SHORT REPORT

Involvement of the human cerebellum during habituation of the acoustic startle response: A PET study

Dagmar Timmann, Christina Musso, Florian P Kolb, Michel Rijntjes, Markus Jüptner, Stefan P Müller, Hans Christoph Diener, Cornelius Weiller

Department of Neurology
D Timmann
M Jüptner
H C Diener

Department of Nuclear Medicine, University of Essen, Hufelandstraβe 55, 45122 Essen, Germany
S P Müller

Institute of Physiology, University of Munich, Pettenkoferstraβe 12, 80336 Munich, Germany
F P Kolb

Department of Neurology, Friedrich-Schiller-University of Jena, Philosophenweg 3, 07740 Jena, Germany
C Musso
M Rijntjes
C Weiller

Correspondence to: Dr Dagmar Timmann, Department of Neurology, University of Essen, Hufelandstraβe 55, 45122 Essen, Germany. Telephone 0049 201 723 2460; fax 009 201 723 5901; email Dagmar.Timmann@uni-essen.de

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Abstract
The present study investigated the involvement of the human cerebellum in the habituation of the acoustic startle response using PET. The startle response was elicited in seven young, healthy subjects by a tone presented via headphones. Startle responses were recorded from the right sternocleidomastoid muscle. Regional cerebral blood flow (rCBF) was assessed in nine scans and one startle stimulus was applied during each scan. The reduction of size of the sternocleidomastoid muscle response was correlated with changes in rCBF during the ongoing process of startle response habituation. A significant decrease of rCBF was found in the medial cerebellum. These data are consistent with an involvement of the medial parts of the human cerebellum in non-associative learning as proposed by previous animal studies.
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Keywords: cerebellum; habituation; startle response; positron emission tomography

The role of the cerebellum for associative and non-associative learning has been intensively studied in animals. Classical conditioning of the nictitating membrane reflex has often been used to study the role of the cerebellum for associative learning and habituation of the acoustic startle response for non-associative learning.[1 2] Deficits in classical conditioning of the eyeblink and limb flexion reflex have been demonstrated in patients with cerebellar disorders and in healthy subjects using PET.[3 4]

In normal subjects, unexpected auditory stimuli produce a startle response which usually involves flexion of the neck and trunk and brief closure of the eyes.[5] The activity responsible for the startle response originates within the lower brain stem.[5 6] Animal studies have shown that the medial cerebellum is part of the essential circuitry for long term habituation of the acoustic startle response.[6–8] So far, the role of the cerebellum in habituation of the startle response has not been investigated in humans. In the present study, involvement of the human cerebellum in habituation of the acoustic startle response was investigated in healthy subjects using PET.

Materials and methods

With the approval of the local ethics committee the study was performed in seven healthy subjects (five men and two women; age from 25 to 63 years, mean 35.5 years) without any hearing loss, without neurological and orthopaedic diseases, and not receiving any medication. All subjects were right handed. All gave their informed consent.

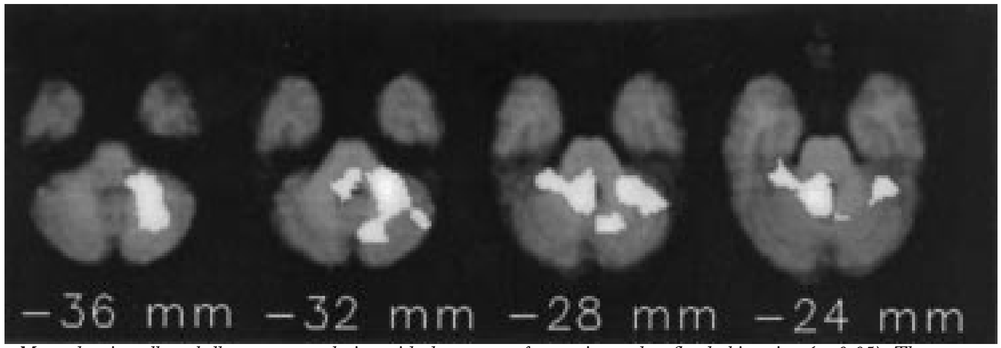
STARTLE RESPONSE HABITUATION

Subjects were lying supine and with their eyes closed. Head position was maintained by use of an individually molded foam headrest to minimise involuntary head movements during the scans. The startle stimulus consisted of an auditory tone pulse (1000 Hz; 95 dB; 50 ms) applied bilaterally via headphones. This tone was superimposed on continuous white noise (50 dB SPL) to mask environmental noise.

Electromyography recordings were performed from the right sternocleidomastoid muscle with surface electrodes (sampling rate=1 kHz). The EMG traces were amplified, full wave rectified and bandpass filtered (10 Hz<f<10 kHz). Latency and integrated EMG activity (iEMG) were calculated after visual identification of the onset of the sternocleidomastoid muscle activity using a commercial analysis program (axograph®). Due to difficulties in defining the end point of the sternocleidomastoid muscle burst the EMG integral was calculated using a fixed interval of 100 ms after the onset of the EMG burst. Sternocleidomastoid muscle iEMG was normalised by assigning an arbitrary value of 100% to each subject's iEMG in the first trial and referencing changes in their iEMG to that value. The ongoing process of habituation was defined as the trial by trial reduction in size of the sternocleidomastoid muscle response.

SCANNING METHODS

Regional cerebral blood flow (rCBF) was measured during nine consecutive scans using the $H_2^{15}O$ injection technique with an ECAT



t Maps showing all cerebellar areas correlating with the process of acoustic startle reflex habituation (p<0.05). The average PET data (n=7) were superimposed on to the MRI template. The highest correlation was seen in the intermediate parts of the left cerebellar hemisphere (p=0.014).

953–15 PET scanner (CTI Inc, Knoxville, TN, USA) over 90 seconds. Ten seconds after the beginning of each scan one startle stimulus was applied (= total of nine auditory tone bursts). Data acquisition and analysis have been described previously in detail.[4] In brief, assessment of significant rCBF change was performed using statistical parametric mapping (SPM, Wellcome Department of Cognitive Neurology, Queen Square, London, UK). The scans were transformed into the standard stereotactic anatomical space of Talairach and Tournoux.[9] Because of the restricted field of view of our camera (5.4 cm) we decided to scan the lower parts of the brain which included the cerebellum. Scans were performed from −56 mm below the AC-PC line for cerebellar planes to +24 mm above the AC-PC line for the frontal lobes, as the camera was tilted.

The rCBF of each test scan (1–9) was correlated pixel by pixel with normalised sternocleidomastoid muscle iEMG of each individual subject elicited by the startle stimulus during the corresponding scan using the general linear model.[10] Significant changes in rCBF were assumed at $p<0.05$ (corrected for multiple comparisons). Results are displayed as statistical parametric maps showing the significance level of areas of significant change of rCBF.

Results

Subjects habituated the acoustic startle response over the course of nine startle stimuli. Mean sternocleidomastoid muscle activity was reduced to 50.1% (SD=19.0) of its initial size by the end of the PET session. Decrease of sternocleidomastoid muscle iEMG was roughly linear (linear regression: R=0.70, slope=−3.5 , $p<0.05$).

The correlation analysis showed an area of significantly decreased blood flow in the cerebellum correlating with the ongoing process of startle response habituation ($p<0.05$, corrected for multiple comparisons). The cerebellar area of significantly decreasing rCBF extended from the vermis to intermediate regions of the cerebellar hemispheres predominately on the left side (figure). The highest correlation was seen in the left intermediate part of the cerebellum (maximum: x=−20 mm, y=−44 mm, z=−32 mm; Z=4.44; p=0.014 (corrected for multiple comparisons)). Local maxima in the area of significant change of regional cerebellar blood flow correlating with startle response habituation are given in the table. There were no brain areas outside the cerebellum which showed significant change of rCBF correlating with the process of habituation. However, it has to be emphasised that the view of our camera was restricted (5.4 cm) and only the lower parts of the brain were scanned.

Local maxima in the area of significant decrease (p<0.05) of regional cerebellar blood flow correlating with startle response habituation

Cerebellar location	*x*	*y*	*z*	*Z value*
Intermediate cerebellum (left)	−20	−44	−32	4.44*
Vermis (right)	8	−54	−24	3.44
Vermis (left)	−10	−70	−28	3.37
Intermediate cerebellum (left)	−20	−60	−36	3.17
Intermediate cerebellum (right)	24	−42	−26	3.03
Intermediate cerebellum (left)	−36	−46	−22	2.71
Intermediate cerebellum (left)	−40	−60	−28	2.52

*p=0.014.

Discussion

We showed significant decreases in rCBF correlating with the process of habituation of the human acoustic startle response in the medial parts of the cerebellum. These data are consistent with an involvement of the human cerebellum in the habituation of the startle response.

The cerebellar vermis has been shown to be a key region of a circuit essential for the acquisition of long term habituation of the acoustic startle response in rats.[6–8] However, medial cerebellar damage did not disrupt the basic neural circuitry for the acoustic startle response itself or the short term habituation mechanism which is presumed to be intrinsic to that pathway within the caudal brainstem.[11] Because the cerebellum is not involved in the basic neural circuit for the acoustic startle response, involvement in the modulation of the response size seems likely.[12] The significant decrease of cerebellar activity during the process of habituation might reflect a decrease in tonic activity on reticular neurons subserving startle responses during the process of habituation.[13] In the present study, effects of short term and long term habituation were not separated with the subjects receiving a total of nine startle stimuli within one 2 hour test session. The significant change of rCBF might reflect activity related to short term or long term habituation.

It has to be emphasised that PET studies alone cannot tell us whether a particular locus is essentially involved in a function or informed about a function located elsewhere. The presented data of activity changes may most simply reflect the monitoring of peripheral events, one of the cerebellar tasks generally.[14] As the size of muscular responses is diminished during the process of habituation the reduced activity in the cerebellum might simply reflect the reduced motor output.

The present study provides further evidence for the idea of functional compartments within the human cerebellum. A previous study from this laboratory showed a significant change of activity of the ipsilateral intermediate part of the human cerebellum during classical conditioning of the human flexion reflex.[4] During startle response habituation, the area of significantly decreased rCBF included the vermis and adjacent intermediate parts of the cerebellum. The vermis seems predominantly to be involved in non-associative learning (habituation of the startle response) whereas different parts of the intermediate cerebellum contribute to non-associative and associative learning (classical conditioning). These findings are supported by animal studies which showed different localisation for specific aversive classical conditioning and non-specific fear habituation or conditioning: Cerebellar vermal lesions prevented long term startle reflex habituation and heart rate conditioning but not eyelid conditioning, whereas lateral cerebellar lesions prevented eyelid conditioning but not heart rate conditioning.[1 6–8 15]

In summary, involvement of the medial cerebellum in the habituation of the startle response has been demonstrated in human subjects by changes in rCBF, monitored via PET. These data are consistent with an involvement of the human cerebellum in non-associative learning as suggested by previous animal studies. Further studies are needed to clarify if the human cerebellum is involved in short term, or long term habituation,or both, and whether its involvement is essential or not.

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