

FIVE healthy male subjects participated in a classical conditioning experiment, and positron emission tomography (PET) was used to compare regional cerebral blood flow before and after conditioning. The subjects participated in three different experimental phases. In the first (habituation) phase they listened to 24 repetitions of a tone with random intervals. In the second (acquisition) phase, the tone was paired with a brief shock to the wrist. In the third (extinction) phase, the tone was presented alone again. ^{15}O PET scans were taken during the habituation and extinction phases. Because the habituation and extinction phases were similar, any difference in blood flow to the tones presented during extinction probably reflected conditioning that occurred during the acquisition phase. Statistical parametric mapping (SPM) analysis of the PET data showed significantly increased activation in the right hemisphere in the orbito-frontal cortex, dorsolateral prefrontal cortex, inferior and superior frontal cortices, and inferior and middle temporal cortices. The only activated areas in the left hemisphere were area 19 and the superior frontal cortex. The results are interpreted as evidence for the involvement of cortical areas in human classical conditioning.

Key words: Blood flow; PET; Classical conditioning

Brain mechanisms in human classical conditioning: a PET blood flow study

Kenneth Hugdahl, Annamaria Berardi,¹
William L. Thompson,¹
Stephen M. Kosslyn,¹ Robert Macy,¹
David P. Baker,¹ Nathaniel M. Alpert²
and Joseph E. LeDoux³

Department of Biological and Medical Psychology, University of Bergen, Årstadveien 21, N-5009 Bergen, Norway; ¹Department of Psychology, Harvard University, Cambridge, MA; ²Department of Radiology, Massachusetts General Hospital, Boston, MA; ³Center for Neural Science, New York University, New York, NY, USA

Introduction

One of the simplest forms of learning is Pavlovian, or classical, conditioning. This form of learning occurs when a previously neutral stimulus is paired with a stimulus that produces a response; after pairing, the neutral stimulus then comes to produce the response. Conditioning is, thus, learning of relationships between events, allowing the organism to represent its environment.¹ Recent work has emphasized that the classical conditioning of different response systems involves different brain systems.^{2,3} A particularly interesting form of classical conditioning is fear conditioning, in which a neutral CS, such as a tone or light, is paired with an aversive UCS, such as a brief electric shock or intense noise.

Although the neural pathways mediating the acquisition of fear conditioning have been studied extensively in animals, relatively little work has been conducted in humans. The only data that pertain to the neural mechanisms underlying human conditioning rely on event-related potentials (ERPs)^{4–7} and the results suggest that cortical areas of the brain are involved in conditioning, especially with regard to the elicitation of slow negative shifts in the ERP. This may indicate the build-up of cortical expectancy that is linked to the presentation of the CS. However, the ERP effects are small, and not always replicated. In

addition, although ERP findings provide good information about temporal coding of information, they are less useful for specifying the spatial location of the brain regions involved.

Here we report a preliminary attempt to delineate the brain mechanisms that underlie human classical conditioning. We have studied this phenomenon using positron emission tomography (PET), which allowed us to measure blood flow distribution in the brain. The ^{15}O PET technique we used allows average blood flow in various regions of the brain to be recorded within a 70 s recording window for each scan, with a 10 min resting period between scans. Ideally, we would have liked to have compared patterns of PET activity on a trial-by-trial basis,⁸ using one CS that is paired with the UCS (CS+), and one which is not paired (CS–). However, because of the technical limitations imposed by the measurement technique, it was necessary to develop a novel between-subjects design (Fig. 1). The design consists of three phases; a pre-conditioning (habituation) phase, a conditioning (acquisition) phase and a post-conditioning (extinction) phase. PET scanning was performed during the first and third phases, with the second scan starting immediately after the last acquisition trial and continuing through the extinction phase. During the habituation phase, tones were repeatedly presented at random intervals. During the

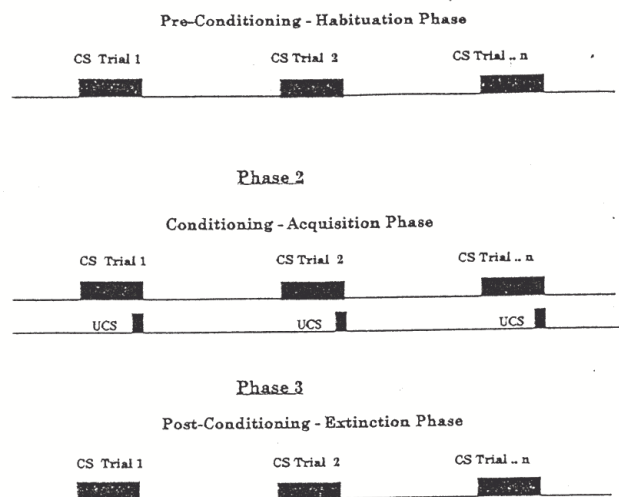


FIG. 1. Outline of the experimental design, featuring the three phases of the experiment. Note that PET scans were taken only during Phase 1 and 3.

acquisition phase, the tones were paired with a brief electric shock to the wrist. Finally, during the extinction phase, the tones were presented alone in a similar arrangement to that in the habituation phase. Because the habituation and extinction phases were similar, any difference in blood flow to the tones presented during the extinction phase would probably be due to the pairing of the CS tone with the UCS shock during the acquisition phase. Thus, to examine the neural mechanisms underlying conditioning, we subtracted blood flow during the habituation phase from that during the extinction phase.

Subjects and Methods

Subjects: The subjects were five right-handed male students with a mean age of 22 years (range 18–28). All subjects were healthy as determined from a health questionnaire. Potential subjects were tested on the Eysenck Personality Inventory⁹ (Neuroticism subscale) before the experiment, as a screening tool over the telephone. The Neuroticism subscale was used because high scores on this subscale could be taken as index of conditionability.¹⁰ Only subjects with scores ≥ 10 were included (mean = 17, range = 13–21). The Spielberger State-Trait Anxiety Inventory was administered just before the experiment to determine state and trait anxiety levels. The Trait Anxiety mean was 46 (range 32–59), and the State Anxiety mean was 41 (range 32–51). The Trait Anxiety scores indicated that subjects had higher anxiety levels than would be expected by the normative values (mean = 38, s.d. = 10).¹¹ The State anxiety scores were also higher than would be expected from male college students (normative sample mean = 36, s.d. = 10).

Apparatus and stimuli: Electric shocks were administered by a Grass Instruments S-44 stimulator, with an Isolation Unit SIU-6, and a constant current unit CCU1 (all by Grass Instruments, Quincy, MA). The shock level to be used during the experiment was determined in a pre-study phase while subjects sat comfortably on a chair. The shock electrodes (also made by Grass Instruments) were placed on the back of the right wrist. Subjects were instructed that their task was to determine a shock level that was 'uncomfortable but not painful'. Following this, shock levels were incremented by 0.5 mA, starting at 0 mA, until the subject decided that the shock level was uncomfortable but not painful. If subjects hesitated at a particular level, they were encouraged to try a higher current level, which was then decreased to the lower setting if the level was found to be too uncomfortable.

The CS tones had a duration of 5 s, with an intensity of 1000 Hz/70 dB, and with 5 ms rise and decay time. The UCS had a duration of 250 ms, with a steady state shock with a mean intensity of 1.8 mA (range 1.0–2.5 mA). The UCS was administered during the last 250 ms of CS tone presentation. The tones were generated using the tone generator option under the effects file menu of the SoundEdit program for Macintosh computers (Farallon Computing, Berkeley, CA). Rise time was controlled by increasing the tone amplitude by 20% every ms until after 5 ms the tone had full amplitude (100%). Decay time was controlled in a similar manner during the last 5 ms of tone presentation. Amplitude was decreased by 20% every ms until after 5 ms the tone was off (0% amplitude). The subjects listened to the tones through AIWA loudspeakers placed on a wooden table raised above the scanner bed. The tones were presented by a PC. Tone frequency was controlled by a sound meter that was held where the subject's head would be placed in the scanner.

There were 24 trials during the habituation phase of the experiment, with 12 trials during acquisition and extinction. We included more trials during habituation in order to habituate every aspect of the orienting response before acquisition began. The inter-trial interval was either 5 or 9 s (half of the intervals were 5 s, the other half 9 s, randomly interspersed) for all three phases of the experiment.

Conditioning procedure: Subjects remained on the PET scanner bed during all phases of the experiment. In Phase 1, subjects were instructed that they would soon hear a series of tones, and that they should close their eyes and keep them closed. They were instructed to empty their minds and not do anything other than listen to the tones. Before Phase 2, the acquisition phase, subjects were provided similar instructions as in Phase 1, but in addition they were instructed that at

times, they would be administered an electric shock at the level they previously had chosen to be uncomfortable but not painful. The subjects were told not to speak during the entire experiment. Ambient noise was kept to the minimum and lights were turned off in the PET room during the experiment.

Phase 2 (acquisition) shifted to Phase 3 (extinction) without any indication to the subject. In Phase 2, the 12 tones were presented paired with the shock UCS. In Phase 3, the shocker was turned off when the PET scanner was turned on, and subjects continued to listen to the tones. The subjects were not informed about the contingency between the tones and shock during Phase 2. All stimulus presentations and timing between events were controlled by a Macintosh computer using the MacLab software program.¹²

For technical reasons we could not record autonomic measures when the subjects were being scanned. However, before beginning the PET study, we tested six subjects off-line in the conditioning procedure. The details of the procedure were adjusted until we obtained reliable conditioning, as indicated by characteristic changes in skin conductance before and after conditioning. The paradigm we used in the scanning study was thus validated off-line, and we are confident that it would lead to conditioning.

PET procedure: Subjects were tested individually. After being informed of the conditioning phase that was to follow, each subject was fitted with a thermoplastic custom-moulded face mask (True Scan, Annapolis, MD). The subject was then positioned in the scanner, head aligned relative to the cantho-meatal (CM) line. The mask was placed in position to stabilize the subject's head, then a pair of nasal cannulae was placed in the subject's nose. The cannulae were attached to a tube that was connected to a gas inflow by which the radiolabelled isotope ^{15}O was delivered. A plastic vacuum mask was then placed over the subject's nose. Three transmission scans were taken with an orbiting rod prior to the ^{15}O emission scans. Following this, the experiment began, with 20 measurements during each PET scan; the first three over a 10 s period, then the following 17 measurements each over 5 s periods.

In Phase 1 of the experiment, subjects first listened to 15 tones. This required approximately 3 min. Immediately following this initial tone presentation period, the PET camera acquisition program was started (at this point, the scanner measured only residual background activity from previous studies). Following the initiation of the acquisition program, four more tones were presented; on the fourth trial (30 s after the PET acquisition program was turned on), delivery of ^{15}O began. Subjects continued listening to the tones for an additional 60 s, while

being scanned. Following this 60 s scan period ^{15}O delivery was stopped, and the subjects continued to listen to the remaining tones for an additional 12 s.

During Phase 2 of the experiment, no PET scanning was performed. In this phase, the subjects listened to the 12 tones paired with shock. After eight tones, (approximately 96 s), the PET acquisition program was initiated once again, activating the camera, and with tones continuing throughout. Thirty seconds after the PET camera was turned on, ^{15}O delivery resumed while subjects continued to hear tones which were now no longer paired with shock. This marked the beginning of Phase 3 of the experiment. This scanning phase lasted 60 s while subjects listened to six tones. After a 60 s period, the flow of ^{15}O was stopped, while the subjects continued to listen to an additional six tones. The concentration of the delivered [^{15}O]CO₂ was 2800 MBq l⁻¹ at a flow rate of 2 l min⁻¹. The ^{15}O was diluted by mixing with room air so that the measured peak count rate from the brain was 100 000–200 000 events s⁻¹.

Scanning was performed by a GE Scanditronix PC4096 15-slice whole-body tomograph, which was used in its stationary mode.¹³ The scanner yielded contiguous slices separated by 6.5 mm center-to-center; the axial field was equal to 97.5 mm. The axial resolution was 6.0 mm full width at half maximum (FWHM). The PET machine is located in a room built specifically for scanning. Every effort was made to ensure that ambient conditions were controlled across all scans.

PET image reconstruction: The blood flow images were computed based on scans 4–16, which were summed after reconstruction. Using radial artery cannulation, we have found that integrated counts over periods up to 90 s are a linear function over the flow range of 0–130 ml min⁻¹/100 g⁻¹. Thus, an arterial line was not required to ensure that data could be described in units of flow relative to the whole brain. The images were reconstructed using a measured attenuation correction and a Hanning-weighted reconstruction filter; the filter was set to a spatial resolution of 8.0 mm in-plane (FWHM). For the image reconstruction process, effects of random coincidences, scattered radiation, and counting losses due to dead time in the camera electronics were also corrected.

Each slice of the scan data was summed across both scanned behavioral conditions, and the co-ordinates of midline structures were marked on all slices. Parameters of the midsagittal plane were estimated by applying a least squares procedure to the above-mentioned coordinates. We then re-sliced the images parasagittally at 5.1 mm intervals. The countour of a sagittal slice 10.2 mm from midline was outlined by

hand at the 50% threshold level (nominal). In some cases, missing data from the parasagittal emission images were completed with the data from sagittal transmission images, which covered a broader range of the brain surface. The PET data were then translated into the Talairach coordinate system by deforming the 10 mm sagittal planes specified by Talairach and Tournoux¹⁴ until we obtained the best fit to a standard template (with 'best' being defined in a least-squares sense; see Ref. 15). This method allowed us to estimate the positions of the frontal and occipital poles, vertex, anterior (AC) and posterior (PC) commissures, as well as angle of tilt. Piecewise linear conversion to Talairach space was accomplished using the estimated loci of these regions as well as that of the midsagittal plane. The quality of the transformation was assessed both by inspecting the parameters' standard errors, and by visually comparing the manually drawn brain contour to the atlas contour produced by the program.

To ensure that the brain regions represented in the converted image were accurately translated to Talairach coordinates, we superimposed a computerized version of the Talairach and Szikla¹⁶ atlas onto the transformed image data. The computerized rendering of the atlas projects silhouettes of brain areas that then can be visually matched to the actual PET emission data images, and examined for goodness of fit.

PET statistical analysis: The mean concentration in each slice for each run was specified as an area-weighted sum, which we adjusted to a nominal value of 50 ml min⁻¹/100 g⁻¹. The images were then scaled and smoothed with a two-dimensional Gaussian filter (20 mm wide, FWHM). We then summed the images across subjects within each condition, with one image (condition) being subtracted from the other; these images were subtracted within subjects. The results were represented as images of the mean differences, standard deviations, and a *t*-value for each pixel. Each *t*-statistic image was then submitted to a statistical parametric mapping (SPM) analysis.¹⁷ This process generates 'omnibus subtraction images', which correspond to standardized normal deviates and ensures the minimization of Type I errors since artifactual areas of activation are very unlikely to reach significance. Image smoothness partly addresses the problem of multiple comparisons. This value was measured employing the method of Friston *et al*¹⁷ and was found to be 14.2 mm. In these analyses, *Z*-scores of 3.0 correspond to *t*-scores that without correction for multiple comparisons achieve the *p* < 0.001 level. This criterion has been used in previous articles reporting similar types of data (see Ref. 18). For additional details on the PET methods and analysis procedures, see Ref. 19.

Results

Two sets of analyses were performed on the PET data. First, Phase 1 (habituation) data were subtracted from Phase 3 (extinction) data, and then the reverse subtraction was performed. Co-ordinates of the centroid of activation are presented in Table 1 along with maximal *Z*-scores for each region.

Figures 2 and 3 illustrate the significant findings and the loci relative to the anterior-posterior commissures. When we asked which areas had more blood flow in the extinction phase than in the habituation phase, we found activation in the following regions in the right hemisphere: orbito-frontal cortex, dorsolateral prefrontal cortex, inferior and superior frontal cortices, as well as inferior and middle temporal cortices. In the left hemisphere, only area 19 and the superior frontal cortex were activated. When we asked which areas had more blood flow in the habituation phase than in the extinction phase, we found that only the left temporo-occipital junction/area 19 was activated. Contrary to our expectations, we did not find activation in the amygdala.

Discussion

Classical conditioning resulted in increased activation in frontal and temporal regions of the brain, especially in the right cerebral hemisphere. This is suggested by the increased blood flow to these regions in extinction relative to habituation. In addition, there was a deactivation in the left temporo-occipital junction (see Table 1 and Fig. 3). The present findings are in contrast to the brain pathways of fear conditioning revealed by studies of experimental

Table 1. Coordinates (in mm, relative to the anterior commissure) and *Z*-scores for regions in which there was more activation in the extinction condition than in the habituation condition, and vice versa. Regions are presented from posterior to anterior. Seen from the rear of the head, the X coordinate is horizontal (with positive values to the right), the Y coordinate is in depth (with positive values anterior to the anterior commissure) and the Z coordinate is vertical (with positive values superior to the anterior commissure). Values within 5 mm of the inter-hemispheric midline are considered to be 'midline regions'

	x	y	z	Z score
Extinction minus habituation				
Left hemisphere regions				
Area 19	-33	-65	24	3.15
Right hemisphere regions				
Inferior temporal	55	-18	-12	3.36
Middle temporal	58	-17	-8	3.70
Orbito-frontal	18	15	-16	3.56
Inferior frontal	34	29	-16	3.13
DLPFC	26	63	-12	3.63
Midline regions				
Superior frontal (area 9)	4	52	32	3.53
Habituation minus extinction				
Left hemisphere regions				
Temporo-occipital junction	-39	-65	12	3.44

DLPFC, dorsolateral prefrontal cortex (area 10).

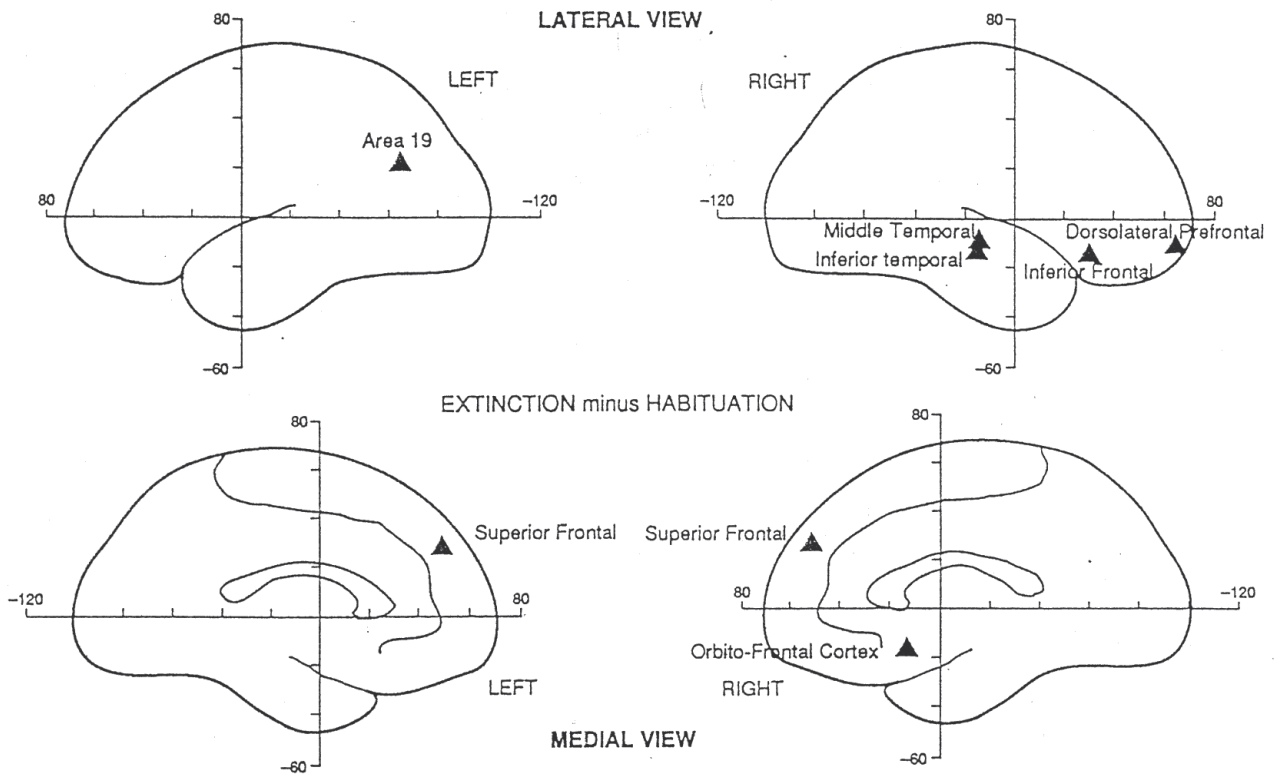


FIG. 2. Areas with significant increases in blood flow (▲) mapped on the lateral and medial surfaces of the brain for the extinction minus habituation subtraction. The tick marks on the axes represent 20 mm increments relative to the AC-PC line.

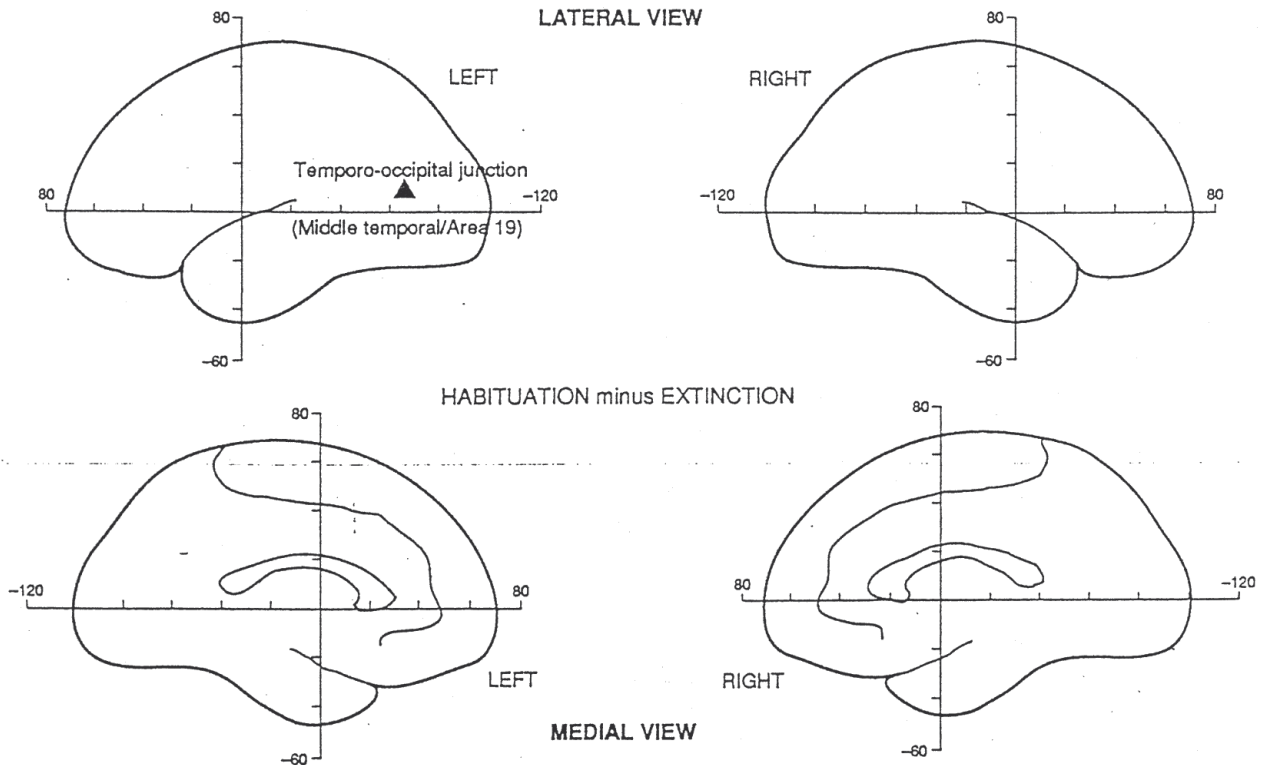


FIG. 3. Same as for Figure 2, for habituation minus extinction subtraction.

animals.^{2,20,21} For example, the amygdala has been implicated in fear conditioning in animal studies, although we found no evidence of increased blood flow in the amygdala. Other conditioning studies in e.g. cats and guinea pigs with auditory CSs have found evidence for neuronal changes in areas of the auditory cortex and auditory thalamus that are specific for the frequency of the CS (see Ref. 22 for review). Our Z-scores for the thalamus were in most instances close to zero, with the exception of the dorsomedial thalamus (Z-score = 3D 1.35: the coordinates for this point were -1, -12, 8 (x, y, z)). It should be pointed out, however, that areas 9 and 10, which both showed increased activation, have principal connections to the thalamus, the other cortical lobes, and the hypothalamus.

A possible explanation for these differences between our findings and those in animals may be related to the fact that our results are most accurately considered measures of extinction than conditioning, since we scanned only the habituation and extinction phases, leaving out the acquisition phase (for methodological reasons). In light of this it is interesting that we found significant increases in blood flow in the frontal areas, areas that animal studies also have shown to be crucial to extinction processes in fear conditioning. Further, a recent study of evoked potentials during human conditioning found localized frontal activation, during the extinction of the conditioned response.⁵ In addition, the increase in blood flow in fronto-temporal areas in our study may reflect the involvement of certain higher functions that are called into play in the human brain during conditioning. For example, activation of the inferior frontal, orbito-frontal, and dorsolateral prefrontal cortex may reflect the activity of an 'expectancy circuit'. Furthermore, the frontal cortical areas that were activated during extinction are similar to those that comprise the 'anterior attention system' postulated by Posner.²³ Although this circuitry may not be necessary for the acquisition, or performance, of a conditioned response, it is likely that the processing of the CS during extinction will result in the directing of attention to the CS.

It is also worth noting the apparent asymmetry in fronto-temporal activation, with more activation on the right side. A right hemisphere basis for classical conditioning has previously been suggested by Hugdahl and Johnsen.²⁴ This suggestion was based on studies with unilateral visual input, using autonomic responses. The present PET data support the right hemisphere asymmetry previously suggested. Subtraction of habituation from extinction showed that the only area in the left hemisphere that was significantly activated during extinction was area 19,

which lies in the occipital-temporal 'visual association' cortex. Thus, the right hemisphere asymmetry effect seems empirically valid, and should be further explored in future studies.

We close with an important word of caution. The present results are based on a single conditioning group. Thus, possible effects of UCS sensitization were not controlled. It is possible that some of our results may reflect perceptual processing of the UCS, rather than the association between the CS and UCS. However, it is very unlikely that all the observed significant effects pertain to sensitization and perceptual sensitivity. A reasonable speculation is that some of the activation over the sensory areas may be due to sensitization, rather than to CS-UCS associations. The validity of these speculations will however have to await further empirical evidence.

References

1. Rescorla RA. *Am. Psychol* 43, 151-160 (1988).
2. LeDoux JE. *Annu Rev Psychol* 46, 209-235 (1995).
3. Thompson RF. *Trends Neurosci* 11, 152-155 (1986).
4. Begleiter H and Platz A. *Science* 166, 769-771 (1969).
5. LaBar KS, Phelps EA, McCarthy et al. Event-related potential indices of fear conditioning in humans. Paper presented at the First Annual Meeting of the Cognitive Neuroscience Society, San Francisco, CA, March.
6. Lammers WJ. Evoked potentials in conditioning. Paper presented at the Sixth International Congress of Psychophysiology, Berlin, September, 1992.
7. Nordby H and Hugdahl K. Event-related potentials and hemispheric asymmetry of conditioned associations. *J Psychophysiol*, 9, 56-64 1995.
8. Prokasy WF and Kumpfer KL. Classical conditioning. In: Prokasy WF and Raskin DC (eds.) *Electrodermal activity in psychological research*. New York: Academic Press, 1973: 157-196.
9. Eysenck HJ and Eysenck SEG. *Manual for the Eysenck Personality Inventory*. Educational and Industrial Testing Service, San Diego, CA, 1968.
10. Hugdahl K, Fredrikson M and Ohman A. *Behav Res Ther* 15, 345-353 (1977).
11. Spielberger CD, Gorsuch RL, Lushene R et al. *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologists Press Inc., Palo Alto, CA, 1983.
12. Costin D. *Behav Res Methods Instrum Comp* 20, 197-200 (1988).
13. Rota-Kops E, Herzog HH, Schmid A et al. *J Comp Tomogr* 14, 437-445 (1990).
14. Talairach J and Tournoux P. *Co-planar stereotaxic atlas of the human brain*. New York: Thieme Medical Publishers Inc, 1988.
15. Alpert NM, Berdichevsky D, Weise et al. Stereotaxic transformation of PET scans by nonlinear least squares. In: Uemura K, Lassen NA, Jones T et al. (eds.) *Quantification of brain function: Proceedings of PET 1993*. Amsterdam: Elsevier Science Publishers, 1993: 459-463.
16. Talairach J and Szikla Z. *Atlas of stereotaxic anatomy of the telencephalon*. Paris: Masson and Cie, 1967.
17. Friston K, Frith C, Liddle P et al. *J Cereb Blood Flow Metab* 11, 690-699 (1991).
18. Rauch SL, Savage CR, Alpert NM. *Arch Gen Psychiatry* 52, 20-28 (1995).
19. Kosslyn SM, Alpert NM, Thompson, WL et al. *J Cogn Neurosci* 5, 263-287 (1993).
20. Kapp B, Whalen PJ, Supple WF et al. Amygdaloid contributions to conditioned arousal and sensory information processing. In: Aggleton J. (ed.) *The amygdala: Neurobiological aspects of emotion, memory, and mental dysfunction*. New York: Wiley-Liss, 1992: 229-254.
21. Davis M, Falls WA, Campeau S et al. *Behav Brain Res* 58, 175-198 (1993).
22. Weinberger NM. Retuning the brain by fear conditioning. In: Gazzaniga MS (ed.) *The cognitive neurosciences*. Cambridge, MA: MIT Press, 1995: 1071-1090.
23. Posner NI. Attention as a cognitive and neural system. *Curr Dir Psychol Sci* 1, 11-14 (1992).
24. Hugdahl K and Johnsen BH. Brain asymmetry and autonomic conditioning: Skin conductance responses. In: Roy JC, Boucsein W, Fowles DC et al. (eds.) *Progress in electrodermal research*. London: Plenum Press, 1993.

ACKNOWLEDGEMENTS: The present research was financially supported by the John D. and Catherine T. MacArthur Foundation, Min-Body Network, Chicago, IL. The comments and suggestions by members of the mind-body network core group are gratefully acknowledged. Thanks to Arve Asbjørnsen for preparing the auditory stimuli.

Received 9 May 1995;
accepted 9 June 1995