)). Exclusion criteria were prior and current neurological and psychiatric diagnoses; pregnancy; intake of any medication (except for oral contraceptives) or psychotropic drugs including excessive consumption of alcohol (regular intake of > 7 units/week), cigarettes (> 1 pack/day) and caffeine (> 5 cups/day) and general contraindications against MRI examinations. After each session, subjects were asked in a structured interview on tiredness, general feelings, specific experiences and the strategies used for regulation (table S1). The mean period between sessions was 7.46 days (SD = 1.39, range = 3 – 17). The study was approved by the ethics committee of the canton of Zürich and conducted in compliance with the Declaration of Helsinki (World Medical 2014). All participants gave written informed consent and received financial compensation.

Experimental task and scanning procedure

Prior to the first session, all participants were given written instructions. The regulation group was informed about the feedback delay of about 5-6 seconds due to the delay of the hemodynamic response function. The amygdala was first localized functionally for each participant in each session. In the localizer task, participants were presented negative and neutral pictures from the International Affective Pictures System (IAPS, Lang 2005). Pictures were presented in a blocked design with 8 pictures in each block, each shown for 3 sec (total block time 24 s). After each block a baseline period (fixation cross) of 26 sec allowed the blood oxygen level dependent (BOLD)-signal to level off before the next condition. Subjects were instructed to passively look at the pictures. In total, 4 blocks of negative and 4 neutral blocks were presented (total duration of the localizer: 6 min). Based on the

contrast negative versus neutral, we localized the area, responding to negative emotional stimulation in the anatomical area of the left amygdala.

The feedback task (fig. 1) was constructed similar to the localizer task in a blocked design, but only containing negative IAPS stimuli. A run consisted of 14 blocks of 24 sec, comprising 8 pictures shown for 3 sec each. Prior to each block, the instruction to "view" or "regulate" (reg) was given on the screen for 1 sec. For the regulate-condition, the participants were instructed to apply cognitive reappraisal by using reality checking and were provided examples such as "these are only pictures", "I am lying in the scanner", "I am participating in an experiment" (Herwig et al. 2007). In the view-condition they were instructed to just regard the pictures.

Stimulation blocks were separated by a baseline period (fixation cross) of 25 sec (baseline + instruction = 26 sec). Each run consisted of six "view" conditions and ten "regulate" conditions. Participants performed two feedback runs in sessions 1 and 4 and three runs in session 2 and 3. In some cases, participants completed only 2 runs in those session due to high self-reported drowsiness (mean number of feedback runs per session: 2.42). Picture sequences were randomized and in each session 50% of the pictures were new, prior unseen pictures to prevent habituation and effects of familiarity. Amygdala activity was recorded from the region identified in the localizer task and was given as real-time feedback to the participant in form of colour-changing blocks laterally on both sides of the pictures (fig. 1). Colour-blocks were positioned bilaterally and in the middle of the vertical axis to avoid distraction to either side or away from the centre of the negative pictures.

The control group completed the same task, except that the colour-blocks changed in the same colour range but in a random fashion, not associated with the individual's amygdala activation. Participants in the control group received the same instructions to regulate as the feedback group. The changing colour-blocks were not explained or mentioned as containing feedback of any sort.

Finally, in order to detect a possible transfer effect of the training to another emotion regulation task and without amygdala feedback, participants performed prior the first session and after the last session a regulation task with stimuli consisting of videos displaying negative emotional facial expressions (anger, fear, sadness, disgust, embarrassment; van der Schalk et al. 2011). The task consisted of 5 view and 5 regulate blocks, displaying 5 short videos (length between 3 and 6 sec) within a total time of 24 sec. Total duration for the transfer task was 6 minutes, 54 sec.

Image acquisition

Imaging was performed with a 3.0 T Philips Achieva Scanner (Philips Medical Systems, Best, The Netherlands, equipped with an 8-channel receive head-coil array). Echo-planar imaging was performed for functional MR imaging (repetition-time (TR)/echo-time (TE) 2000/25 ms, 30 sequential axial slices, whole brain, slice thickness: 3.0 mm, field of view (FOV): 240x240 mm, resulting voxel size: 3x3x3 mm, axial orientation, SENSE-factor: 2.0). The localizer run consisted of 190 volumes, the feedback runs of 330 volumes, transfer run 207 volumes. At each session, high-resolution 3-D T1

weighted anatomical volumes were acquired (TR/TE 6.73/3.1 ms; voxel size 1x1x1 mm, 145 slices, axial orientation) for coregistration with the functional data. Stimuli were presented via digital goggles (Resonance Technologies, Northridge, CA).

FMRI analysis and statistics

Online real-time analysis and statistics: Functional data were analysed online during fMRI with TurboBrainvoyager™ (TBV) Version 3.2 (Brain Innovation, Maastricht, NL). The processing has been described previously (Caria et al. 2010; Goebel 2001). Real-time data analysis comprised incremental 3D motion detection and correction and drift removal and resulted in incrementally computed statistical maps based on the General Linear Model (GLM) and event-related averages. After the localizer scan, a region of interest (ROI) was individually placed in the anatomical region of the right amygdala extending over 3 slices (= 9 mm) using a t-value threshold of 2.0. The size and centres of these individual localizer ROIs are given in table S3.

Compared to studies aiming at upregulating brain regions, the down-regulation of a brain region is particularly sensitive to individual differences in the total reactivity of the BOLD signal. Therefore, we computed the individual reactivity of the amygdala from the localizer using the average % signal change from baseline in the chosen amygdala ROI. This was entered as the maximum value (= bright orange) for the range of colours during feedback blocks. The signal changes during feedback were computed as percent of the individual maximum change. The feedback was first normalized based on the percent signal increase from the previous baseline condition (last five volumes), then three-point averaged (averaging the current value with the previous two) to reduce noise and strong fluctuations of the feedback (in parallel to Sulzer et al. 2013b). This feedback signal was computed and presented by a custom made software running on VisualStudio™ (Microsoft, Redmond, WA, USA).

Offline analysis and statistics: After scanning, the acquired images were processed offline using BrainVoyagerQX™ 2.8 (Brain Innovation, Maastricht, NL, Goebel et al. 2006). Standard preprocessing with BrainVoyagerQX included motion correction, slice scan-time correction, high-frequency temporal filtering and removal of linear trends. All individual functional datasets were checked for excessive head movements. We excluded runs with > 3mm head movement in at least one direction. In cases where there was one single spike > 3mm in an otherwise steady run, we discarded the part of the run (containing the spike) from analysis (resulting in halve runs of 155 Volumes). In cases where we had less than 1 run (or 2 halve runs) for a session, we excluded the subject from our analysis.

Functional data were co-registered with the individual T1-weighted 3D-structural data. Structural and functional data were transformed into Talairach space and spatially smoothed with a 4 mm full-width half-maximum Gaussian kernel for subsequent within- and between-subject analysis. Since we were interested in feedback-guided emotion regulation we modelled the *regulate condition* as a box car function starting 10 s after feedback-onset, to take into account the delay in the feedback signal

and an estimated time for applying emotion regulation based on the feedback. The instruction period and first 10 seconds of the feedback were modelled as a separate predictor but it was not further analyzed. We modelled the *view condition* correspondingly, with the same phase of the block (11-24 s) as the basis for comparison with the regulate condition. Further, our model contained box car functions for the baseline periods between blocks. Box cars were convolved with the standard hemodynamic response function provided by BrainVoyager adapted to the duration of the blocks.

Three-dimensional statistical parametric maps were calculated for *regulate* and *view* conditions for all four sessions. These datasets were combined into individual fixed effects general linear models (GLMs) and random fixed effects group GLMs. We extracted for each subject and each session the mean beta-weights of the individual amygdala ROI identified in this session's localizer for the conditions *view*, *regulate* and the contrast *regulate* versus *view* for comparison between sessions, subjects, and groups. Subsequent analyses were done in SPSS 22 (IBM, Armonk, NY, USA).

To investigate training effects we computed a repeated measures ANOVA, with the within-subject factor *session* (4 levels) and *condition* (3 levels: *regulate*, *view*, *regulate-view*) for both groups including confirmation of sphericity (Mauchly). Where the main effect of *session* was significant, we conducted post-hoc paired t-tests, to test our directed hypothesis of decreased amygdala activity in session 4 compared to session 1. To directly compare the training effects between groups, we further conducted a two-sample t-test with the difference in the regulation effect between the first and last trainings-session (*regulate– view* in session 4 minus *regulate-view* in session1) as the dependent variable. We hypothesized a bigger regulation effect in session 4 compared to session 1 for the feedback group.

Regarding the transfer task, we also analysed each individual's amygdala ROI activation during regulate and view and compared the difference between sessions in a repeated measures ANOVA, with the within-subject factor *session* (2 levels, session 1 and 4) and *condition* (3 levels: regulate, view, regulate-view) in the feedback and the control group.

As a control region for the feedback group, hypothesized to be not involved in brain activation associated with emotion regulation (Buhle et al. 2014), we selected a ROI in the primary sensorymotor cortex (x/y/z = ±33/-24/62; 10 mm diameter) and performed the same analysis as done with the amygdala ROIs.

Results

The primary study question addressed the effect of repeated training sessions on amygdala regulation in the feedback group: In this group, the repeated measures ANOVA on the beta weights of the contrast *regulate>view* in the right amygdala revealed a significant main effect of *session* [$F(3,14) = 3.29$, $p = 0.030$, partial $\eta^2 = 0.190$]. The effect of *session* on amygdala activity during *regulation* alone (beta weights of regulation versus baseline) was highly significant [$F(3,14) = 5.44$, $p = 0.003$,

partial $\eta^2 = 0.280$], while it was not significant for the *view* condition alone (against baseline) [F(3,14) = 0.507, $p = 0.679$, partial $\eta^2 = 0.035$].

The post-hoc paired t-test directly compared amygdala activation between session 4 and 1 for the conditions *regulate* (versus baseline) and *regulate>view*. Amygdala activation during *regulate* (versus baseline activation) was significantly lower in S4 (M=0.068, SD=0.22) compared to S1 (M=0.26, SD=0.15); t(14)=-3.56, p = 0.003 (Cohen's d: 1.9029, Effect size: 0.6893). Amygdala activation during the contrast *regulate > view* was also significantly lower in S4 (M=-0.17, SD=.26) compared to S1 (M=0.013, SD=0.11); t(14)=-2.45, p = 0.028 (Cohen's D: 1.31, Effect size: 0.55). In the control group, the repeated measures ANOVA on the beta weights of the contrasts '*regulate>view*', *'regulate>baseline'* or *'view>baseline'* in the right amygdala revealed no significant main effects of the factor *session* (*View*: F(3,10) = 0.72, p = 0.51; *Regulate*: F(3,10) = 1.93, p = 0.15; *Regulate-view*: F(3,10) = 1.43, p = 0.25).

The direct comparison between feedback and control group revealed significantly higher regulation effects in terms of a down-regulation of amygdala activity in session 4 compared to session 1 in the feedback group (beta weights feedback-group: M=0.15, SD 0.31, control-group: M=-0.18, SD=0.29, result: t=2.80, p=0.010).

We did not observe a decrease of the amygdala response in the *view* condition, in the sense of a possible habituation, over the four stimulation sessions regarding comparisons between '*view*' in S1 and in S4 in either the feedback (t-test p=0.84) or control group (p=0.51).

The control ROI in the feedback group within the sensorymotor cortex (Brühl et al. 2014: x/y/z = ± 30/-24/62; 10 mm diameter) did not show differences between session 1 and 4 in the '*regulate* versus *view'* condition (t=-0.58, p=0.57).

We analysed the transfer task before and after the training in the feedback group analogous to the analysis of the feedback data. We found a regulation success already in the task prior training, but we did not found a difference after the training in right amygdala activity during the conditions *regulate* [F(3,14) = 0.40, p = .54, partial $\eta^2 = 0.03$], *view* [F(3,14) = 0.95, p = .35, partial $\eta^2 = 0.07$], or the contrast *regulate > view* [F(3,14) = 0.87, p = .37, partial $\eta^2 = 0.06$].

The individual localizer ROI did not differ in size between sessions. However, there was a difference between groups (Feedback-group: M=846 mm^3, SD=252 mm^3, Control-group: M=431 mm^3, SD=165 mm^3, t(24)=-4.74, p<0.001, two-tailed).

The structured interview after the scanning confirmed that the subjects in both, the feedback and control group, mainly applied the instructed reappraisal strategy, whenever in the feedback group also other feedback strategies such as mindfulness or attention shift were tried occasionally.

Discussion

We assessed whether training of emotion regulation based on neurofeedback of one's own amygdala activity as the main target of cognitive emotion regulation might improve amygdala down-

regulation. Indeed, our results indicate that it is possible to improve the down-regulation of one's own amygdala activity based on the feedback of this activity. This was demonstrated in the experimental group, which obtained feedback of its amygdala activity, when comparing the *view* and the *regulate* condition with an increasing regulation success in the course of the training as shown by down-regulation of the amygdala. A control region, the sensorymotor cortex, showed no such effect, supporting the assumption of a specific effect of the training on the amygdala but not brain areas not involved in emotion regulation. This result was further supported by the comparison with a control group that also trained emotion regulation, but without obtaining feedback. This group showed no improvement of amygdala down-regulation. The data further confirm and extend earlier findings from a pilot study with a comparable design on six subjects (Brühl et al. 2014). This method could support training of emotion regulation strategies in the frame of psychotherapy of affective, anxiety and personality disorders, which should be further subject of investigation.

Providing feedback of one's own neurobiological or psychophysiological parameters has a relatively long history in the field of neuropsychiatry (review in Sulzer et al. 2013a). However, until now no neurofeedback application has been established in clinical psychiatry. This may be due to low immediacy or low specificity of the feedback signal in relation to the function to modify, for instance when using skin conduction and also electroencephalography. This issue might be better addressed by providing direct feedback of brain activity which is more closely associated with a mental act or a psychological function. Rt-fMRI can provide direct feedback information from the activity of selected brain regions, networks (Sitaram et al. 2011) or from connectivity measures (Koush et al. 2013; Lee et al. 2012). Subjects can use this information to acquire better control over the underlying neural activity (Cox et al. 1995; Sulzer et al. 2013b). This may have particular impact for disorders of mental functions that might be consciously modified in a health promoting way.

An important advance of the presented approach consists in applying a mode to effect on the emotion processing by using a cognitive element of psychotherapy compliant with a well-established model of emotion regulation. In the field of emotional disturbances it is particularly relevant to control negative emotions such as fear, anger or sadness. These emotions are associated with increased amygdala activation, the control of which is the main target of cognitive emotion regulation (Buhle et al. 2014). Earlier studies on rt-fMRI mostly demonstrated the up-regulation of the activity within a certain brain region, which however also can have impact on emotions: Greer et al. (Greer et al. 2014) reported an increase of nucleus accumbens activation through rt-fMRI neurofeedback as an indicator of reward related brain activation; Linden et al. (2012) and Young et al. (2014) found an increase of amygdala activity through induced positive emotions in depression; Veit et al. (Veit et al. 2012) demonstrated coping with threatening stimuli due to up-regulation of the anterior insula. Down-regulation through rt-fMRT has also been demonstrated in the anterior cingulate cortex for regulating craving in smokers (Li et al. 2013). Another recent report of neurofeedback assisted amygdala down-regulation (Paret et al. 2014) demonstrated an effect in a single session, however without training.

Our additional aim, to show the transfer of the training effect into another related function, did not result in an improvement over the training and it did not reveal any difference between the groups. There might be a couple of reasons for this: already prior the feedback scanning, the healthy participants were able to regulate, such that the task may have been too simple to discriminate training effects due to a ceiling effect. The emotional reaction due to the short video clips might have been easier to down-regulate compared to the series of negative emotional pictures, also because it was much shorter than the feedback sessions. Further, the initial transfer task was performed *prior* the first scanning session and the final one was done imminently *after* the last scanning session. Yet, after the last scanning the subjects were tired and could have been less capable and motivated to concentrate on the task and therefore corroborating the effect. However, despite this lack of a transfer into the other task, this does not exclude a relevant effect when performing the rt-fMRI training, for instance, in patients where the improvement might rather be stronger than in healthy, well-regulated volunteers.

While it might be of course attractive also for healthy people to train emotion regulation, the important impact refers to potentially supporting treatments of emotional and affective disorders in psychiatry. Scientific applications of rt-fMRT in the field of psychiatry addressed till now particularly depression (Johnston et al. 2011; Linden et al. 2012; Young et al. 2014) but in a pilot approach also schizophrenia (Ruiz et al. 2013). A practical application further to investigate is the training of emotion regulation in disorders with primary emotional disturbances or mood alterations such as anxiety disorders, borderline personality disorder with emotional instability and depression. Training of emotion regulation, supported and improved by rt-fMRI, may help to cope with emotionally challenging situations. Considering potential practical application one might think about implementation in for instance cognitive behavioural psychotherapy (Disner et al. 2011; Holmes et al. 2014) as an augmentation method for learned cognitive techniques of emotion regulation. Whenever it may take long time, linking psychotherapy with neurobiology would mean a new era in clinical practice.

One might also consider experiments in the field of rt-fMRT from a general perspective. Humans explore deeper the capabilities of brain functions and, based on that, learn to improve and to support their mental abilities. A major general development in the evolution of the brain is to gain mental control over own brain and body functions and to influence neural representations of inner and exterior processes from a hierarchically higher neural level (Damasio and Carvalho 2013). Real-time fMRI adds on such strategies with an own quality as it uses directly neurobiological feedback signals from circumscribed relevant brain regions to guide and promote mental processes. As such, rt-fMRT, among others, may generally serve developing mental capabilities to gain control over own brain functions.

With respect to limitations of this study, we first have to mention the sample sizes: with 15 included participants in the feedback group and 11 in the control group a bigger sample size might be desirable. Nevertheless, given the data basis of 104 fMRI scans altogether, it can be regarded as a

substantial amount, and it was sufficient for providing meaningful results. Furthermore, this study confirms the findings of the pilot study (Brühl et al. 2014) and shows even a higher effect size, which is usually not the case in bigger sample sizes if the initial statistical power was low (Button et al. 2013). We also had to encounter relatively high drop-out rates of 31 and 37 % in both groups due to technical problems over the scanning sessions, due to movement artefacts, or in case we could not identify appropriate amygdala activation in the localizer task, and because some subjects did not complete the four sessions. A central issue refers to control conditions in rt-fMRI investigations. Whenever the major aim was to assess a training effect in the feedback group, which could be demonstrated, the comparison with a control group is important. We decided for a control group that should also train emotion regulation in a similar design but without obtaining feedback. Such, we did not apply a really sham controlled approach, however, we consider our control as being valid as it controls for the condition feedback assisted training versus non-feedback assisted training.

Conclusion

The results indicate that a training of emotion regulation supported be amygdala-feedback is possible and that it is more efficient than training without this feedback. The finding has basic implications for learning emotion regulation strategies with potential relevance for mental disorders, where better emotion regulation represents a key capability for symptom improvement. Neuroimaging-feedback has translational potential for introducing scientific neuroimaging knowledge into clinical application, providing an integrative link of psychotherapy and neurobiology.

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Figures

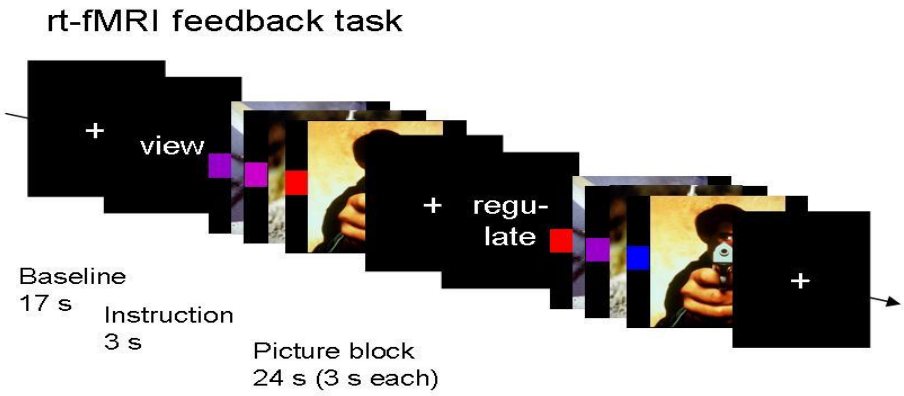


Fig. 1a: Feedback task, the pictures were randomized changing

Fig. 1b: Example of feedback of decreasing amygdala activity

Fig. 1 Experimental Task

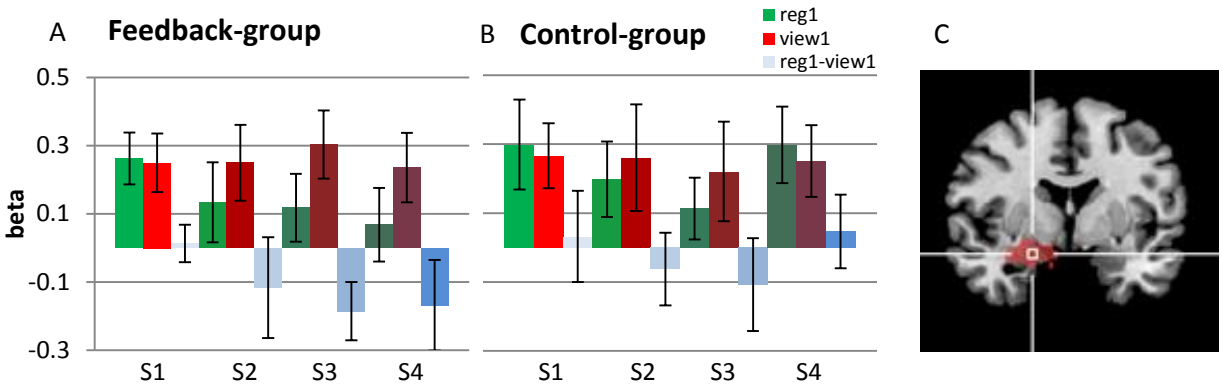


Fig. 2 A. Mean beta value from each subjects' localizer ROI for conditions regulate (green), view (red) and the difference between regulate and view (blue) over the four sessions (later sessions are represented in darker colors). Error bars represent 1 SD. Difference between regulate and view increases. B. The same graph for the control group. C. Mask of area in the amygdala region covered by all individual ROIs. D. Correlation of amygdala down-regulation with subjective regulation success.

Supplementary Material

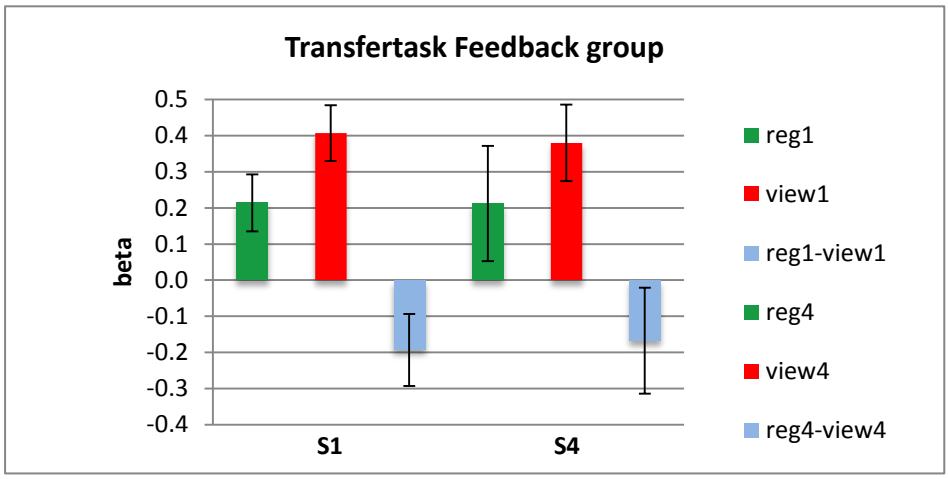


Fig. S1 Results of the transfer task with already regulation success prior scanning.

2. General discussion

We investigated distinct domains of environmental stimuli such as cues that indicate potential threat or healthy/unhealthy food regarding the associated neural information processing. The aim was to elucidate brain regions that are functionally involved in risk evaluation. Such regions are supposed to contribute to a neural network for risk processing. A general hypothesis consisted in supposing a close relationship between risk and emotion processing.

The common methodological denominators of our risk studies were based on the application of functional neuroimaging. Subjects were presented with external stimuli, which they had to identify, evaluate and upon which they had to react. In the fourth study, subjects applied a mental regulation strategy in order to control their emotional reaction on the presented stimuli. In the same time, the associated neural activation was provided in an individual realtime-feedback to the subjects in order to train their regulation-skills.

Findings

In the first study, our aim was to investigate neural correlates associated with high-risk estimation of environmental and technological hazards for the society. We found distinct brain regions that were activated with high-risk estimation: Prefrontal, insular, and posterior cortical regions including precuneus, as well as medial thalamus and caudate head. Regarding the explorative deconvolution analysis and considering the time courses on a descriptive level, we found the anterior insula, medial thalamus and anterior cingulate to be active in the earlier periods of risk evaluation. Interestingly, the amygdala was, whenever activated, not discriminatively active with high or low risk estimation. This might be because we asked for risk estimation for society in general, but not individually. Furthermore, risk estimation was based on written terms, not on pictures; thus, a rather cognitive evaluation might have taken place. The amygdala might be differentially involved, when facing a personal imminent risk.

In the study that investigated neural responses to food stimuli, we found a positive association between healthiness evaluation and activity in the PMC/DLPFC, Brodmann areas (BA) 6/8/9, and in the precuneus in the whole brain analysis. We further revealed activity in the ventral striatum and the orbitofrontal cortex in the ROI analysis regarding the rating period. Furthermore, we found a negative association between food healthiness and activation in the amygdala. The negative association was also found bilaterally in more anterior and lateral regions of the DLPFC (BA 46) as well as in primary and secondary visual areas. Gender differences were represented by a higher activation in the midbrain in females when presented with healthy stimuli. These results indicate the close association of food evaluation with emotion processing. Particularly, the activation of the amygdala in the association with low health might be interpreted as a warning signal and the activation of the ventral striatum with high healthiness as a reward signal. The amygdala signal might serve in order to better reflect eating

behaviour and to finally promote healthy nutrition. The same might hold true for the ventral striatal reward signal, which possibly elicits approaching behaviour.

Moreover, we investigated the effect of threatening stimuli on the activation of neural networks that differentiate between modern and evolutionary content. By contrasting threatening with neutral pictures in both domains, we revealed regions typically found in emotion processing. Evolutionary-threatening pictures evoked significantly stronger activations than modern-threatening pictures in most regions of the network that is involved in processing threatening stimuli: In the bilateral amygdala, left inferior frontal gyrus, right middle frontal gyrus, right parietal lobe, right precuneus, left thalamus, bilateral fusiform gyrus and bilateral superior parietal lobule. However, this finding was controversial with the behavioral part of the experiment; the post-scan rating of the IAPS pictures. Subjects rated modern-threatening stimuli as significantly more negative in valence than evolutionary-threatening pictures, indicating a higher level of perceived threat or fear for stimuli such as guns, knives, and car accidents. The two threatening conditions did not differ in the arousal rating, which implies no relevant association of subjective arousal with the difference in neural activity between the two threatening conditions. Thus, one may argue that the evolutionary preparedness for evolutionary-threatening stimuli is the actual driver of the found neural activity. This has also implications on a methodological level. When presenting emotional stimuli to subjects, a differentiation between evolutionary and modern threatening image might be done in order not to bias the results.

Finally, we assessed whether training of emotion regulation based on neurofeedback of one's own amygdala activity as the main target of cognitive emotion regulation might improve amygdala down-regulation. The results indicate that it is possible to improve the down-regulation of one's own amygdala activity based on the feedback of this activity. This was demonstrated in the experimental group that obtained feedback of its amygdala activity. When comparing the 'view' and the 'regulate' condition we found an increasing regulation success in the course of the training as shown by down-regulation of the amygdala over sessions. This method could support the training of emotion regulation strategies in the frame of psychotherapy, of coping with threatening situations or of decision-making, and, therefore, shall further be investigated.

In the following section, the neuro-systemic findings serve as a basis for a cybernetic model of central nervous information processing upon risk evaluation of external stimuli.

Risk and emotion processing

The functional imaging results of the three risk studies were used in order to draft a neural network model for risk processing, also based on models of emotion regulation (fig. 3.1). This simplified model shall highlight important nodes of the information processing stream. The starting point for the course of risk evaluation onto an external stimulus is the perception of the stimulus. Given a visual stimulus, as in our tasks, the information is processed via retina and optical nerve to the first relay station in the posterior thalamus respectively the pulvinar. From there, two streams of information

flow shall be regarded: The first stream quickly flows to the amygdalae via medial thalamus (Tamietto 2011). We found the medial thalamus activated whilst high-risk estimation, evolutionary risk perception, and food healthiness evaluation, irrespective of whether the food is healthy or not. The amygdalae perform a pattern detection and, in case of threat, initiate a vegetative reaction of sympathetic arousal, as increase of heart rate, via hypothalamus and brain stem nuclei. In our studies, amygdala activity was associated with risk and food evaluation, whereby low healthiness and evolutionary threats led to higher activity. In the first study, in which terms of risk were presented, we found enhanced amygdala activity on risk, but no discrimination between high and low risk. As outlined above, this implicates a cognitive evaluation, whereby the presentation of pictures might have evoked higher amygdala activity with higher risk as observed in the study with evolutionary versus modern threats compared to the neutral stimuli.

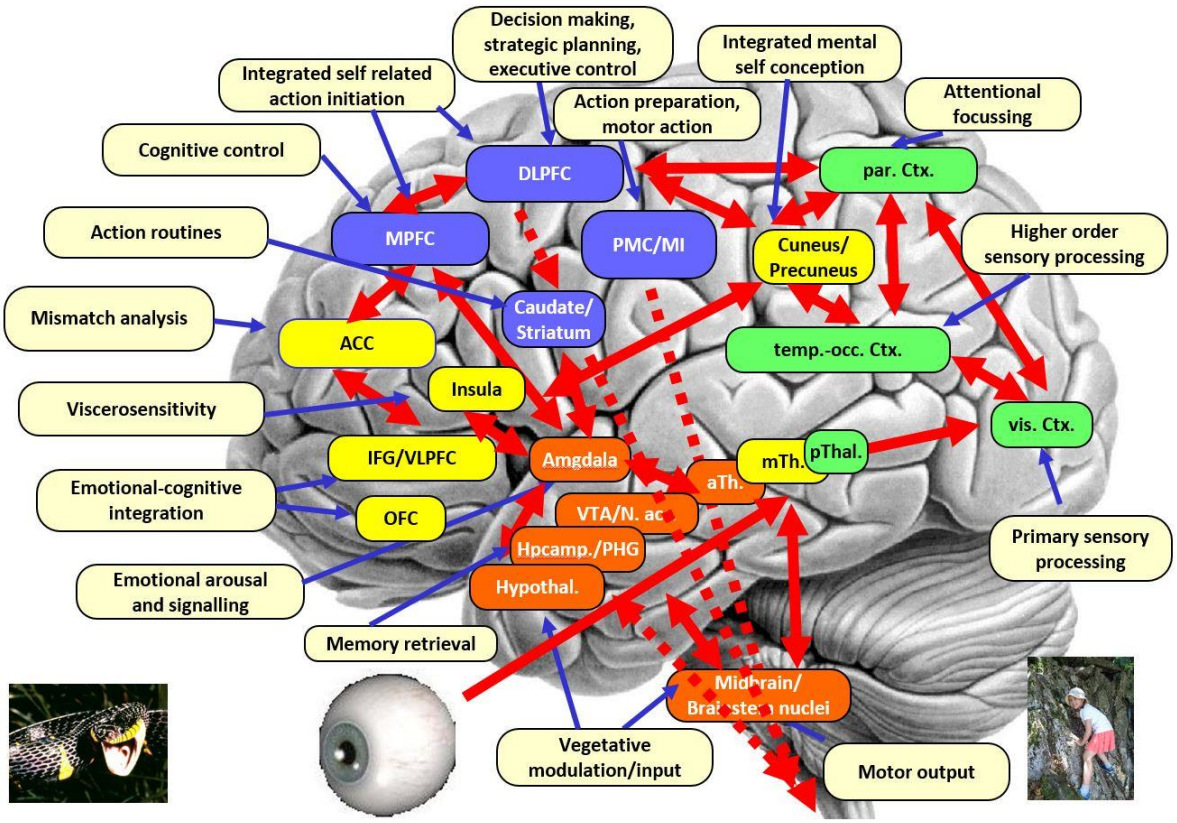


Fig. 3.1 Risk information processing model based on emotion processing models: LeDoux 2000, Drevets et al. 2001, Seminovicz et al. 2004, Pessoa et al. 2008, Tamietto 2010 and others. Abbreviations: ant. Cing. Anterior cingulum, MPFC medial prefrontal cortex, DLPFC dorsolateral prefrontal cortex, par. Ctx. parietal cortex, temp.-occ. Ctx. temporo-occipital cortex, vis. Ctx. visual cortex, aTh. Anterior thalamus, mTh, medial thalamus, p.Thal. posterior thalamus, Hypothal. hypothalamus, Hpcamp. hippocampus, PHG parahippocampaler Gyrus, VTA ventral tegmental area, N. acc. nucleus accumbens, IFG inferior frontal gyrus, VLPFC ventrolateral prefrontal cortex, OFC orbitofrontal cortex. Source of snake picture: International Affective Picture System (IAPS) database, Lang et al. 2008, source of child climbing picture: private.

Furthermore, quickly prepared action routines can be activated that lead to the preparation for fight, fight or freeze behaviour. These action routines, or 'algorithms for survival', are stored in striatal areas (Arnstein et al. 2009; Bach and Dayan 2017). In this context, the caudate nucleus might work as a relay between cortical evaluation and thalamic signalling which contributes the classification of the presented terms and the selection of implicit behavioural coping strategies (Cavanna and Trimble 2006). We found the caudate to be particularly involved in the assessment of high risk. The detection of positive stimuli with personal value for the subject activated the reward system, comprising the ventral tegmental area and nucleus accumbens, and ventral striatum (Ruff and Fehr 2014). The ventral striatum was particularly active when estimating food to be of high healthiness, thus, when reflecting the reward aspect.

In parallel, the amygdalae promote information upstream to emotional-cognitive integration areas such as the inferior frontal gyrus (IFG, or ventrolateral prefrontal cortex, VLPFC) and orbitofrontal cortex; regions that are also involved in downstream impulse control (Pessoa 2008, Pessoa and Adolphs 2010). In our food study, the IFG was active along with high healthiness evaluation. The hippocampus serves for memory retrieval. The parahippocampal gyrus (PHG), as part of the neocortex, serves together with the limbic system for linking between emotion processing and episodic memory subserving contextual analyses (Aminoff et al. 2013). We found the PHG to be more active particularly with modern threatening stimuli. We interpreted that the enhanced activity might be due to the degree of involvement in the retrieval and the contextual integration of the learned stimuli.

The viscero-sensitive part, including upstream information via brainstem and medial thalamus, is processed in the insula and associated with a conscious emotional signal as well as salience processing (Uddin 2014). This information is crosschecked with a representation of internal needs in the anterior cingulate cortex (ACC). The ACC promotes information about a mismatch detection (target/reality) along with a risk situation again upstream to dorsolateral and medial prefrontal areas (Summerfield and de Lange 2014). In our risk studies, the insula and ACC were active in high-risk estimation and in healthiness evaluation, irrespective the degree of healthiness. This is in line with recent studies that assessed risk processing associated networks (Kohno et al. 2017).

The second stream, in contrast to the first stream, initiated a more profound and detailed feature analysis and flows to the secondary and tertiary higher order sensory processing areas in the temporo-occipital cortex (Tamietto et al. 2011). We observed an involvement of these regions also in our studies: For instance, the temporo-occipital cortex was activated with higher risk or higher healthiness estimation. Need of attentional direction is promoted by parietal areas. Such areas, in our studies, for instance, were activated with evolutionary threatening stimuli.

The somato- and viscero-sensory analysis is matched with a mental self-concept in sensory and cognitive domains. It is also linked to autobiographical memory and to an assessment of self-relevance, with a neural representation in cuneus and precuneus (Tang et al. 2015, Sumiyo et al. 2017). We found the precuneus to be involved in risk as well as in healthiness evaluation processing. The

information of the analyses is then further processed in prefrontal cortex areas (Tamietto et al. 2011).

In the medial and dorsolateral prefrontal cortex (MPFC/DLFPFC), the next step of cognitive-sensory-emotional information integration takes place, which leads to a final evaluation of the stimulus (Fuster 1999; Arnstein 2009; a.m.o.). Based on the evaluation of the meaning for the self or the subject respectively, a decision of whether the initiation of a further action is desirable or not is done. In case that a further action is desirable, an appropriate action strategy and its execution are planned. The detailed planning and preparation of the action on the motor level is performed in the premotor cortex and the action is finally executed by the motor cortex (Fuster 1999).

Thereby, the MPFC hosts the representation of self-related aspects and contributes to the conscious self in the context of evaluation and action (Northoff et al. 2006). The MPFC is primarily involved in the conscious control and in directing, as well as initiating, internal and external action. Internal action may consist in the cognitive control of impulses, which might result from the level of emotional processing in the amygdala (Herwig et al. 2010). External action means the observable response to the perceived stimulus. The MPFC and the DLPFC were generally activated throughout our risk and our healthiness evaluation tasks. Both were more active associated with higher risk and higher healthiness estimation as well as with perceiving evolutionary threatening pictures.

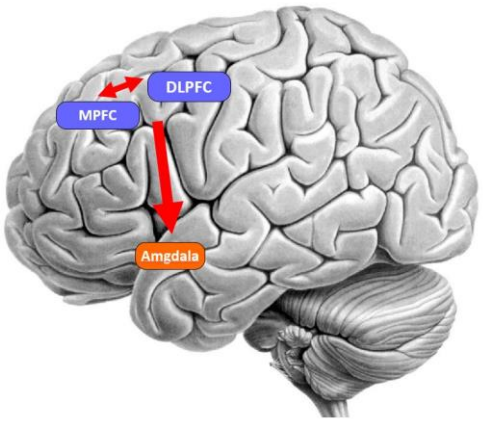
Taken together, the studies indicate a neural network that is involved in the perception and evaluation of the emotional meaning of important environmental incidents and items. This was demonstrated in the domains of risk assessment, health worthiness of food and evolutionary versus modern threats.

Self-guiding of mental processing

Given the knowledge about psychological and neural foundations of risk processing, the question raises whether a new mode of guiding one's own mental processes may be feasible. In our risk studies, we demonstrated major contributions of central emotion processing brain regions, such as the amygdala, as well as central cognition processing areas, such as the medial and dorsolateral prefrontal cortex. In this context, the cognitive control of emotions, also to withhold possibly disadvantageous impulses, is an important functional domain. Emotion regulation and related cognitive interventions are well investigated (Ochsner and Gross 2005, Herwig et al. 2007, Herwig et al. 2010, and many others). Emotion regulation also has a central role in risk evaluation and processing in cases in which no emotion driven actions are required. On the neuro-system level, an adequate mental intervention is associated with activation in the M/DLPFC and able to down-regulate amygdala activity (fig. 3.2).

We assessed and demonstrated the capability for actively influencing the neural processing of environmental emotional stimuli in the sense of emotion regulation and of training this capability. Training emotion regulation by means of neurofeedback of the amygdala activity on presented aversive emotional pictures leads to improved down-regulation of the amygdala.

Fig. 3.2 Cognitive emotion regulation techniques with activation in M/DLPFC are suitable to down-regulate amygdala activity.



This finding also has implications for potential approaches to deal with risk in the future. The here identified brain regions and networks may serve as basis for developing new mental interventions to guide the processing of environmental stimuli, serving a secure, health promoting and sustainable coping with risks.

Outlook

Future risk evaluation and decision making processes may be founded additionally to the current algorithms on knowledge about basic central-nervous processing and about individual predispositions potentially influencing the process disadvantageously. Therefore, decision making upon potential threats or dealing with risks may be trained, particularly in professionals, giving more attention to the internal mental emotional and cognitive processes and signals. This meta-level of awareness may be actively considered as a new domain in risk management.

In summary, becoming more aware of the inner mental processes may support risk evaluation. As a result, we may become also aware, that in case of a risk or threat we *are* not threatened, in the sense of an existential *being*, but that we *realize* a threat; a meta-cognitive approach that opens more opportunities to cope with risk and also other every-day emotionally laden situations.

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3. Summaries

3.1. English summary

Our perception of the environment and of environmental incidents is accompanied by a complex central nervous information processing. A key issue of this processing represents the evaluation of the meaning of the incidents for the organisms with associated neural signalling. Understanding the associated information processing may help to deal with noxious or threatening environmental incidents or to cope with contra productive impulses for actions. Exemplary environmental incidents are potentially threatening items or also food items, that may be healthy or not, of which the adequate evaluation is promoting health or even survival. The aim of the presented study series is identifying neural correlates of the mental evaluation of environmental incidents or items as well as on a basic level the possibility to influence mentally the associated basal information processing. The results may serve developing a higher level of awareness for environmental threats as a basis for actively initiated mental interventions for a secure and sustainable coping with the environment.

In a first study, we assessed the evaluation of risks. In personal and in society related context, people often evaluate the risk of environmental and technological hazards. Previous research addressing neuroscience of risk evaluation assessed particularly the direct personal risk of presented stimuli, which may have comprised for instance aspects of fear. Further, risk evaluation primarily was compared to asks of other cognitive domains serving as control conditions, thus revealing general risk related brain activity, but not such specifically associated with estimating a higher level of risk. We here investigated the neural basis on which lay-persons individually evaluated the risk of different potential hazards for the society. Twenty healthy subjects underwent functional magnetic resonance imaging while evaluating the risk of fifty more or less risky conditions presented as written terms. Brain activations during the individual estimations of ‘high’ against ‘low’ risk, and of negative versus neutral and positive emotional valence were analyzed. Estimating hazards to be of high risk was associated with activation in medial thalamus, anterior insula, caudate nucleus, cingulate cortex and further prefrontal and temporo-occipital areas. These areas were not involved according to an analysis of the emotion ratings. In conclusion, we emphasize a contribution of the mentioned brain areas involved to signal high risk, here not primarily associated with the emotional valence of the risk items. These areas have earlier been reported to be associated with, beside emotional, viscerosensitive and implicit processing. This leads to assumptions of an intuitive contribution, or a “gut-feeling”, not necessarily dependent of the subjective emotional valence, when estimating a high risk of environmental hazards.

The second study dealt with neural signalling associated with food healthiness. The ability to differentiate healthy from unhealthy foods is important in order to promote good health. Food, however, may have an emotional connotation, which could be inversely related to healthiness. The neurobiological background of differentiating healthy and unhealthy food and its relations to emotion

processing are not yet well understood. We addressed the neural activations, particularly considering the single subject level, when one evaluates a food item to be of a higher, compared to a lower grade of healthiness with a particular view on emotion processing brain regions. Thirty-seven healthy subjects underwent functional magnetic resonance imaging while evaluating the healthiness of food presented as photographs with a subsequent rating on a visual analogue scale. We compared individual evaluations of high and low healthiness of food items and also considered gender differences. We found increased activation when food was evaluated to be healthy in the left dorsolateral prefrontal cortex and precuneus in whole brain analyses. In ROI analyses, perceived and rated higher healthiness was associated with lower amygdala activity and higher ventral striatal and orbitofrontal cortex activity. Females exerted a higher activation in midbrain areas when rating food items as being healthy. Our results underline the close relationship between food and emotion processing, which makes sense considering evolutionary aspects. Actively evaluating and deciding whether food is healthy is accompanied by neural signalling associated with reward and self-relevance, which could promote salutary nutrition behaviour. The involved brain regions may be amenable to mechanisms of emotion regulation in the context of psychotherapeutic regulation of food intake.

The third approach assessed differential neural processing of evolutionary and modern image content on the emotional basis. From an evolutionary perspective, environmental threats relevant for survival constantly challenged human beings. Current research suggests the evolution of a fear processing module in the brain to cope with these threats. Recently, humans increasingly encountered modern threats (e.g., guns or car accidents) in addition to evolutionary threats (e.g., snakes or predators) which presumably required an adaptation of perception and behavior. However, the neural processes underlying the perception of these different threats remain to be elucidated. We investigated the effect of image content (i.e., evolutionary vs. modern threats) on the activation of neural networks of emotion processing. During functional magnetic resonance imaging (fMRI) forty-one participants watched affective pictures displaying evolutionary-threatening, modern-threatening, evolutionary-neutral, and modern-neutral content. Evolutionary-threatening stimuli evoked stronger activations than modern-threatening stimuli in left inferior frontal gyrus and thalamus, right middle frontal gyrus and parietal regions as well as bilaterally in parietal regions, fusiform gyrus and bilateral amygdala. We observed the opposite effect, i.e. higher activity for modern-threatening than for evolutionary-threatening stimuli, bilaterally in the posterior cingulate and the parahippocampal gyrus. We found no differences in subjective arousal ratings between the two threatening conditions. On the valence scale though, subjects rated modern-threatening pictures significantly more negative than evolutionary-threatening pictures, indicating a higher level of perceived threat. The majority of previous studies show a positive relationship between arousal rating and amygdala activity. However, comparing fMRI results with behavioral findings we provide evidence that neural activity in fear processing areas is not only driven by arousal or valence, but presumably also by the evolutionary content of the stimulus. This has also fundamental methodological implications, in the sense to suggest a more elaborate

classification of stimulus content to improve the validity of experimental designs.

Finally, we investigated whether neurofeedback may serve training emotion regulation. Being in control of one's emotions is not only desirable in many everyday situations but is also a great challenge in a variety of mental disorders. Intentionally applying emotion regulation strategies is a basic social skill and a key element of psychotherapy. Successful intentional regulation of one's own emotions has its neurobiological correlates in a down-regulation of amygdala activity. Training emotion regulation strategies supported by neurofeedback of one's own amygdala activity by means of real-time (rt-)fMRI might be beneficial for mental health and well-being. Fifteen healthy participants underwent four sessions of rt-fMRI neurofeedback of their own amygdala activity while applying a cognitive emotion regulation strategy during the stimulation with emotional pictures. During the four weekly sessions the participants trained emotion regulation skills as measured by a down-regulation of amygdala activity compared to simply viewing the pictures. Eleven other participants trained emotion regulation without feedback in four sessions in the scanner and served as a control group. Amygdala feedback resulted in a significant reduction of amygdala activity compared to simply viewing the pictures. This effect improved over the training sessions and it was superior to the control group with no such training effect. Training of emotion regulation supported by rt-fMRI-neurofeedback of amygdala activity was more efficient than training without this feedback. The finding has basic implications for learning emotion regulation strategies in health with potential relevance for mental disorders, where better emotion regulation represents a key capability for clinical improvement. Rt-fMRI neurofeedback bears potential for clinical application, linking psychotherapy and neurobiology.

Taken together, the studies demonstrate neural networks involved in the perception and evaluation of the emotional meaning of important environmental incidents and items. This was demonstrated in the domains of risk assessment, health worthiness of food and evolutionary versus modern threats. We further demonstrated the capability to actively influence the neural processing of environmental emotional stimuli in the sense of emotion regulation and to train this capability. The identified brain regions and networks may serve as basis for developing mental interventions for guiding of the processing of environmental stimuli, serving a secure, health promoting and sustainable coping with them.

3.2. German Summary (Zusammenfassung)

Unsere Wahrnehmung der Umwelt und von Ereignissen in der Umwelt geht immer mit einer komplexen zentralnervösen Informationsverarbeitung einher. Ein Schwerpunkt dieser Verarbeitung liegt in der Bewertung der Bedeutung der Umweltreize für den Organismus mit entsprechenden neuronalen Signalen. Ein vertieftes Verständnis von der assoziierten zentralnervösen Informationsverarbeitung kann helfen, mit zum Beispiel bedrohlichen oder schädlichen Umweltreizen besser umzugehen oder eine kontraproduktive bzw. nicht zielführende Reaktion auf diese Reize in den Griff zu bekommen. Beispiele sind potentiell riskante und bedrohliche Gegebenheiten oder Nahrungsmittel, welche für den Organismus nützliche wie auch nachteilige Aspekte haben und eine entsprechende Erfassung wichtig ist für den sicheren Umgang. Ziel der vorliegenden Arbeit ist, neuronale Korrelate bei der mentalen Verarbeitung von Umweltreizen zu identifizieren und grundsätzlich kognitive Einflussmöglichkeiten auf diese Verarbeitung zu untersuchen. Hieraus könnten Grundlagen für die Entwicklung von bewussten und aktiv intendierten mentalen Interventionen für einen sicheren und nachhaltigen Umgang mit Umweltgegebenheiten abgeleitet werden.

Wir führten vier neurowissenschaftliche Studien mit gesunden Probanden zu der Thematik durch. Allen Studien gemeinsam war methodisch die Anwendung der funktionellen Magnetresonanz-Tomographie (fMRT) zur Erfassung der neuronalen Aktivität während der experimentellen Aufgaben. In der ersten Arbeit präsentierten wir 20 Probanden Wörter von verschiedenen Umweltreizen mit mehr oder weniger bedrohlichem Charakter. Die Probanden sollten die den Wörtern entsprechenden Umweltgegebenheiten hinsichtlich des Risikos einschätzen und die Einschätzung dann auf einer visuellen Analog-Skala festhalten. Wir vergleichen die Hirnaktivität während der Einschätzung eines hohen versus niedrigen Risikos und identifizierten Hirnregionen, welche für die Risikoeinschätzung, aber nicht für die emotionale Valenz.Einschätzung differentiell aktivierten. Hohes Risiko war assoziiert mit medial thalamischer, anterior insulärer, cingulärer, caudatärer, swoie temporo-occipitaler und präfrontaler Aktivität. Die Aktivierung insbesondere der für Viszerosensitivität bekannten Hirnareale wie die Inselregionen bei Einschätzung eines hohen Risikos unabhängig von der bewussten Einschätzung der emotionalen Valenz spricht für ein evolutionär altes „Bauchgefühl“ bei und eine intuitive Verarbeitung von Risiko-Reizen.

In der zweiten Studie untersuchten wir die Hirnaktivität während der Betrachtung und Einschätzung von Bildern von Nahrungsmitteln hinsichtlich hoher oder niedriger Gesundheitswertigkeit insbesondere in Hirnregionen, welche für emotionale Informationsverarbeitung bekannt sind. Siebenunddreissig gesunde Probanden gaben während der fMRT auf einer visuellen Analogskala an, für wie gesund sie insgesamt 40 verschiedene Nahrungsmitteln hielten. Wir fanden in der Whole-brain-Analyse insbesondere den linken dorsolateralen präfrontalen Kortex und den Präcuneus bei Einschätzung hoher Gesundheit aktiv, während in der Region-of-interest-Analyse die Mandelkerne geringer aktiviert und ventral striatale wie orbitofrontale Region stärker aktiviert waren.

Frauen zeigten ausgeprägtere Mittelhirnaktivität, wenn sie Nahrungsmittel als gesund einschätzten. Die Ergebnisse weisen auf eine enge Verknüpfung emotionaler Informationsverarbeitung mit der Gesundheitseinschätzung hin, was auch aus evolutionären Erwägungen sinnhaft ist. Dabei waren auch Belohnungs- und Selbstreferenz-assoziierte Areale aktiviert. Die entsprechenden Hirnregionen könnten einem psychotherapeutischen Einfluss bei Essstörungen zugänglich sein.

In der dritten Studie widmeten wir uns der neuronalen Verarbeitung von Umweltreizen mit bedrohlichem Charakter. Dabei untersuchten wir Unterschiede in der Verarbeitung von modernen versus evolutionär alten Reizen. Insgesamt einundvierzig Probanden sahen wären einer fMRT Bilder von evolutionär bedrohlichem, modern bedrohlichem, evolutionär neutralem und modern neutralem Inhalt. Die evolutionär-bedrohlichen Stimuli gingen im Vergleich zu den modern bedrohlichen Bildern mit stärkerer Aktivierung im linken inferioren und mittleren frontalen Gyrus, im Thalamas, in parietalen Regionen, im fusiformen Gyrus und beidseitig in den Amygdalae einher. Modern-bedrohliche Bilder waren dagegen mit ausgeprägerer Aktivierung im posterioren Cingulum und parahippocampalen Gyrus assoziiert. Dabei fanden wir keine Unterschiede in den Arousal-Ratings beim Vergleich der beiden Stimuliarten. Auf der Valenzskala waren die modern-bedrohlichen Stimuli allerdings negativer eingeschätzt als die evolutionären. Die Ergebnisse sprechen für eine differenzielle neuronale Verarbeitung von modernen und evolutionär alten bedrohlichen Stimuli, wobei letztere trotz gleichem Arousal und geringerer negativer Valenz mit mehr Aktivierung in zentralen emotionsverarbeitenden Strukturen wie den Amygdalae einherging. Dies hat neben der neurobiologischen Differenzierung auch eine methodische Implikation für fMRI Studien in der Hinsicht, dass emotionale Stimuli auch in dieser Hinsicht klassifiziert werden sollten, um die Validität der Studien zu steigern.

Die vierte Studie adressierte die Frage, ob die emotionale Informationsverarbeitung auf neuronaler Ebene mittels Rückmeldung der aktuellen Amygdala-Aktivität durch mentale Interventionen gezielt beeinflusst werden kann. Bestimmte mentale Interventionen zu erlernen kann hilfreich sein, die emotionalen Reaktionen auf Umweltreize wenn gewünscht zu steuern. Insgesamt fünfunddreissig gesunde Probanden, von welchen letztlich sechsundzwanzig in die Analyse eingeschlossen werden konnten, führten während der Betrachtung emotionaler Bilder während einer fMRT einen reality-check als kognitive Kontrolle für die Emotionsregulation durch. Dabei wurde einer Gruppe die eigene Amygdala-Aktivität als Erfolgskontrolle für die Emotionsregulation online rückgemeldet, eine Kontrollgruppe erhielt eine unspezifische Rückmeldung, machte aber dieselbe Aufgabe. Die Probanden trainierten die Emotionsregulation über vier Sessions im Abstand von einer Woche hinweg. Die Feedback-Gruppe zeigte über die Sessions hinweg eine zunehmende Fertigkeit, die Amygdala-Aktivität durch die mentale kognitive Kontrolle herunter zu regulieren, anders als die Kontrollgruppe. Die Ergebnisse weisen auf die Möglichkeit hin, mithilfe von Neurofeedback mentale Interventionen zu trainieren, welche für Emotionsregulation eingesetzt werden können. Dies kann auch für den Umgang mit Umweltreizen geprüft werden.

Insgesamt zeigen die Studien auf neuronaler Ebene Netzwerke auf, welche in die Verarbeitung der Wahrnehmung und emotionalen Bedeutung von wichtigen Umweltaspekten eingebunden sind. Dies wurde am Beispiel von Risikoeinschätzung, Gesundheitswertigkeit und evolutionär älterer wie moderner Bedrohungsfaktoren untersucht. Weiterhin wurde die grundsätzliche Möglichkeit aufgezeigt, die neuronale Verarbeitung von Umweltreizen aktiv mental auf der Ebene der Emotionsregulation zu steuern und diese Fertigkeit auch erfolgreich zu trainieren. Die identifizierten Hirnregionen und Netzwerke könnten als Ansatzpunkte für eine gezielte aktive mentale Steuerung der Verarbeitung von Umweltreizen im Sinne eines sicheren, gesundheitsbewussten und nachhaltigen Umganges dienen.

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