Associative Learning, Acquired Equivalence, and Flexible Generalization of Knowledge in Mild Alzheimer Disease

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Background: Acquired equivalence is a phenomenon in which prior training to treat 2 stimuli as equivalent increases generalization between them. Previous studies demonstrated that the hippocampal complex might play an important role in acquired equivalence associative learning. In this study, we tested the possibility that acquired equivalence learning is a sensitive marker of mild Alzheimer disease (AD).

Methods: In the associative learning test, antecedent stimuli were cartoon faces and consequent stimuli were different colored cartoon fishes. Each cartoon character had some pet fish and the task was to learn these face-fish associations using feedback provided after each decision. In the transfer phase, knowledge about face-fish pairs had to be generalized to new associations.

Results: AD patients exhibited mild impairments in the training phase, whereas they were profoundly impaired on the acquired equivalence test. Associative knowledge could not be transferred to a more flexible retrieval condition.

Conclusions: These results suggest that acquired equivalence learning is specifically impaired in early AD, which may indicate the pathology of the hippocampal complex.

Key Words: Alzheimer disease, habit learning, acquired equivalence, hippocampus

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Alzheimer disease (AD) is characterized by a marked dysfunction of declarative memory, which refers to the conscious recollection of facts and events. This deficit is present even in very early stages of the disease and is associated with structural alterations of the medial temporal lobe, including the hippocampus.[1,2] Although traditional associative learning paradigms, such as the learning of face-name pairs, are related to the hippocampal region and are sensitive markers of AD,[3] recent evidence suggests that the hippocampal region is dispensable for the feedback-guided acquisition of associations. Previous studies tested nondemented elderly individuals with and without hippocampal atrophy on stimulus-response associative learning tasks.[4,5] Individuals with hippocampal atrophy, who are at risk for AD, were able to learn stimulus-response associations and were able to perform a series of object discriminations. However, they were impaired on a subsequent generalization task in which familiar features and objects were recombined.[4,5] The results of these studies suggest that the hippocampal region may not be critical for simple feedback-guided stimulus-response associative learning, but is indispensable for the generalization of this knowledge.[6,7] This raises the possibility that such generalization deficits may be a sensitive behavioral marker of hippocampal pathology and may be present in early AD.

To test this hypothesis, we investigated acquired equivalence associative learning in mild AD. Acquired equivalence is a phenomenon in which prior training to treat 2 stimuli as equivalent increases generalization between them, even if the stimuli are superficially very dissimilar. Acquired equivalence associative learning is markedly impaired in individuals with the atrophy of the hippocampal region,[5] as predicted from computational modeling of cortico-hippocampal representations,[7] which is based on animal studies of odor discrimination and generalization.[8] In rats, Coutureau et al[9] demonstrated that the lesion of the entorhinal cortex, but not of the hippocampus proper, resulted in impaired acquired equivalence learning.

In our human acquired equivalence associative learning test, antecedent stimuli were cartoon faces and consequent stimuli were different colored cartoon fishes[5,10] (Fig. 1). Each cartoon character had some pet fish, and the task of the participant was to learn these face-fish associations using feedback provided after each decision. There were 4 stages of the task, as shown in Table 1. First, 2 antecedent stimuli A1 and A2 were associated with the same consequent stimulus X1, whereas 2 antecedent stimuli B1 and B2 were associated with consequent Y1 (stages 1 and 2). Next, A1 was associated with a new consequent X2 whereas B1 was associated with a new consequent Y2. Finally, a transfer phase tested

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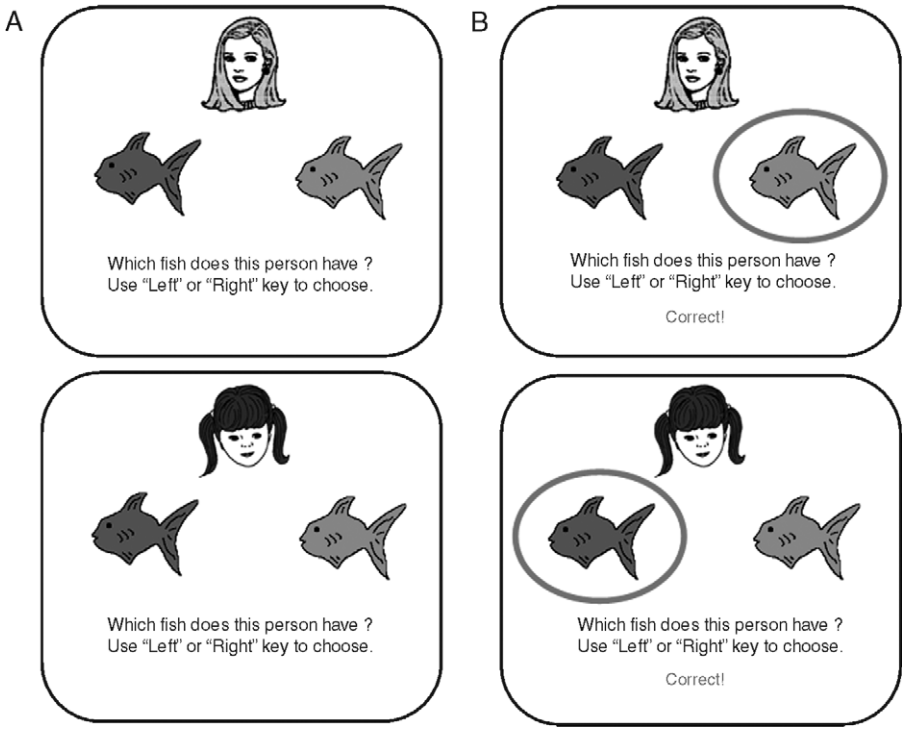

FIGURE 1. Example screen events during 1 trial. A, Stimuli appear. B, Participant responds and corrective feedback is given.

whether patients would show acquired equivalence and associate A2 with X2 and B2 with Y2, even though these particular stimulus pairings had never been trained.

We investigated 3 issues and tested the following hypotheses:

1. Feedback-guided learning of face-fish associations: We hypothesized that patients with AD are able to learn these associations using feedback and retain this knowledge.
2. Acquired equivalence: The hypothesis was that, despite the fact that patients with AD are able to learn associations, they show severe deficits when associations must be generalized.
3. Flexible application of associative knowledge: In this test, participants are requested to retrieve associations by pairing faces and fishes printed on cards instead of making forced-choice judgments. We hypothesized that patients with AD are impaired on this task requiring a more flexible application of knowledge.

TABLE 1. Acquired Equivalence Learning

Acquisition Stage 1: Shaping	Acquisition Stage 2: Equivalence Training	Acquisition Stage 3: New Consequents	Transfer Phase: Equivalence Testing
A1→X1	A1→X1A2→X1	A1→X1 A2→X1 A1→X2	A2→X2?
B1→Y1	B1→Y1B2→Y1	B1→Y1 B2→Y1 B1→Y2	B2→Y2?

METHODS

Participants

Twenty-five patients with mild AD and 20 healthy elderly controls participated in the study. Patients and controls were matched for age, sex, and education. The diagnosis of probable AD was made according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria.[11] Participants were evaluated with the Mini-Mental State Examination[12] and Global Deterioration Scale.[13] Neuropsychologic assessment included the Clock test (constructional and visuospatial functions), the Ray Auditory Verbal Memory Test, the FAS (letter) fluency test (executive functions), and the Rey-Osterrieth Complex Figure Test (drawing and visual memory).[14]

Clinical information included medical history, laboratory tests, brain imaging findings (head MRI), neurologic examination, and neuropsychologic test results. Exclusion criteria consisted of vascular lesions on MRI scans and prior neurologic and psychiatric disorders. The clinical, demographic, and background neuropsychologic data are shown in Table 2.

Associative Learning Test

Stimuli were presented and responses were collected using a Macintosh Power-Book laptop. The antecedent stimuli were 4 drawings of faces (man, woman, girl, and boy). The consequents were drawings of fish colored red, orange, purple, and pink. For each participant, stimuli

TABLE 2. Clinical and Demographical Characteristics of the Participants

	Controls (n = 20)	Alzheimer's Patients (n = 22)
Age (y)	70.1 (4.8)	69.8 (6.9)
Male/female	12/8	15/7
Education (y)	13.7 (3.2)	13.6 (3.8)
MMSE	29.4 (0.7)	24.0 (1.3)*
GDS	—	3.7 (0.5)
Clock test	9.8 (1.3)	7.7 (2.1)*
RAVLT	7.6 (3.8)	1.5 (1.7)*
Rey figure-copy	31.8 (5.6)	30.2 (6.9)
Rey figure-recall	15.9 (4.9)	5.6 (7.3)*
FAS fluency	14.3 (6.3)	12.9 (8.7)

*$P < 0.05$.

GDS indicates Global Deterioration Scale; MMSE, Mini-Mental State Examination; RAVLT, Ray Auditory Verbal Learning Test, trial 7.

were randomly assigned as antecedent and consequent stimuli. At the start of the experiment, the following instruction appeared on the screen: "Welcome to the experiment. You will see drawings of people who each have some pet fish. Different people have different kinds of fish. Your job is to learn which kinds of fish each person has. At first, you will have to guess." The experimenter read the instruction aloud to the participant and then clicked the mouse button to begin the acquisition phase. On each trial, a face and 2 fish drawings were displayed on the computer screen along with the prompt: "Which fish does this person have? Use the Left or Right key to choose." The participant responded with pressing 1 of the 2 separate keys labeled as "LEFT" and "RIGHT" to indicate whether the fish on the left or the fish on the right was associated with the face. The selected fish drawing was circled and corrective feedback was given (Fig. 1). In the case of an incorrect response, an alert beep sounded. The left-right ordering of the fish drawings was randomized within and across the subjects. There were 3 stages in the acquisition phase (Table 1). Stages 1 and 2 terminated after 8 consecutive correct responses, whereas stage 3 terminated after 12 consecutive correct responses. The participant was not informed on the beginning of a new stage. After the termination of the acquisition phase, a new instruction appeared on the screen, informing the participant that the task would remain the same but feedback would no longer be provided. The participant was not informed of the presence of new associations. The transfer phase consisted of 48 trials of which 12 trials were new associations for the testing of learned equivalence and 36 trials were old associations trained during the acquisition phase. The dependent measures were the mean number of errors in the acquisition phase and the proportion of incorrect responses in the transfer phase (for methodologic details, see Ref. 5).

After the computer-administered testing phase, participants received cards (size: $5 \times 5\,cm^2$) depicting the faces and fishes. The task was to pair fishes and faces as learned during the test. The dependent measure was the percentage of correctly retrieved face-fish associations. After the card-sorting test, participants were asked to read a newspaper article for 5 minutes. After this, the original computer-administered testing phase was repeated.

Data Analysis

The number of errors in the training phase of the associative learning test and the clinical parameters were analyzed with 2-tailed t tests and Mann-Whitney U test (this nonparametric analysis was used for Mini-Mental State Examination values which showed non-Gaussian distribution). Errors from the testing phase were analyzed with a 3-way repeated measures analysis of variance (ANOVA), which had the following design: 2 (group) by 2 (immediate vs. delayed testing) by 2 (old vs. new associations). A 2-way ANOVA was used for the analysis of errors from the card-pairing test with a 2 (group) by 2 (old vs. new associations) design. Tukey Honestly Significant Difference (HSD) Test was used for post hoc analysis. Neuropsychologic scores were compared with 2-tailed t tests. The level of significance was $\alpha < 0.05$.

RESULTS

Background Neuropsychology

Table 2 shows that patients showed profound verbal and visual declarative memory impairments, whereas visuospatial and executive functions were less severely affected.

Training Phase of the Associative Learning Test

Twenty-two AD patients out of the original sample of 25 patients were able to complete the training phase. Patients with AD committed more errors (mean: 14.8, SD = 7.0) compared with controls (mean: 8.5, SD = 3.6), $t(40) = -3.64$, $P < 0.01$.

Transfer Phase of the Associative Learning Test

The ANOVA indicated significant main effects of group, $F(1,40) = 40.76$, $P < 0.0001$, and type of associations (old vs. new associations), $F(1,40) = 61.60$, $P < 0.0001$. There was a significant interaction between group and type of associations, $F(1,40) = 33.16$, $P < 0.0001$. All other main effects and interactions, including the delay phase, were not significant, $F < 1$, $P > 0.5$. Tukey HSD tests revealed that patients with AD were severely impaired in the case of new associations (acquired equivalence) ($P < 0.001$) but not in the case of old associations, $P > 0.5$ (Fig. 2).

Card-pairing Test

The ANOVA indicated significant main effects of group, $F(1,40) = 40.76$, $P < 0.0001$, and type of association, $F(1,40) = 61.6$, $P < 0.0001$. The 2-way interaction between group and type of association was also significant, $F(1,40) = 33.16$, $P < 0.0001$. Tukey HSD tests revealed that patients with AD showed lower performance than the controls in the case of old and new associations ($P < 0.001$) (Fig. 3). This suggests that

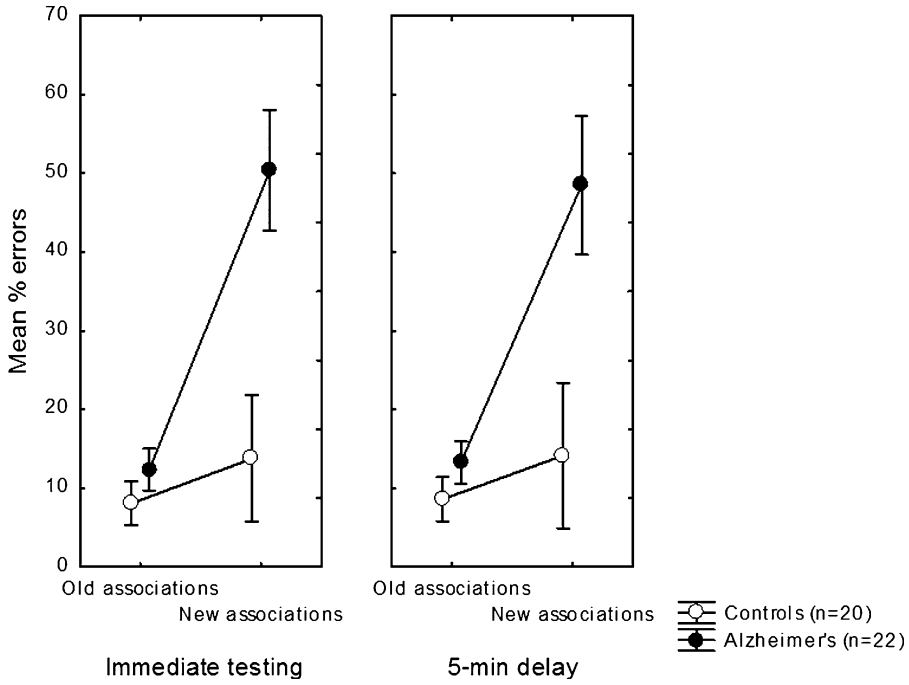

FIGURE 2. Performance in the transfer phase of the task (immediate and delayed testing). Old associations refer to fish-face pairs exposed in the training phase. New associations refer to never trained pairs learned during acquired equivalence. Error bars indicate 95% confidence intervals.

changing the response requirement to card matching resulted in the impairment on the old associations. To confirm this finding, we tested the interaction between group and response requirement. This ANOVA revealed a significant interaction between group and response requirements, $F(1,40) = 26.18$, $P < 0.0001$.

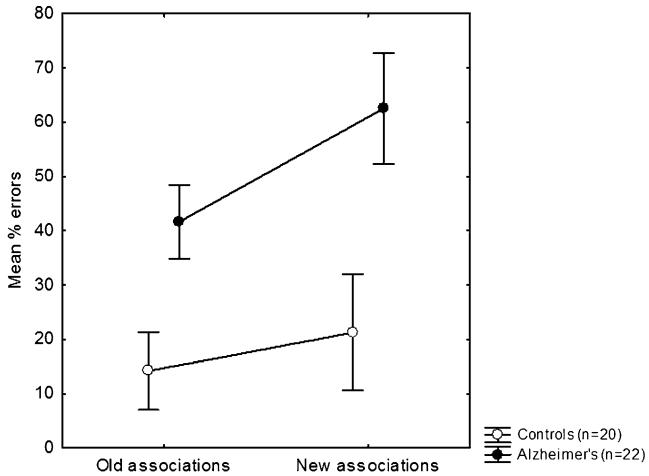

FIGURE 3. Performance in the card selection task. Old associations refer to fish-face pairs exposed in the training phase. New associations refer to never trained pairs learned during acquired equivalence. Error bars indicate 95% confidence intervals.

DISCUSSION

The findings of the present study demonstrate that the deficit of acquired equivalence associative learning is impaired in mild AD, which was characterized by severe declarative memory dysfunctions and better visuospatial and executive functions (Table 2): the patients performed at the chance level in the case of new associations, whereas they were able to learn old associations similarly to controls. However, well-trained old associations, which were correctly retrieved in the computer-assisted forced-choice test, were lost in the card-pairing test in patients with AD. In other words, even though AD patients could perform the old pairs on the computer, they could not perform them flexibly in a different (card) format. This is especially striking because, after the card-pairing test, patients again performed the computer test for old associations and controls. This suggests that the representation of old associations is less flexible in AD patients and cannot be transferred to new retrieval conditions. This feature of old associations is characteristic for habit learning. As proposed by Dickinson,[15] overtraining results in the development of behavior autonomy and to the formation of habits. Converging evidence from animal studies, human neuropsychology, and functional neuroimaging indicates that the basal ganglia play a crucial role in habit formation.[16] Indeed, Myers et al[5] found that patients with Parkinson disease tested on their normal dopaminergic medication failed to learn associations during the training phase or committed a large

number of errors. The most plausible explanation of this phenomenon is that the impairment of the fronto-striatal dopamine system in medicated Parkinson's patients disrupts feedback-guided learning of associations and forming of habits. Intriguingly, those Parkinson's patients who were able to learn the associations showed normal acquired equivalence, that is, their performance was spared in the case of new associations in the transfer phase.[5] This suggests that their intact hippocampal formation made them enable to generalize knowledge.

Our results are consistent with the findings of Eldridge et al[17] who demonstrated intact habit learning in the incremental implicit learning of associations in AD. More recently, Klimkowicz-Mrowiec et al[18] examined implicit habit learning on a probabilistic classification task (weather prediction task). Unexpectedly, these authors found that AD patients with moderate explicit memory impairment performed the task significantly better than those with mild AD and controls. The authors interpret their results as supporting evidence for the hypothesis of competition between declarative (explicit) and procedural (implicit) memory systems in humans. These observations are in good accordance with classic findings demonstrating severely affected declarative memory and relatively preserved procedural memory in AD.[19–22]

However, it is not entirely valid to claim that feedback-guided associative learning is a pure implicit or procedural function (especially in the case of absolute and not probabilistic associations), given that participants make conscious effort to memorize associations at the beginning of the test. Bozoki et al[23] pointed the possibility that, during similar tasks, the comparison of patient and control groups is confounded by the contribution of more than 1 memory systems. Using functional MRI, Johnson et al[24] examined the dynamic neural response during associative learning over trials. Results revealed hippocampal signal attenuation associated with learning in healthy participants, which may indicate that the role of the hippocampal memory system became less evident over trials. Intriguingly, patients with amnestic mild cognitive impairment, a clinical risk condition for AD, did not show such attenuation, which may be a compensatory phenomenon of inefficiently functioning of the hippocampal formation.[24]

A second problem is that neurodegenerative processes often cross the boundary of classic diagnostic categories.[25] Colla et al[26] identified a subgroup of AD patients with altered metabolism in the basal ganglia who showed deficits on the learning of probabilistic associations. Similarly, Ferraro et al[27] demonstrated impaired implicit learning of associations during a serial reaction time task in both AD and Parkinson disease patients. In our study, only 3 AD patients were not able to complete the feedback-guided training phase and, although the completer patients still committed more training errors than controls, their performance was much better than that of patients with Parkinson disease reported in the literature.[5] A related problem is that even in early AD neurodegenerative processes may affect the multimodal association cortex, which may contribute to impaired generalization.

In conclusion, our results suggest that the impairment of acquired equivalence associative learning is selectively impaired in mild AD: whereas feedback-guided associative learning was only mildly affected, AD patients performed at the chance level in the acquired equivalence condition. These data allow new insight into the functioning of the hippocampal complex in early AD and may provide a new tool for the refinement of the neuropsychologic characterization of AD.

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