Neuroscience Letters 604 (2015) 173–177

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Cerebellar vermis contributes to the extinction of conditioned fear

A. Utz[a], M. Thürling[a], T.M. Ernst[a], A. Hermann[b], R. Stark[b], O.T. Wolf[c], D. Timmann[a,*], C.J. Merz[c,d]

[a] Department of Neurology, University Clinic Essen, University of Duisburg-Essen, Hufelandstrasse 55, 45147 Essen, Germany
[b] Department of Psychotherapy and Systems Neuroscience and Bender Institute of Neuroimaging, Justus Liebig University Giessen, Otto-Behaghel-Str. 10H, 35394 Giessen, Germany
[c] Department of Cognitive Psychology, Institute of Cognitive Neuroscience, Ruhr-University Bochum, Universitätsstr. 150, 44780 Bochum, Germany
[d] Department of Biological and Clinical Psychology, University of Trier, Johanniterufer 15, 54290 Trier, Germany

HIGHLIGHTS

- Re-analysis of a human fMRI study on fear extinction focusing on the cerebellum.
- The anterior vermis plays a role in the extinction of conditioned fear.
- The cerebellum is likely part of the neural circuitry underlying extinction of conditioned fear.

ARTICLE INFO

Article history:
Received 8 June 2015
Received in revised form 17 July 2015
Accepted 21 July 2015
Available online 26 July 2015

Keywords:
Cerebellum
Fear conditioning
Fear extinction
fMRI
Lobule VI
Vermis

ABSTRACT

The cerebellum is known to contribute to the acquisition and retention of conditioned motor and emotional responses. Eyeblink conditioning and fear conditioning have been studied in greatest detail. Whereas a considerable number of studies have shown that the cerebellum is also involved in extinction of conditioned eyeblink responses, the likely contribution of the cerebellum to extinction of conditioned fear responses has largely been ignored. In the present study, we analyzed functional brain imaging data (fMRI) of previous work investigating extinction of conditioned fear in 32 young and healthy men, in which event-related fMRI analysis did not include the cerebellum. This dataset was analyzed using a spatial normalization method optimized for the cerebellum. During fear acquisition, an unpleasant electric shock (unconditioned stimulus; US) was paired with one of two pictures of geometrical figures (conditioned stimulus; CS+), while the other picture (CS−) was never paired with the US. During extinction, CS+ and CS− were presented without the US. During the acquisition phase, the fMRI signal related to the CS+ was significantly higher in hemispheric lobule VI in early compared to late acquisition ($p < .05$, permutation corrected). During the extinction phase, the fMRI signal related to the contrast CS+ > CS− was significantly higher within the anterior vermis in early compared to late extinction ($p < .05$, permutation corrected). The present data show that the cerebellum is not only associated with the acquisition but also with the extinction of conditioned fear.

1. Introduction

In cued fear conditioning, the association of an initially neutral conditioned stimulus (CS, for example a sound) with an unpleasant unconditioned stimulus (US, often an electric shock) is learned. After successful acquisition, the presentation of the CS already provokes the fear response, which is the conditioned response (CR, e.g. freezing, change of heart rate). During extinction learning the CS is presented alone leading to a decrease of fear responses [1].

In classical eyeblink conditioning an involvement of the cerebellum during acquisition is well known [2,3]. There is also animal and human data showing that the cerebellum is equally involved

Abbreviations: BOLD, blood oxygenation level dependent; CR, conditioned response; CS, conditioned stimulus; EPI, echo planar imaging; fMRI, functional magnetic resonance imaging; FWHM, full width at half maximum; LTP, long-term potentiation; MPRAGE, magnetization prepared rapid acquisition gradient echo; PET, positron emission tomography; SCR, skin conductance response; SPM, statistical parametric mapping; SUIT, spatially unbiased infratentorial (and cerebellar) template; SVC, small volume correction; US, unconditioned stimulus.

* Corresponding author.
E-mail address: dagmar.timmann-braun@uni-due.de (D. Timmann).

http://dx.doi.org/10.1016/j.neulet.2015.07.026
0304-3940/

in extinction of conditioned eyeblinks [4]. The cerebellum is also known to play a role in acquisition of conditioned fear, although this has been studied in less detail. Whereas the intermediate cerebellum appears to be most important in acquisition of conditioned eyeblinks, the vermis plays a role in fear conditioning [5]. For example, Supple and Leaton [6] showed that lesions of the vermis severely attenuated the acquisition and retention of conditioned fear responses in rats, with no weakening of the fear response to the US. Sacchetti et al. [7] found that the vermis is important in consolidation of learned fear. Likewise, impaired acquisition of fear conditioning has been shown in humans with vermal lesions [8].

Because the cerebellum is known to contribute both to the acquisition and extinction of conditioned eyeblink responses, and there is evidence that the cerebellum contributes to acquisition of conditioned fear, it is reasonable to assume that the cerebellum is part of the neural circuitry underlying extinction of conditioned fear as well. The contribution of the cerebellum to extinction of learned fear, however, has only rarely been assessed. For example, there are no animal lesion or recording studies examining the possible contribution of the cerebellum to fear extinction. Likewise, no studies in humans with cerebellar lesions have been performed. As yet, brain imaging studies focused on the contributions of the amygdala and other fear-related cerebral areas on extinction of conditioned fear. The cerebellum has largely been ignored. Earlier brain imaging studies did not include the cerebellum in their field of view, in later studies the cerebellum was scanned but usually not considered a region of interest [9–11]. Linnman et al. [12] report fear extinction related activation in the cerebellum in supplementary materials, but do not discuss their observation. As yet, only Kattoor et al. [13] focused on cerebellar activation related to acquisition and extinction. Different to most other fear conditioning studies, however, they used rectal extensions as US.

In the present study a previously published data set of classical fear conditioning in young and healthy subjects was analyzed using optimized normalization methods of the cerebellum [11]. In the study of Merz et al. [11] cerebral areas, known to be involved in fear conditioning, that is the amygdala, anterior cingulate gyrus, hippocampus, medial frontal cortex, nucleus accumbens and orbitofrontal cortex, were analyzed. The cerebellum, however, was not considered a region of interest. In the present study, we hypothesized that the activation of the cerebellum is also associated with the extinction of learned fear.

2. Materials and methods

Previously published data by Merz et al. [11] were analyzed. The experimental procedure is presented in short. The normalization procedure of the cerebellum, which has additionally been performed in the present study, will be described in more detail.

Thirty-two healthy men (mean age 24.6 years, standard deviation 3.9 years, range 18–35 years) participated in this fMRI experiment. In the original study, half of all participants were given cortisol after acquisition, the other half placebo, to measure its effect on extinction learning. Since no significant between group differences (cortisol vs. placebo) during extinction were observed in the cerebellum, data analyses were based on all 32 subjects.

One of two pictures (a gray rhomb or square displayed on a black background) was used as CS+ and was paired with an unpleasant but not painful electrical stimulation (US) applied to the left shin. The CS− consisted of the other picture and was not paired with the US. In extinction trials, neither the CS− nor the CS+ was paired with the US. 16 CS+ and 16 CS− were presented for 8 s for acquisition as well as for extinction. 7.9 s after CS+ onset the US was presented for 100 ms during acquisition. The intertrial interval was randomly jittered and lasted between 9.5 and 12 s. Each phase lasted about 10 min in total. To slow down acquisition and extinction and to make learning non-trivial a partial reinforcement schedule was used. Only in 10 out of 16 trials the CS+ was linked with the US. The first half of acquisition was defined as early acquisition and the second half as late acquisition. The same was done for extinction. After the acquisition phase participants had to decide whether the CS+ or CS− preceded electrical stimulation to confirm contingency awareness (for details see [11]). Skin conductance responses (SCRs) were obtained as a behavioral measure of fear responses (for details see [11]).

During acquisition and extinction fMRI data was obtained using a 1.5 T whole body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural imaging included 160 T1-weighted sagittal images (MPRAGE; 1 mm slice thickness). Functional imaging encompassed 245 volumes for fear acquisition and 245 volumes for fear extinction. They were registered using a T2*-weighted gradient echo planar imaging sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice order; TA = 100 ms; TE = 55 ms; TR = 2.5 s; flip angle = 90°; field of view = 192 mm × 192 mm; matrix size = 64 pixel × 64 pixel). The axial slices were oriented parallel to the orbitofrontal cortex-bone transition and a gradient echo field map sequence was measured before both functional runs to get information for unwarping B_0 distortions [11].

Due to an incomplete steady state of magnetization the first three volumes of each session were discarded. Realigned EPI images were co-registered to the T1 volume but otherwise unsmoothed. First level single subject analysis was calculated using an event related design. Individual normalizations were calculated using the SUIT toolbox (version 2.7) in SPM8 (http://www.icn.ucl.ac.uk/motorcontrol/imaging/suit.htm; [14]). For the T1-weighted images a brain extraction was performed, and the resulting images were segmented using the isolate function provided by the SUIT toolbox. Segmented images were used to calculate the normalization to the spatially unbiased atlas template (SUIT) of the human cerebellum [14]. For each subject the normalization was applied to the first level contrast images, and images were subsequently smoothed by a three-dimensional convolution with an isotropic Gaussian kernel of 12 mm full width at half maximum (FWHM). Relevant first level contrast images included CS+ and CS+ > CS− in early and late acquisition, and CS+ and CS+ > CS− in early and late extinction (for details on the complete statistical model see [11]). CS+ alone was included in accordance with eyeblink conditioning studies, in which a CS− is usually not presented [4,5]. Normalized contrast images were used for second-level random-effects analysis. Time-dependent changes in fMRI signal between early and late acquisition as well as extinction were compared using the flexible factorial design with group (cortisol vs. placebo) as between subjects factor, stimulus (CS+ vs. CS− or CS+ alone) and phase (early vs. late) as within subjects factor. The “explicit masking” option was used in SPM. Small volume correction (SVC), based on the probabilistic atlas of the cerebellar cortex (Cerebellum_SUIT.nii), was performed. A height threshold of $p < .05$ (permutation corrected, 1000 permutations) was used. To depict the localization of cerebellar activation the maximum probability atlas of the cerebellum cortex was used (Cerebellum_SUIT_maxprob.nii) [15].

3. Results

SCRs were significantly higher for the CS+ than for the CS− during acquisition. Likewise, SCRs were higher for the CS+ than for the CS− during early extinction, although the difference was less compared to the acquisition phase. SCRs and the difference between CS+ and CS− showed a significant decline both during late