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Amygdala habituation: A reliable fMRI phenotype

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ABSTRACT

Amygdala function is of high interest for cognitive, social and psychiatric neuroscience, emphasizing the need for reliable assessments in humans. Previous work has indicated unsatisfactorily low within-subject reliability of amygdala activation fMRI measures. Based on basic science evidence for strong habituation of amygdala response to repeated stimuli, we investigated whether a quantification of habituation provides additional information beyond the usual estimate of the overall mean activity. We assessed the within-subject reliability of amygdala habituation measures during a facial emotion matching paradigm in 25 healthy subjects. We extracted the amygdala signal decrement across the course of the fMRI run for the two test–retest measurement sessions and compared reliability estimates with previous findings based on mean response amplitude. Retest-reliability of the session-wise amygdala habituation was significantly higher than the evoked amygdala mean amplitude (intraclass correlation coefficients (ICC) = 0.53 vs. 0.16). To test the task-specificity of this finding, we compared the retest-reliability of amygdala habituation across two different tasks. Significant amygdala response decrement was also seen in a cognitive task (n-back working memory) that did not per se activate the amygdala, but was totally unreliable in that context (ICC ~ 0.0), arguing for task-specificity. Together the results show that emotion-dependent amygdala habituation is a robust and considerably more reliable index than the mean amplitude, and provides a robust potential endpoint for within-subject designs including pharmaco-fMRI studies.

Introduction

Amygdala function is of high interest for cognitive, social and psychiatric neuroscience, emphasizing the need for reliable assessments in humans (Adolphs, 2010; Sander et al., 2003; Schaefer and Gray, 2007; Schumann et al., 2011). In a previous study we have compared the retest reliability of three different tasks (emotional, motivational and cognitive) comprising an fMRI task battery (Plichta et al., 2012). We found that each of these three tasks robustly activated their particular target regions and that the group-level profiles were all highly stable across sessions with a retest interval of ~2 weeks. The within-subject reliability, however, varied considerably for the different tasks and the regions-of-interest (ROIs). Both the motivational (monetary reward anticipation) and the cognitive task (n-back working memory) exhibited fair to good within-subject reliability (reward task: ICCs = 0.56–0.62; n-back: ICCs = 0.44–0.57). Consistent with other recent reports in the literature (Lipp et al., 2014; Sauder et al., 2013; van den Bulk et al., 2013), the facial emotion-matching task also showed stable group-mean response amplitudes across the two sessions but worse within-subject reliability (ICC = −0.02–0.16), indicating that this paradigm might be better suited for a between-subjects design. However, because of the more advantageous statistical power profile of within-subject designs and their possible application in individualized medicine including pharmaco-fMRI, it is of interest to seek more reliable within-subject summary features for tasks probing emotional responses.

Most fMRI analyses model the response to a repeated block design stimulus using a single regressor of constant amplitude, an approach that yields an estimate of the mean response amplitude along the fMRI run. However, measures of differential response amplitude in the amygdala (i.e., habituation) to repeated stimuli have also been reported. Differences in amygdala habituation have been suggested as a sensitive assay sometimes even more meaningful than differences in magnitude (Kleinhans et al., 2009). Moreover, it is also possible that apparent magnitude differences between two groups may result from differences in habituation (Phillips et al., 2001).

In general, habituation is a fundamental form of a biological system's plasticity and is defined as a response decrement due to stimulus repetition (Rankin et al., 2009). Habituation can be found across animals from *Aplysia* to humans and can be understood as an evolutionary

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advantageous mechanism that limits the utilization of attentional resources for stimuli with no meaningful consequences. Previous human studies have shown that (among other neural structures) the amygdala rapidly habituates to diverse stimuli including neutral and emotional faces (Breiter et al., 1996; Fischer et al., 2000, 2003; Fisher et al., 2009; Ishai et al., 2004; Phillips et al., 2001; Shin et al., 2005; Wright et al., 2001). Several studies have also demonstrated hemispheric lateralization with stronger habituation in the right versus left amygdala (Denny et al., in press; Lonsdorf et al., 2011; Phillips et al., 2001; Wedig et al., 2005; Wright et al., 2001). Of particular interest from the clinical perspective, amygdala habituation has been shown to be negatively correlated with trait anxiety (Hare et al., 2008), increased risk for social anxiety disorder (Blackford et al., 2013) and autism spectrum disorder (Kleinhans et al., 2009; Swartz et al., 2013; Wiggins et al.,2014). Furthermore, it is sensitive to genetic variants linked to depression, anxiety, aggression and neuroticism (Fisher et al., 2009; Lonsdorf et al., 2011; Wiggins et al., 2014). However, a test–retest reliability study of neural habituation measures has not yet been reported.

Therefore, we reanalyzed the fMRI face task data with special focus on amygdala habituation to both neutral and emotional stimuli. We tested the within-subject reliability of amygdala habituation across the course of the experiment. In order to control specificity of the findings we also compared the reliability of amygdala habituation across two different tasks. By this, we sought to test whether the reliability of amygdala habituation is task specific or whether it is simply a consequence of subjects being more aroused at the start of an fMRI experiment as compared to at the end. In other words, we tested whether it is necessary to perform an emotion specific task like the faces task in order to extract reliable amygdala habituation. Therefore, we also extracted the amygdala signal from an n-back working memory task, tested for habituation and calculated its reliability. Reliable amygdala habituation extracted from a task that does not target emotional processing would argue against the need for a specific emotional task. Face task specific reliability of amygdala habituation would argue for the need to significantly engage amygdala function as a prerequisite.

To summarize, the aim of the present study is to profile amygdala habituation during a facial emotion matching paradigm as an alternative fMRI phenotype and to test whether its within-subject reliability is superior to the usual estimate of the overall amygdala mean response.

Methods

Subjects

As previously reported (Plichta et al., 2012), N = 25 young healthy volunteers (15 F/10 M; mean age 24.4 years, standard deviation 2.8 years, range 20–32 years) were scanned on two occasions (mean interval between scanning sessions 14.6 days, standard deviation 2.1 days, range 12–21 days). During each scanning session, they performed three tasks in the same fixed order (n-back, faces, reward). For the present report, only the faces and the n-back task are analyzed. Exclusion criteria included regular use of any medication, presentation with DSM-IV axis I and II disorders and any history of neurological disorders. We assessed hours of sleep, number of cigarettes smoked and caffeine intake (cups of coffee or caffeinated tea) at the first scanning visit and provided this information to the subject before the second session with a request that they arrive in a comparable state with respect to these measures (all p-values > 0.10).

All participants were informed of the nature of the study and the MR scanning procedure before providing written informed consent. The study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Mannheim medical faculty of the University of Heidelberg).

Paradigm

The emotive face task is designed to engage emotional processing systems in the brain and in particular to bilaterally activate the amygdala (Hariri et al., 2002). The paradigm comprises visual presentation of faces conveying negative emotions (either fearful or angry), representing the experimental condition, or geometric shapes, representing the control condition, in alternating blocks of ~30 s each. Within each block, each trial was presented for 5 s. In each trial, the visual presentation comprises three pictures: the target image centered above two test pictures positioned left and right below it. In each trial, one of the test pictures is identical to the target image and the subject's task is to identify it via a button press (left or right). Four blocks of each condition were presented, giving a total fMRI scan length for this task of 4 min 28 s.

For comparison reasons we also extracted amygdala habituation from an n-back working memory task which bilaterally activates the parietal cortex as well as the dorsolateral prefrontal cortex, predominantly on the right (rDLPFC) (Callicott et al., 1998). In this paradigm, a series of digits (1–4) was presented visually in a sequence of frames, with each frame shown for 500 ms and an inter-stimulus interval of 1500 ms. In each frame, one of the digits was highlighted and represents the target number to be maintained in memory. As the paradigm sequence progresses, the subject's task is to indicate the highlighted number corresponding either to the currently displayed frame (0-back, control condition) or two frames previously (2-back, experimental condition) via a button press. The stimuli are presented in a block design that is very similar to the faces task. Each block was of 28 s duration, and four blocks were presented for each condition in an alternating fashion. The total fMRI scan length for this task was 4 min 16 s. We chose the n-back working memory task for comparison because of its similarity to the face task with regard to the number of stimulation blocks and block length, while it targets a completely different neural system.

Image acquisition

All MR scanning used a 3.0-T whole body scanner (Magnetom Trio, Siemens Medical Solutions, Erlangen, Germany). A high-resolution T1-weighted 3D MRI sequence was acquired first (ascending slices, slice thickness = 1.1 mm, FOV = 256 mm × 256 mm, matrix = 256 × 256), followed by the fMRI scans. For each paradigm, the anatomical coverage was identical and covered the whole-brain as well as scalp, cerebellum, eyes and nose in order to avoid wrap-around artifacts. Functional data were acquired using an echo planar imaging (EPI) sequence (TR/TE = 2000/30 ms; flip angle = 80°; 28 axial slices (slice-thickness = 4 mm + 1 mm gap) ascending, FOV = 192 mm × 192 mm, matrix = 64 × 64).

fMRI data analysis — preprocessing

The fMRI data were analyzed using statistical parametric mapping (SPM8; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, United Kingdom). fMRI data preprocessing was identical for the two tasks and included motion correction, spatial normalization into Montreal Neurological Institute [MNI] space with resampling to $2 \times 2 \times 2$ mm^3, and spatial smoothing with an 8-mm full-width at half maximum (FWHM) Gaussian kernel. Spatial normalization employed both linear (12-parameter affine) and nonlinear transformations, calculated for the mean EPI image from each time series with respect to the SPM EPI template in MNI space, and then applied to the full time series.

First-level temporal modeling within a general linear model (GLM) framework was performed to generate 3D maps of estimated regressor response amplitudes. For both tasks, the design matrices included 8 block regressors, which were convolved with the default SPM hemodynamic response function (HRF) computed as a 2-parameter gamma function. Motion parameters were included as additional covariates of no interest and were not convolved with the HRF. A high-pass filter

with a cut-off frequency of 1/262 Hz was used to attenuate only the lowest frequency components (linear scanner drifts). The filter was tailored to the low frequency characteristics of each single block regressor (1 stimulation in 260 s, i.e. 1/260 Hz) and the particular effect of interest (i.e. habituation). All analyses were corrected for serially correlated errors by fitting a first-order autoregressive process (AR[1]) to the error term. Separate ROI masks for the left and right amygdalae were defined from the WFU-PickAtlas (Version 2.5, Wake Forest University, School of Medicine, Winston-Salem, North Carolina; www.ansir.wfubmc.edu), atlas = "human-atlas aal".

Temporal signal-to-noise (tSNR)

Since amygdala fMRI signals may be particularly affected by susceptibility artifacts induced by the magnetic field inhomogeneities in the ventral part of the brain (Merboldt et al., 2001), we calculated block-wise tSNR (Welvaert and Rosseel, 2013) — see supplementary material for details and results.

Habituation analyses

For the face task, we modeled each stimulation block separately and extracted a total of 8 amygdala response estimators per subject (four face blocks and four form blocks). Exactly the same procedure was applied to the n-back task (four 0-back and four 2-back blocks). To facilitate interpretation of the beta estimates, each stimulation block was contrasted to the mean of all remaining blocks, i.e. the block-wise activation estimates are scaled to the overall mean.

We calculated two different amygdala habituation indices: (1) the amplitude difference between the first and last stimulation block (FmL; Blackford et al., 2013) and (2) modeling of habituation by means of the regression (REG) approach.

The second approach is based on the regression

$$Y = bX + a$$

where the mean amygdala response (Y) is predicted by the log-transformed block number (X). That is, blocks 1, 2, 3 and 4 were transformed (natural logarithm) to 0, 0.69, 1.10 and 1.39. Within this framework the intercept a of the regression line is an estimate of initial reactivity, and the regression coefficient b is an estimate of the rate of habituation. Since b has been shown to be dependent on a, we calculated absolute habituation according to Montagu (1963) as

$$b' = b - c(a - \bar{a})$$

where c is the slope of b on a, and $\bar{a}$ is the mean of a. The absolute habituation index (b') is a measure independent of initial amplitudes.

We tested whether amygdala habituation is differentially modulated by stimulus category (emotional vs. neutral) and applied a repeated measures ANOVA (alpha-level = 0.05) with factors *condition* (face, forms), *session* (first, second), *method* (FmL, REG) and *hemisphere* (left, right) to the extracted amygdala BOLD response data. We report habituation separated for conditions (face, forms) and across conditions. Our primary tests are based on the ROI-average of all amygdala voxels (one-tailed; alpha = 0.05 — uncorrected), following temporal modeling performed voxel-wise.

Reliability assessment

We assessed reliability using two ICC variants, namely ICC(2,1) and ICC(3,1). These were defined by Shrout and Fleiss (1979) as:

$$ICC(2,1) = BMS - EMS / (BMS + (k-1) * EMS + k * (JMS - EMS)/N) \quad (1)$$

$$ICC(3,1) = BMS - EMS / BMS + (k-1) * EMS \quad (2)$$

where BMS = between-subjects mean square; EMS = error mean square; JMS = session mean square ("J" originally stood for "Judge"); k = number of repeated sessions and n = number of subjects. Thus, in the current study, k = 2 and n = 25.

The calculation of these different ICC variants allowed the reliability to be assessed in terms of both relative (consistent measures = ICC(3,1)) and absolute agreement (ICC(2,1)). Note that the sample analyzed in the present report is identical to the sample described in Plichta et al. (2012) wherein amygdala mean response was tested for reliability. Using the same sample allows a meaningful comparison of ICCs obtained from the habituation summary measures to those obtained previously using the mean response.

Results

Behavioral data

Analyses of the behavioral data showed that the subjects' response data were stable across sessions (Table 1).

Amygdala habituation analysis (voxel-wise and ROI)

Left and right amygdala habituation is shown in Fig. 1. Both analysis approaches (FmL and REG) indicate significant amygdala habituation to face stimuli of the amygdala in session 1 while only the REG approach showed significant habituation in both sessions at the ROI level (see Table 2). Amygdala habituation was found to be significant in the right amygdala ROI, whereas only the REG approach revealed habituation also in the left amygdala (see Table 2). Habituation to form stimuli was revealed only by the REG approach for both hemispheres. Reliability maps of the left and right amygdala response to faces and forms across the stimulation blocks are shown in Fig. 2.

The amygdala habituation parameter was of moderate effect size (ES) in the case of the FmL approach (ES = 0.52–0.94). In the case of the REG approach the effect sizes were moderate to large (0.59–2.41) — see Table 2. Repeated measures ANOVAs indicated a trend-wise condition effect for right amygdala analyzed with the REG approach [$F(1,24) = 3.89$; $p = 0.06$] but not with the FmL approach [$F(1,24) = 2.74$; $p = .11$].

Within-subject reliability of amygdala habituation

Right amygdala: Analyses of the within-subject habituation showed fair-to-good (all ICCs > 0.40) independent of the analysis approach (FmL or REG) for the face condition and the average of faces and forms. ICCs > 0.40 for the form condition were indicated only by the FmL approach. Nominally, the global habituation index (i.e. average of

Table 1
Behavioral data.

Behavioral measure	Session #1	Session #2	t/p (df = 24)	ICC(2,1) (95%-CI)	ICC(3,1) (95%-CI)
RT (total) in ms (±SD)	1091 (205)	1062 (177)	1.33/.20	.83 (.66 .92)	.84 (.66 .92)
RT (faces) in ms (±SD)	1150 (244)	1131 (217)	0.73/.47	.85 (.68 .93)	.84 (.68 .93)
RT (forms) in ms (±SD)	1039 (186)	997 (161)	1.58/.13	.69 (.42 .85)	.70 (.43 .86)
Missed (total) in %	0.42 (1.04)	0.42 (0.85)	0.00/.99	–	–
Incorrect (total) in %	1.33 (1.69)	1.08 (1.60)	0.53/.60	–	–

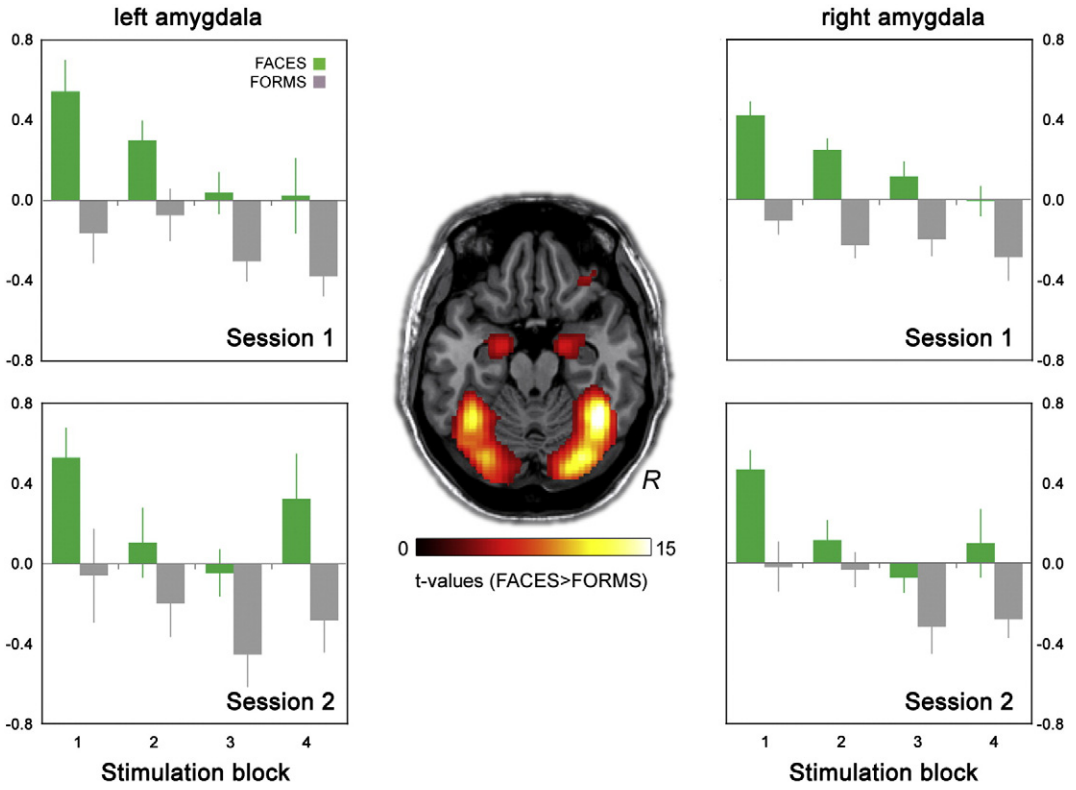

Fig. 1. FMRI group-level map for the contrast faces > forms and estimated block-wise left and right amygdala responses for session 1 and session 2. Bilateral habituation of amygdala responses toward face stimuli across blocks can be observed with a more consistent pattern in the right amygdala. Furthermore, compared to the form condition the beta estimates for the face condition are predominantly within the positive range of the scale (i.e. above grand mean of all blocks) — for further fMRI statistics, see supplementary material.

faces and forms) as quantified by FmL outperformed the condition-dependent indices (ICC = 0.53 versus ICC = 0.47 [forms] and ICC = 0.48 [faces]) while the REG approach yielded equally sized ICCs for faces and the average of both conditions. The REG approach nominally outperformed the FmL approach in terms of the face effect: ICCs of 0.53 vs. 0.48 (global) but not for the form effect (0.29 vs. 0.47). Left amygdala: habituation to faces analyzed with the FmL approach was associated with ICC > 0.40. All other ICCs for this hemisphere were <0.40.

When correcting for multiple testing (n = 12 tests), only the right amygdala [global habituation (FmL and REG) and the face habituation (REG)] effects survived. Finally, habituation ICCs (FmL-global; REG-face and REG-global) were significantly higher than the previously reported ICCs (<0.16) for the original activation contrast corresponding to the mean amplitude over the full fMRI run (Plichta et al., 2012).

Specificity of amygdala habituation

To test the task specificity of amygdala habituation and its stability, we contrasted the findings with amygdala habituation during a second task, i.e. an n-back working memory task, that does not significantly engage the amygdala per se. Analyses showed significant bilateral amygdala habituation (see Fig. 3). ICC analyses, however, showed that amygdala habituation during the n-back task was not reliable (ICC = 0[1]).

Discussion

We have demonstrated that amygdala habituation during an emotional face task exhibited significantly higher within-subject reliability than standard analyses of the mean response amplitude. Moreover, we found evidence that this reliable amygdala habituation is task specific, with poor retest reliability observed for the n-back task, despite the fact that significant amygdala habituation was also observed in that paradigm.

Our observation of significant habituation of the amygdala response – particularly in the right amygdala as indicated by the FmL approach – across repeated exposure is consistent with previous reports (Denny et al., in press; Lonsdorf et al., 2011; Phillips et al., 2001; Wright et al., 2001). It has been hypothesized that the right amygdala is involved in rapid, dynamical emotional stimulus detection whereas the left amygdala might be specialized for a more sustained evaluation (Baas et al., 2004; Wright et al., 2001).

Our finding of a significantly more stable amygdala habituation characteristic than mean amplitude response to emotional tasks activating the amygdala within-subjects has some important implications. As described by Phillips et al. (2001), under some circumstances, modeling of constant amygdala amplitudes and comparing the means can be an oversimplification — i.e., analyses that estimate only the mean response amplitude may not accurately model the underlying BOLD time courses. Finding amygdala mean differences and concluding that a hypo- or hyperresponsiveness is an important characteristic for a group might thus be invalid. For example, when comparing two groups with regard to amygdala evoked amplitudes, it might well be that enhanced mean amygdala reactivity results from less habituation over time or is a mixture of both absolute and relative magnitude changes during the fMRI run (Lonsdorf et al., 2011). Moreover, a finding of no difference in the mean amygdala response may be misinterpreted as a lack of differential amygdala response to the task. For example, an absence of significant amygdala amplitude differences before and after cognitive behavioral therapy in a group of patients with spider phobia has been reported (Schienle et al., 2009); this may simply reflect that amygdala amplitude is a less sensitive parameter and that habituation might differ. Given the higher within-subject reliability of the amygdala habituation, these

[1] The ICCs for both amygdalae were negative and therefore set to zero.

Table 2
FMRI statistics for amygdala habituation during the faces task.

ROI	Method	Condition	Session	MNI [x y z]	t-max	p (FWE-corr.)	t_{ROI} (df = 24)	p_{ROI}	$Mean_{ROI} \pm SE_{ROI}$	ES_P	ES_R
Amy – L	FmL	Forms	1	−22 −8 −12	2.21	0.194	–	–	–	–	–
	FmL	Forms	2	−18 0 −12	2.92	0.056	–	–	–	–	–
	FmL	Faces	1	−22 −8 −12	3.02	0.058	–	–	–	–	–
	FmL	Faces	2	−18 0 −12	2.37	0.144	–	–	–	–	–
	FmL	Forms + faces	1	−22 −8 −12	2.89	0.069	–	–	–	–	–
	FmL	Forms + faces	2	−18 0 −12	2.79	0.072	–	–	–	–	–
Amy – R	FmL	Forms	1	32 −2 −12	3.02	0.061	–	–	–	–	–
	FmL	Forms	2	20 −2 −12	2.68	0.099	–	–	–	–	–
	FmL	Faces	1	30 0 −14	3.27	0.043	2.64	<0.01	0.51 (0.19)	0.65	0.53
	FmL	Faces	2	22 −4 −12	3.55	0.020	2.14	0.02	–	0.71	–
	FmL	Forms + faces	1	30 0 −14	3.41	0.031	2.60	<0.01	0.89 (0.34)	0.69	0.52
	FmL	Forms + faces	2	22 −6 −12	3.21	0.040	1.97	0.03	–	0.64	–
Amy – L	**REG**	**Forms**	**1**	**−28 −6 −18**	**5.56**	**<0.001**	**2.95**	**<0.01**	**0.14 (0.04)**	**1.11**	**0.59**
	REG	**Forms**	**2**	**−18 0 −12**	**12.04**	**<0.001**	**5.31**	**<0.01**	**0.21 (0.04)**	**2.41**	**1.06**
	REG	**Faces**	**1**	**−20 −6 −14**	**7.95**	**<0.001**	**2.97**	**<0.01**	**0.22 (0.07)**	**1.59**	**0.59**
	REG	**Faces**	**2**	**−18 0 −12**	**5.10**	**<0.001**	**2.35**	**<0.01**	**0.25 (0.11)**	**1.02**	**0.47**
	REG	**Forms + faces**	**1**	**−22 −8 −12**	**7.35**	**<0.001**	**3.08**	**<0.01**	**0.20 (0.07)**	**1.47**	**0.62**
	REG	**Forms + faces**	**2**	**−18 0 −12**	**7.95**	**<0.001**	**3.06**	**<0.01**	**0.26 (0.08)**	**1.59**	**0.61**
Amy – R	**REG**	**Forms**	**1**	**32 −2 −12**	**9.30**	**<0.001**	**6.31**	**<0.01**	**0.26 (0.04)**	**1.86**	**1.26**
	REG	**Forms**	**2**	**22 2 −20**	**10.77**	**<0.001**	**7.86**	**<0.01**	**0.30 (0.04)**	**2.15**	**1.57**
	REG	**Faces**	**1**	**28 0 −16**	**8.33**	**<0.001**	**6.47**	**<0.01**	**0.37 (0.06)**	**1.67**	**1.29**
	REG	**Faces**	**2**	**22 −4 −12**	**8.23**	**<0.001**	**4.64**	**<0.01**	**0.37 (0.08)**	**1.64**	**0.93**
	REG	**Forms + faces**	**1**	**30 0 −14**	**8.96**	**<0.001**	**6.95**	**<0.01**	**0.36 (0.05)**	**1.79**	**1.39**
	REG	**Forms + faces**	**2**	**22 −6 −12**	**9.31**	**<0.001**	**5.69**	**<0.01**	**0.38 (0.07)**	**1.86**	**1.14**

Notes. ROI results are shown only in case the peak voxel survived FWE correction. Alpha for ROI activation was set to 0.01. Rows are shown in bold when peak and ROI data were significant in both sessions.

results suggest that studies targeting amygdala responses – especially when repeated measurements are acquired within the same subjects – should consider both characteristics.

We compared two different habituation indices: first minus last block and absolute habituation based on a regression approach. Although the reliability scores did not significantly differ between these two indices (see Table 3), the REG approach numerically outperformed the FmL score in the case of the face condition (ICC = 0.53 vs. 0.48). Importantly, the REG approach was more sensitive in detecting amygdala habituation in both sessions as compared to the FmL approach. In the context of within-subject fMRI studies, including pharmaco-fMRI, that target emotional processes we therefore suggest the REG approach as a first choice. However, the advantage of the REG approach in statistical power is most likely a consequence of including all blocks whereas the

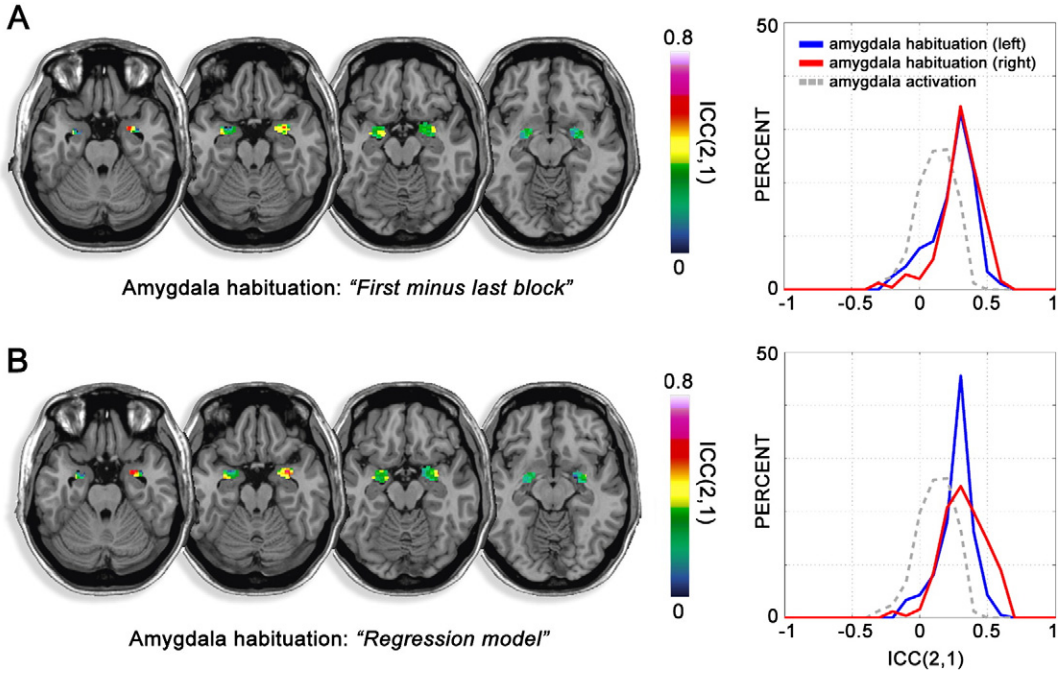

Fig. 2. Within-subject reliability of amygdala habituation for the face task. Panel (A) = first minus last block; Panel (B) = regression model. In each panel, the maps on the left show the voxel-wise anatomical distribution of ICC values in the amygdala, and the histograms on the right show frequency histograms of the voxel-wise ICC values in the left and right amygdala (blue and red lines, respectively). For comparison, the gray dashed lines show the mean response amplitude ICC histogram, for right and left amygdala pooled, from Plichta et al. (2012). See Table 1 for statistics.

Table 3
Within-subject reliability based on the amygdala ROI-mean (m) habituation.

Amygdala parameter	Region	ICC(2,1)m (95%-CI)	ICC(3,1)m (95%-CI)
Amplitude (face > forms) [a]	AMY — L	.16 (−.25 .52)	.16 (−.25 .51)
	AMY — R	−.02 (−.43 .38)	−.02 (−.41 .37)
Habituation-form (FmL)	AMY — L	.20 (−.22 .55)	.19 (−.21 .54)
Habituation-face (FmL)	AMY — L	.41 (.02 .69)	.40 (.02 .68)
Habituation-global (FmL)	AMY — L	.32 (−.09 .64)	.31 (−.09 .63)
Habituation-form (FmL)	AMY — R	.47 (.10 .73)	.46 (.09 .72)
Habituation-face (FmL)	AMY — R	.48 (.10 .73)	.47 (.10 .72)
Habituation-global (FmL)	**AMY — R**	**.53 (.17 .76)[b]**	**.52 (.17 .76)[b]**
Habituation-form (REG)	AMY — L	.22 (−.16 .56)	.23 (−.17 .57)
Habituation-face (REG)	AMY — L	.25 (−.16 .59)	.24 (−.16 .58)
Habituation-global (REG)	AMY — L	.33 (−.07 .64)	.33 (−.07 .64)
Habituation-form (REG)	AMY — R	.29 (−.10 .61)	.29 (−.10 .61)
Habituation-face (REG)	**AMY — R**	**.53 (.17 .76)[b]**	**.52 (.17 .76)[b]**
Habituation-global (REG)	**AMY — R**	**.53 (.17 .76)[b]**	**.52 (.16 .75)[b]**

Significant ICC values are shown in bold.
[a] See Plichta et al. (2012).
[b] $p < 0.05$ (Bonferroni-corrected).

FmL approach only uses two of four blocks. However, in cases of non-linear habituation the FmL approach, or other metrics of habituation, may be more powerful.

We tested whether amygdala habituation is condition-dependent, i.e. whether it is differentially associated with emotional versus neutral stimulation. Previous human studies have shown that the amygdala habituates to both neutral and emotional stimuli. Consistent with our results, amygdala habituation toward neutral stimuli seems to occur in the context of immediate stimulus repetition (Jung et al., 2006; Murray et al., in press). Our ROI results, in particular based on the more sensitive REG approach, indicate a trend toward stronger amygdala habituation to emotional stimuli. This effect, i.e. habituation to faces, was associated with significant retest reliability (ICC = 0.53) while the form condition was not (REG: ICC = 0.29) or did not survive correction for multiple testing (FmL: ICC = 0.41). Taken together, amygdala habituation was a) robustly measured at the ROI level in both sessions only with the REG approach and b) right amygdala habituation toward emotional stimuli quantified by REG was associated with the highest retest reliability (ICC = 0.53).

Furthermore, we tested the task-specificity of amygdala habituation by comparing the habituation rate and its reliability across two different tasks (faces and n-back). We found that significant amygdala habituation was also evident in the working memory paradigm. While at first glance this result seems surprising, it can be explained by task-unspecific arousal effects: subjects seem to be more aroused at the beginning of a measurement (novel stimuli and task demands, etc.) than at the end. However, the most striking finding was that the high reliability of amygdala habituation was solely evident in the face task but not in the n-back task. This suggests that systematic habituation takes place when the neural target structure is significantly and specifically activated by the task.

In line with existing findings (Feinstein et al., 2002; Fischer et al., 2000; Wright et al., 2001) neural habituation during emotion processing was not limited to the amygdala but occurred in a number of different brain areas including the insula, fusiform face area (FFA), cortical and subcortical areas (see supplementary Table 5). Among regions showing significant habituation, the amygdala was nominally the most reliable together with right inferior frontal gyrus (orbital part) and left insula. This is interesting against the background that these neural structures have been proposed to form a network particularly active during emotional face processing (Fusar-Poli et al., 2009; Sabatinelli et al., 2011).

Finally, we controlled for the impact of tSNR within the amygdalae. Interestingly, the only effect was a difference of left and right amygdala tSNR, consistent with previous reports (Johnstone et al., 2005; LaBar et al., 2001) but the exact reason is unknown. A lower tSNR of the left amygdala signal as measured with a standard EPI sequence might explain differences in results of fMRI studies. Importantly, all other main and interaction terms tested indicate that the amygdala tSNR was stable and, in the case of the right amygdala, not systematically different from other brain regions.

Limitations

We did not correct for physiological variation (breathing, heart rate) that might be correlated with the task at least in some participants (Birn et al., 2009; Lipp et al., 2014). In particular the important contribution of Lipp et al. discusses several possibilities how non-neural physiological noise and its correction can impact BOLD responsiveness. They showed that physiological noise correction reduces the repeatability of right amygdala activation and noted that one explanation might be variance reduction. The ICC is highly influenced by the variance of the measured signal, i.e. it decreases when variance is reduced. Another scenario of importance in the present context is that stable non-neural physiological signals are overlaid with neural response and that the pure neural signal is not temporally stable. We cannot rule out the possibility that

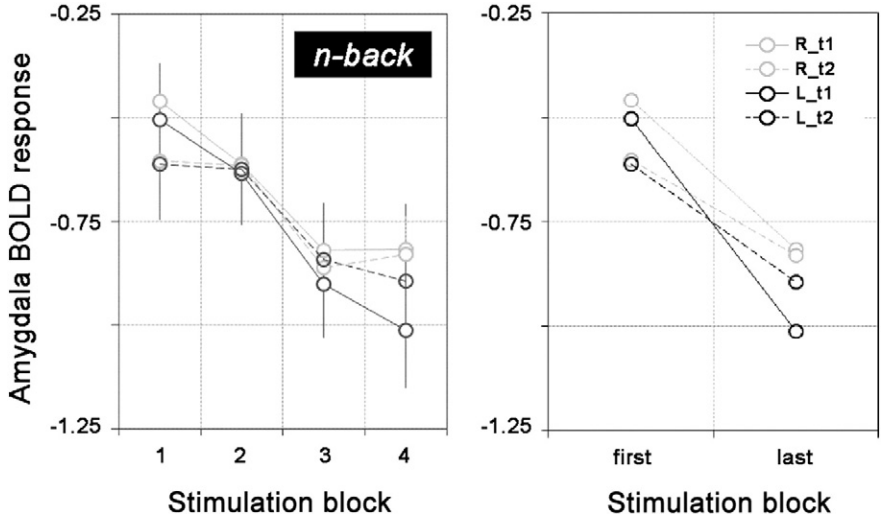

Fig. 3. Time course of amygdala habituation during the n-back task. Significant habituation was detected in the bilateral amygdala in session #1 (right amygdala: $t = 2.91$; $df = 24$; $p < 0.01$; left amygdala: $t = 3.91$; $df = 24$; $p < 0.001$) but not in session #2 ($t = 1.67$; $df = 24$; $p = 0.11$; $t = 1.75$; $df = 24$; $p = 0.09$). R_t1 = BOLD response of right amygdala in session #1, R_t2 = right amygdala in session #2, L_t1 = left amygdala in session one, L_t2 = left amygdala in session two.

presumably time-stable non-neural physiological variation contributed to the high reliability of amygdala habituation toward face stimuli. However, the regional specificity (right amygdala but not left amygdala showed ICC > 0.40) and in particular the fact that amygdala activation (i.e. the mean response) was not reliable argues against this explanation. Nevertheless, these important confounds should be investigated by future studies.

Furthermore, we did not investigate the relationship between amygdala responsiveness and psychometric variables such as (social) anxiety scores. Here differences between low and high scoring subjects have been shown for both amygdala activation (Calder et al., 2011) and habituation (Sladky et al., 2012).

Finally, to optimize the use of the emotive face task to detect habituation, the task design could be modified to include an explicit baseline condition. This would facilitate a direct comparison of condition-specific habituation effects.

Conclusions

We assessed the within-subject reliability of amygdala habituation measures in the facial emotion matching paradigm. Retest reliability of the session-wise amygdala habituation was significantly higher than the evoked amygdala mean amplitude (ICC = 0.53 vs. ICC = 0.16). Although significant amygdala habituation was also seen in a cognitive task (n-back working memory) that did not activate the amygdala per se, the habituation characteristics were totally unreliable in that context (ICC ~ 0.0). This is consistent with the idea that reliable habituation is a feature of tasks that significantly activate the amygdala. Together the results show that emotion-dependent amygdala habituation is a more reliable index than the mean amplitude, and provides a more robust endpoint for within-subject designs including ph-fMRI studies (Patin and Hurlemann, 2011).

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Conflict of interest

Dr. Meyer-Lindenberg has received consultant fees and travel expenses from Alexza Pharmaceuticals, AstraZeneca, Bristol-Myers Squibb, Defined Health, Decision Resources, Desitin Arzneimittel, Elsevier, F. Hoffmann–La Roche, Gerson Lehrman Group, Grupo Ferrer, Les Laboratoires Servier, Lilly Deutschland, Lundbeck Foundation, Outcome Sciences, Outcome Europe, PriceSpective, and Roche Pharma and has received speaker's fees from Abbott, AstraZeneca, BASF, Bristol-Myers Squibb, GlaxoSmithKline, Janssen-Cilag, Lundbeck, Pfizer Pharma, and Servier Deutschland. No other disclosures were reported.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2014.09.059.

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