



Age-Related Reductions in Human Recognition Memory Due to Impaired Encoding

Cheryl L. Grady; Anthony R. McIntosh; Barry Horwitz; Jose Ma. Maisog; Leslie G. Ungerleider; Marc J. Mentis; Pietro Pietrini; Mark B. Schapiro; James V. Haxby

Science, New Series, Vol. 269, No. 5221. (Jul. 14, 1995), pp. 218-221.

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stage or during the plume activities in the geological time. Thus, the transition zone could be a diamond-enrichment zone and, therefore, is a potential reservoir of diamond in Earth.

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21. We thank M. Kitamura and K. Onuma for discussion.

28 February 1995; accepted 18 May 1995

Age-Related Reductions in Human Recognition Memory Due to Impaired Encoding

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The participation of the medial temporal cortex and other cerebral structures in the memory impairment that accompanies aging was examined by means of positron emission tomography. Cerebral blood flow (rCBF) was measured during encoding and recognition of faces. Young people showed increased rCBF in the right hippocampus and the left prefrontal and temporal cortices during encoding and in the right prefrontal and parietal cortex during recognition. Old people showed no significant activation in areas activated during encoding in young people but did show right prefrontal activation during recognition. Age-related impairments of memory may be due to a failure to encode the stimuli adequately, which is reflected in the lack of cortical and hippocampal activation during encoding.

Lesion studies have shown that medial temporal areas, including the hippocampus and adjacent cortices, play a critical role in declarative, or explicit, memory, specifically in the encoding of new memories (1, 2). In brain-damaged patients, the loss of medial temporal structures results in severe anterograde amnesia and in lesser degrees of retrograde amnesia (3). Lesions in animals also

have shown memory performance to be dependent on both the hippocampus and adjacent structures (4). One theory (1) is that the hippocampus participates in the storage of new memories by binding together neural activities in distributed regions of the cortex, so that later presentation of all or part of the stimulus context can reactivate the entire network and facilitate retrieval. The role of the hippocampus is thought to be limited in time, so that at some point after the new memory is processed, it can be retrieved without hippocampal involvement (5). Other regions also participate in memory, such as the prefrontal cortex (6–8), which shows frequency potentiation, similar to that seen in the hippocampus, during conditioning (9), and the inferior temporal cortex (10), which interacts with the prefrontal cortex during encoding of visual associative (11) and delayed match-to-sample tasks (7) in monkeys.

The degree of memory impairment with

aging varies; explicit memory is reduced in old compared with young people, but little change is seen in implicit memory (12). There are changes in the hippocampus with aging, including reductions in long-term potentiation (13), loss of afferent input (14), and loss of neurons (15). Some age-related hippocampal changes such as increased dendritic arborization may be a compensatory response to loss of cells or input (16). There also are age-related changes in other areas of the cortex, such as reductions in neurons (17), dendrites (18), and synapses, particularly in the frontal cortex (19). However, the relation between these changes and age-related reductions in human memory is unknown.

In a previous experiment (20), we used positron emission tomography (PET) to measure regional cerebral blood flow (rCBF) in young people during encoding and recognition of faces. The medial temporal cortex, including the hippocampus, showed increased rCBF (rCBF activation) during encoding of new memories but not during recognition. The prefrontal cortex was activated during both conditions: in the left hemisphere during encoding and in the right hemisphere during recognition. Here we discuss young and old people (Table 1) who underwent repeated PET scans (21) while performing three tasks twice in the following order: memorizing a set of faces (encoding), face matching (perception), and face recognition. A sensorimotor control task also was performed at the beginning and end of each scanning session. During the encoding task, subjects were shown 32 unfamiliar faces and asked to memorize them. Each face was shown for 4 s, and the entire set was shown three times, in a different order each time. The matching task used different faces from the memorization set and was a forced-choice match-to-sample task in which the sample face and two

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choice faces were presented simultaneously. During the recognition task, subjects were shown two choice faces in each trial, one of which was an unfamiliar distractor, and asked to indicate which had been seen previously in the set of 32 faces. There were no age differences in reaction time, but accuracy was reduced in the older people, to a greater degree for the recognition task than for the matching task (Table 1).

Areas of significantly increased rCBF were identified by comparison of rCBF images obtained during encoding and recognition with the images obtained during the matching and control tasks (22). During encoding of faces, relative to both comparison conditions (23), young people showed

rCBF activation in the anterior cingulate and in the left prefrontal cortex, including the orbitofrontal, inferior, and middle frontal gyri (Fig. 1). There also was activation in the left temporal cortex, extending over the middle and inferior temporal gyri. In the right hemisphere, significant activation during encoding, compared with both matching and control tasks, was limited to the medial temporal region, including the hippocampus and the parahippocampal gyrus. The old people (24) had no significant activation in the inferior prefrontal or medial temporal cortex during encoding (Fig. 1). There was an area of increased rCBF in the left ventral temporal cortex similar to that seen in young people during encoding,

but rCBF in this area was increased in comparison only with that observed during the control task, not with rCBF observed during the matching task.

The young people showed four regions of significant activation during the face recognition task, relative to both matching and control tasks (Fig. 1). These areas were in the right prefrontal cortex, including the inferior and middle prefrontal regions; the right parietal cortex; and the bilateral ventral occipital cortices (25). In old people, significant activation during recognition

Table 1. Demographic characteristics of subjects and performance data on the face matching and recognition tasks. Values are mean \pm SD. M, male; F, female. Reaction time shows the significant main effect of task by two-way ANOVA with repeated measures ($F = 119.2, P < 0.001$); other effects are not significant. Accuracy shows the significant main effect of age ($F = 17.1, P < 0.001$) and task ($F = 187.1, P < 0.001$); the accuracy measure also shows a significant interaction of age and task ($F = 6.2, P < 0.025$).

Participant parameters	Young people	Old people
Number	10 (8 M, 2 F)	10 (7 M, 3 F)
Age (years)	25.2 \pm 1.9	69.4 \pm 6.0
Education (years)	16.2 \pm 1.2	16.9 \pm 2.0
Reaction time (ms)		
Face matching	1544 \pm 550	1906 \pm 361
Face recognition	2005 \pm 602	2276 \pm 393
Accuracy (% correct)		
Face matching	99 \pm 2	94 \pm 4
Face recognition	80 \pm 7	66 \pm 9

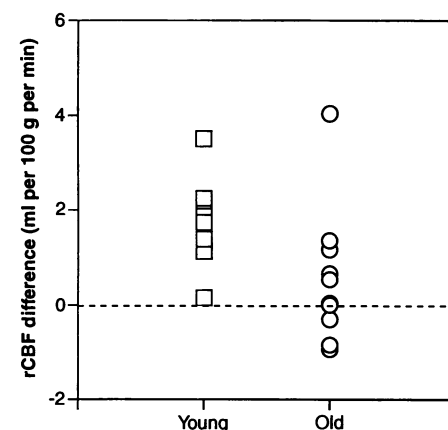


Fig. 2. The rCBF during encoding minus rCBF during the matching task for young and old people in the right hippocampus [at the maximum of activation identified in the young people (34, -24, -12)]. Values are analysis-of-covariance (ANCOVA)-adjusted.

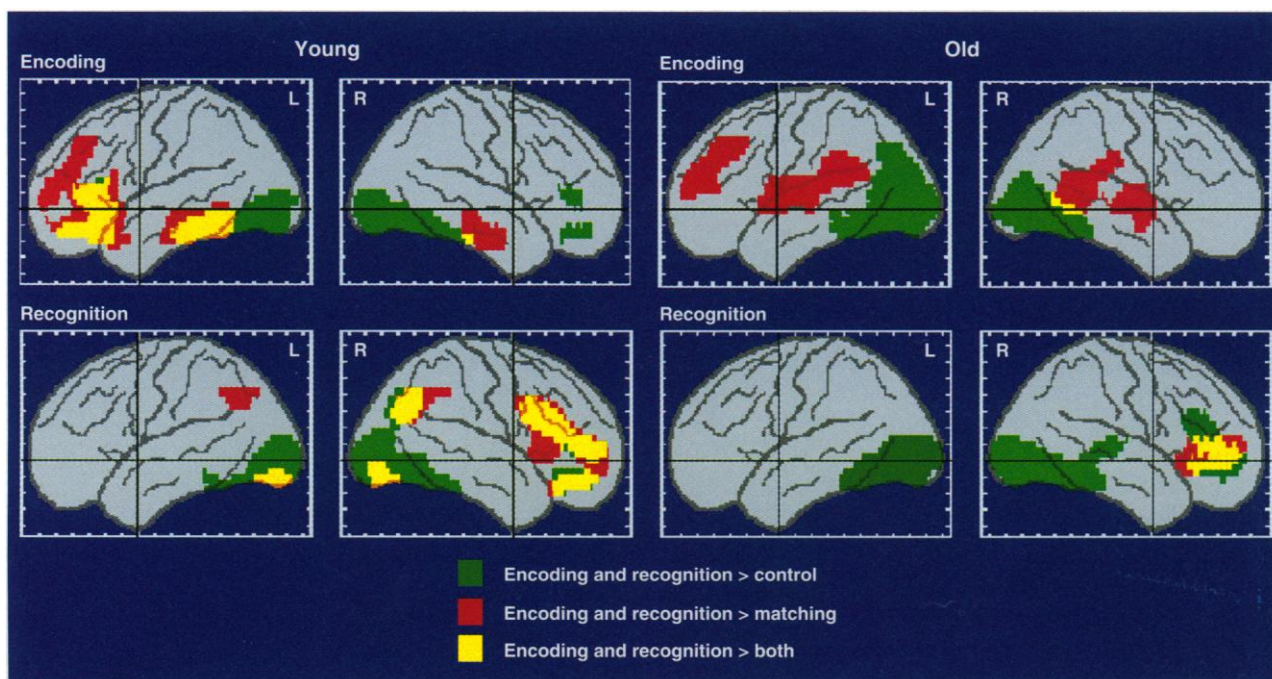


Fig. 1. Lateral schematics of the right and left hemispheres showing areas of significant rCBF increase during the face encoding and recognition tasks in young and old people. All areas in color had significant rCBF activation (22). The horizontal black line denotes the anterior commissure-posterior commis-

sure line, and the intersection of the horizontal and vertical black lines marks the zero point for the coordinate system. The tick marks in the grids are at 1-cm intervals.

was seen only in the right prefrontal cortex (26) (Fig. 1).

Comparison of rCBF activation between groups (27) showed significantly more activation in young than in old people during encoding in the left inferior frontal and inferior temporal cortex and in the anterior cingulate cortex. Despite the significant activation of medial temporal structures in the young people and a lack of significant activation of this region in the old people, there was no significant difference between groups in this area during encoding, mainly because of increased variance in the old people (Fig. 2). During recognition, there was significantly more activation in young than in old people in the right parietal and occipital cortex. There was no difference between groups in the right prefrontal region, in which both showed significant activation during recognition; and for either task, there were no areas in which old people had more activation than young people.

We also tested whether the functional interactions between the hippocampus and other cortical structures were altered in old people during encoding. Correlation coefficients (28) were computed between rCBF in the right hippocampus (at the maximum for the young people) and rCBF in all other brain areas during encoding in young and old people. In young people, the right hippocampal region was most strongly correlated with the anterior cingulate in a region that was activated during encoding (young, $r = 0.94$; old, $r = 0.02$; between-group comparison, $P < 0.002$, two-tailed test), whereas the largest correlation in old people was between the right hippocampus and the left parahippocampal gyrus (young, $r = 0.52$; old, $r = 0.96$; between-group comparison, $P < 0.002$, two-tailed test).

These results show that in young people, dissociable neural systems participate in encoding and recognition. Face memory encoding was associated with activation of the left prefrontal cortex and recognition with activation of the right prefrontal cortex, a hemispheric difference that has been described previously (8, 29). Patterns of rCBF activation were disrupted in older individuals, particularly during encoding, although there also was incomplete activation of the neural system responsible for recognition. The lack of activation in the areas responsible for encoding suggests that impaired recognition performance in older individuals is caused, in part, by a failure to encode the faces sufficiently. This result also suggests that older people employ no particular strategy, beyond that used in perception, in their attempt to memorize the faces. The between-group differences in correlations between the hippocampus and other brain regions suggest that the old people failed to engage the appropriate network for encod-

ing, which in young people includes the hippocampus and anterior cingulate. Recognition in the old people may have been adversely affected by interference from the faces presented during the matching task that preceded the recognition condition, because older individuals are more susceptible to the effects of distracting stimuli (30). However, given the marked differences in rCBF between the two groups during encoding, it is unlikely that poor recognition in the old people was due solely to interference.

In a previous study, we found no difference between young and old people in activation of the fusiform gyrus area that is primarily responsible for the perception of faces (21). Activation in the prefrontal cortex, however, was reduced in older people and was accompanied by increased activation in other regions of the cortex, including the frontal cortex (21). This may reflect an alteration in the cortical networks involved in visual perception by older people in an effort to compensate for reduced efficiency of the prefrontal cortex. This change in the networks appears to be successful in maintaining performance accuracy for this perceptual test, at the cost of a reduction in processing speed. In contrast, during memory processing, old people showed less activation of the areas responsible for task performance than did young people during both encoding and recognition, and there was no evidence for functional compensation in the old people during either task. This suggests that a lack of compensatory changes in cortical networks, coupled with reduced activity of critical areas, results in a more marked age-related reduction in cognitive performance.

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21. Informed consent was obtained from all participants after the nature and possible risks of the experiment were explained. The PET methodology and stimulus arrays have been described previously [C. L. Grady et al., *J. Neurosci.* **14**, 1450 (1994)]. In a separate group of eight young people, we compared the recognition performance after the matching task (incidental learning) with recognition after direct instruction to memorize the faces. Recognition was significantly better ($P < 0.05$) after memorization than after performance of the matching task.
22. Within- and between-group comparisons of rCBF were carried out by means of statistical parametric mapping [R. S. J. Frackowiak and K. J. Friston, *J. Anat.* **184**, 211 (1994)] and a test of significance based on the size of the activated region [K. J. Friston, K. J. Worsley, R. S. J. Frackowiak, J. C. Mazziotta, A. C. Evans, *Hum. Brain Mapping* **1**, 210 (1994)].
23. The following local maxima [as defined in J. V. Haxby et al., *J. Neurosci.* **14**, 6336 (1994), and with the use of coordinates and Brodmann areas (BA) from J. Talairach and P. Tournoux, *Co-Planar Stereotaxic Atlas of the Human Brain* (Thieme Medical, New York, 1988)] are from the comparison of encoding with matching, although these areas also were significantly activated as compared with rCBF during the sensorimotor control task. The left prefrontal region ($P < 0.0001$) had local maxima in BA 11/47 (x, y, z : -33, 27, -12), BA 46 (-26, 42, 16), and the anterior cingulate, BA 32 (2, 26, 36). The left temporal region ($P < 0.0001$) had maxima in BA 20 (-56, -34, -12) and BA 37 (-50, -52, -8). The right medial temporal region ($P < 0.001$) had maxima in the hippocampus (34, -24, -12) and in the parahippocampal gyrus (28, -10, -20).
24. The only area that was activated in old people during encoding, compared with face matching and the control task, was a small area in the right posterior temporal cortex. Although this represented an area of overlap between the two separate comparisons, the local maxima in these comparisons were not located close to this area of overlap.
25. The following local maxima are from the comparison of recognition with matching in young people, although these areas also were significantly activated, compared with rCBF during the sensorimotor control task. The region in the right prefrontal cortex ($P < 0.0001$) had maxima in BA 9/45 (36, 22, 20), BA 10 (34, 54, 4), and BA 8 (44, 8, 32). The maximum of activation in the right parietal cortex ($P < 0.01$) was in BA 7/39 (26, -64, 28). The right ventral region ($P < 0.01$) had maxima in BA 18 (24, -84, -8) and in the cerebellum (14, -78, -20). The left ventral region ($P < 0.001$) had maxima in BA 18 (-36, -84, -12) and in the cerebellum [(-41, -63, -28) and (-4, -66, -28)].
26. The right frontal region that was activated in old people during recognition ($P < 0.0001$), compared with matching and control, had maxima in BA 10/46 (30, 52, 8) and in BA 47 (30, 28, -4).
27. Young people showed greater rCBF activation than old people did during encoding, as compared with face matching, in the left temporal cortex (BA 37) and

in the left frontal cortex (BA 45/47) and showed greater activation as compared with the control task, in the anterior cingulate cortex (BA 32). During recognition, young people showed greater activation than did old people in right BA 7 (compared with matching) and in BA 19 (compared with results from

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21 October 1994; accepted 21 March 1995

Regulation of Human Leukocyte p21-Activated Kinases Through G Protein–Coupled Receptors

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The Rac guanosine 5'-triphosphate (GTP)–binding proteins regulate oxidant production by phagocytic leukocytes. Two Ste20-related p21-activated kinases (PAKs) were identified as targets of Rac in human neutrophils. Activity of the ~65- and ~68-kilodalton PAKs was rapidly stimulated by chemoattractants acting through pertussis toxin–sensitive heterotrimeric GTP-binding proteins (G proteins). Native and recombinant PAKs phosphorylated the p47^{phox} reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase component in a Rac-GTP–dependent manner. The action of PAKs during phagocyte activation by G protein–coupled pathways may contribute to regulation of NADPH oxidase activity.

The oxidative burst of human phagocytic leukocytes, which is critical to the inflammatory response, is mediated by a multicomponent NADPH oxidase regulated by the small guanosine triphosphatase (GTPase) Rac2 (1). Oxidase activation occurs through chemoattractant receptors coupled to pertussis toxin–sensitive G proteins (2). Rac guanine nucleotide exchange factors (3) and the Bcr GTPase activating protein (4) are important components of the activation process. The molecular details of how Rac regulates NADPH oxidase activity remain to be elucidated. Phosphorylation and dephosphorylation events modulate oxidant production, and NADPH oxidase components are substrates *in vivo* for unidentified kinases (5). p65^{pak}, a mammalian protein kinase related to Ste20 kinase of budding yeast (6), binds specifically to the GTP-bound forms of Rac and the related GTPase CDC42 (7). Rac-GTP stimulates autophosphorylation of p65^{pak} and its catalytic activity toward exogenous substrates.

Human neutrophil cytosolic fractions screened in overlay assays with the active forms of p21 Rho-related GTP-binding proteins revealed two proteins with apparent molecular sizes of 65 and 68 kD that bound Rac-GTP or CDC42-GTP or both, but not RhoA-GTP (Fig. 1A). Guanosine 5'-diphosphate (GDP)–bound forms of Rac (Fig. 1A) and CDC42 did not interact with these targets but did bind to the 28-kD Rho GDP

dissociation inhibitor (RhoGDI). Both *Escherichia coli* and baculovirus Sf9 cell-expressed Rac proteins bound p65 and p68. Neither p65 nor p68 was detected in neutrophil membranes when Rac1-GTP was used as a probe (Fig. 1A).

The binding of these cytosolic proteins specifically to Rac-GTP was confirmed with Rac1 and RhoA GST fusion proteins.

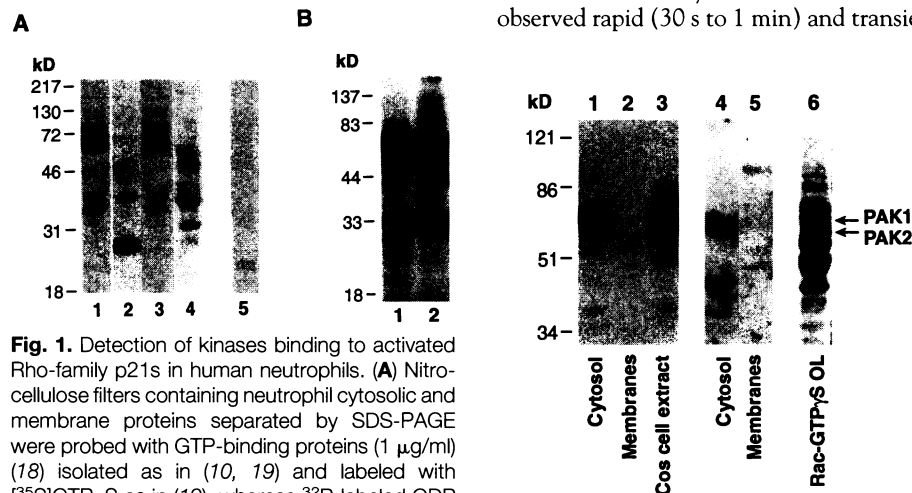


Fig. 1. Detection of kinases binding to activated Rho-family p21s in human neutrophils. (A) Nitrocellulose filters containing neutrophil cytosolic and membrane proteins separated by SDS-PAGE were probed with GTP-binding proteins (1 μg/ml) (18) isolated as in (10, 19) and labeled with [³⁵S]GTPγS as in (19), whereas ³²P-labeled GDP forms were prepared by allowing intrinsic hydrolysis of bound [α-³²P]GTP to take place. Shown is one of three representative overlays with Rac1-GTPγS (lane 1), Rac1-GDP (lane 2), CDC42-GTPγS (lane 3), and RhoA-GTPγS (lane 4) on cytosolic proteins and Rac1-GTPγS on membrane proteins (lane 5). (B) Activated Rac1 (lane 1) and RhoA (lane 2) GST fusion proteins coupled to glutathione-Sepharose beads were incubated with dialyzed neutrophil cytosol for 60 min and washed extensively, and kinase activity was measured (17).

Kinase activity assays revealed that both p65 and p68 became phosphorylated when bound to Rac-GTP resin (Fig. 1B). Antibodies to p65^{pak} (PAK1) revealed a 68-kD protein in neutrophil cytosol that comigrated with recombinant human PAK1, whereas antibodies to a homolog that is 79% identical to PAK1 [termed PAK2 (8)] detected a 65-kD protein (Fig. 2). The proteins observed by immunoblotting of neutrophil cytosol migrated with the same apparent molecular sizes as the p65 and p68 Rac-binding proteins detected in overlays and were not present in neutrophil membranes. Both p65 and p68 were immunoprecipitated by the respective antibodies to PAK2 and PAK1. Thus, human neutrophils contain kinases closely related or identical to PAK1 and PAK2, and both kinases autophosphorylate when Rac-GTP is present.

Because PAKs are direct targets of Rac, we investigated whether PAKs could be activated under conditions in which Rac activation occurs (9). Proteins from neutrophil lysates stimulated with the chemotactic peptide fMetLeuPhe (fMLP) or phorbol myristate acetate (PMA) were immunoprecipitated with antisera to PAK1 or PAK2 and kinase activity was determined. We observed rapid (30 s to 1 min) and transient

Fig. 2. Detection of p65^{pak}-related kinases in neutrophils by immunoblotting. Cytosol (lanes 1 and 4) and membrane fractions (lanes 2 and 5) of neutrophils transferred onto nitrocellulose were probed with antisera to PAK1 (lanes 1 to 3) and PAK2 (lanes 4 and 5) (20). A control lysate of Cos cells overexpressing human PAK1 was used (lane 3); PAK1 partially degraded in this lysate, accounting for the lower band in this lane. The autoradiogram of a Rac1-[³⁵S]GTPγS neutrophil cytosol overlay (OL) (lane 6) is provided for comparison.

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