A total of 140 normal adults participated in one of seven conditions designed to test the hypothesis that memory systems may be distinguished on the basis of their neurobiological substrates. The results revealed a selective disruption of eyeblink classical conditioning (EBCC) when it was performed concurrently with tapping, another cerebellar task. Subjects simultaneously engaged in EBCC and a recognition task or control tasks were relatively unimpaired in EBCC. Results provide evidence for the existence of neurobiologically distinct memory systems, and suggest that the selective disruption of EBCC, when concurrently performed with tapping, may be attributed to cerebellar involvement in both tasks.

Key words: Cerebellum; Classical conditioning; Declarative memory; Dual-task; Hippocampus; Learning; Memory systems; Nondeclarative memory; Timing

Introduction

Memory systems may be distinguished on the basis of their underlying neurobiological substrates. The term declarative has been used to refer to a ‘conscious’ form of memory that relies on the medial temporal lobes. In contrast, nondeclarative refers to a heterogeneous group of ‘unconscious’ memory functions for which the medial temporal lobes are not essential. One form of nondeclarative memory is eyeblink classical conditioning (EBCC). A neutral tone conditioned stimulus (CS) is repeatedly paired with a reflexive corneal airpuff unconditioned stimulus (US). As subjects learn, they begin to blink after presentation of the CS and before onset of the US. This response is called a conditioned response (CR). Evidence from both rabbit and human research has shown that the cerebellum provides the critical substrate for EBCC and the hippocampus is not essential for learning in the delay paradigm. Studies involving amnesic and normal subjects have indicated that subjects are not aware that learning is occurring.

The cerebellum is also critical for tapping tasks requiring subjects to produce timed intervals after presentation of timed stimuli. Subjects with cerebellar damage show deficient tapping compared to control subjects and also show deficits on other tasks requiring timed movements and perception. These findings have led to the supposition that the temporal computation necessary for successful tapping performance may be the same as that which is necessary for successful EBCC performance, and that the cerebellum is involved in both the timing and execution of responses.

In contrast to nondeclarative delay EBCC, for which the hippocampus is not essential, recognition is a declarative memory task that is critically dependent on the hippocampus. Compared with control subjects, patients with damage to the medial temporal lobes show profound deficits in various sensory modalities, as do monkeys with similar lesions. Furthermore, successful recognition performance has been shown to be related to subjects’ awareness of testing procedures.

In the present study, the 400 ms delay EBCC paradigm was used to test the hypothesis that different memory functions rely on different brain memory systems. The role of the cerebellum in EBCC, tapping and timing was also assessed. Subjects were simultaneously engaged in EBCC and either tapping or recognition. Selective disruption of EBCC was predicted for subjects simultaneously engaged in tapping, a task previously shown to depend on the integrity of the cerebellum. EBCC was not expected to be disrupted when subjects were engaged in recognition, a declarative memory task that has been linked to the hippocampus.

Since it was possible that subjects simultaneously engaged in EBCC and tapping or recognition could show EBCC deficits simply as a result of concurrent task performance involving motor responding, a choice reaction time task was included as a control task that presumably would not engage either the cerebellum or the hippocampus. A video viewing group was added as a non-motor control condition.
Three additional control groups were simultaneously engaged in the explicitly unpaired EBCC paradigm and either tapping, recognition, or choice reaction time. In the unpaired EBCC paradigm, the CS and US are not paired. Acquisition of CRs does not occur under such conditions, although subjects still produce a blink following the airpuff US. The purpose of these control groups was to provide baseline secondary task data to determine whether EBCC interfered with performance on these tasks.

Materials and Methods

Using a randomized block procedure, each of 140 young, normal subjects (age 18–29 years) was assigned to one of seven conditions (Fig. 1). The vertical distance between the upper and lower left eyelid was measured (in mm) with a ruler to determine the maximum blink magnitude for data analyses. Subjects were simultaneously engaged in respective tasks (i.e. tapping, recognition, choice reaction time, video viewing) until 90 paired EBCC or explicitly unpaired trials were given. Subjects were told that they would continually hear some tones and feel some airpuffs in their left eye. They were instructed to attend to the respective secondary task. Upon completion of the testing session, subjects were questioned about their awareness of testing procedures and purposes.

**EBCC—paired and explicitly unpaired:** In the 400 ms delay paradigm, a tone conditioned stimulus (CS; 80 dB; 1 kHz; 500 ms duration) was paired with a corneal airpuff unconditioned stimulus (US; 100 ms; 5–7 psi; onset 400 ms after CS onset). In the explicitly unpaired paradigm, either the tone or the airpuff was presented on each trial, but the two stimuli were never paired. A response was considered to be a CR if it occurred between 100 and 400 ms after CS onset. Response latency was the number of milliseconds to the first response that was >1/20 of the maximum blink magnitude. Each 90-trial session was organized into 10 blocks of nine trials for analysis. In the paired EBCC paradigm, the first trial of each block was a CS-alone trial and was not included in the analysis.

**Tapping:** Subjects viewed red LEDs presented at a rate of 500 ms (50 ms duration) and were instructed to tap in synchrony with the stimulus. Once the subject emitted a series of 12 taps, the lights stopped flashing and the subject's task was to continue tapping at the same rate until a message on the monitor indicated the end of the trial. Completion of each trial occurred once the subject emitted a series of 31 unpaced taps. All subjects completed at least 40 trials.

**Recognition:** This task consisted of a battery of word, picture, and digit recognition tests. A 30-word (1.5 s duration, 0.5 s interval) and a 40-picture (2.0 s duration, 0.5 s interval) target list was presented one time prior to recognition testing. Each subsequent test of word recognition consisted of 60 words (30 targets, 30 distractors) and each picture recognition test consisted of 80 pictures (40 targets, 40 distractors). A different series of five digits (1.15 s duration, 0.5 s interval) was presented prior to each series of 20 digit recognition test items (5 targets, 15 distractors). Each digit recognition test consisted of a total of three target lists and 60 test items.

As each test stimulus was presented, subjects indicated whether the presented stimulus was a target stimulus by pressing a 'Yes' or 'No' response key, as appropriate. Stimuli remained on the screen

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**FIG. 1.** Schematic representation of experimental and control groups and rationale for each condition. EBCC=eyeblink classical conditioning; "/" symbolizes dual-task.
until the subject responded. A 1.0 s interval ensued between the subject’s response and presentation of the next work or picture stimulus and a 0.5 s interval ensued for digit recognition. The total recognition battery consisted of 12 subtests presented in the same order for all subjects. All subjects completed at least seven subtests: three word recognition, three digit recognition and one picture recognition.

Choice reaction time: The test consisted of 14 60-trial (30 ‘Yes’, 30 ‘No’) choice reaction time subtests. Stimuli were presented at random with a 1.0–3.5 s response-presentation interval. Subjects were instructed to press the ‘Yes’ or ‘No’ response key, as appropriate, as quickly as possible. The apparatus was the same as that used for the recognition task. All subjects completed at least seven choice reaction time subtests.

Video: The video was entitled ‘Great Escapes’, and illustrated wildlife action sequences. Subjects were told that they would later be tested on their knowledge about information presented in the film, although, in reality, they were not tested.

Results

The results indicated a selective disruption of EBCC in subjects simultaneously engaged in the tapping task (Fig. 2). A multivariate analysis of variance (MANOVA) including percentage of CRs and response latency showed that subjects who simultaneously completed the cerebellar tapping task were impaired in EBCC compared with subjects who simultaneously completed the hippocampal recogni-

![Graph A](image1.png)

**Fig. 2.** The mean percentage of CRs (conditioned responses; A) and response latency (B) across 10 blocks of eight paired conditioned stimulus-unconditioned stimulus eyelid conditioned classical conditioning (EBCC) trials. The dashed horizontal line (B) illustrates the US (unconditioned stimulus) onset at 400 ms. Responses occurring prior to 400 ms are indicative of CRs. For both panels, subjects ($n=20$; $n=80$) simultaneously performed EBCC and tapping (●), recognition (△), choice reaction time (▲), video viewing (□).

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lyses indicated that the groups differed in reaction time during some parts of the session, F(1, 38) = 4.14, p < 0.05. Responses on the choice reaction time task were slower by 34.3 ± 8.72 ms for those subjects who underwent conditioning compared with those who received unpaired tones and airpuffs. Subsequent post hoc EBCC analyses indicated that subjects who simultaneously performed the choice reaction time task were impaired during the first three EBCC blocks compared to control subjects, and performed similarly to subjects in the tapping group (p < 0.05; see Fig. 2). However, by the fourth block, subjects in this group performed better than subjects in the tapping group and similarly to subjects in the other two groups (p < 0.05).

Discussion

The results support the existence of neurobiologically distinct memory systems. Studies with both brain-damaged\(^5,6\) and normal\(^13\) populations have revealed dissociations between nondeclarative EBCC and declarative memory. The present study provides further evidence of neurobiologically distinct memory systems by showing that simultaneous activation of presumed declarative and nondeclarative memory systems is possible, and does not result in deterioration in performance of either task. In contrast, simultaneous engagement of overlapping neural substrates did result in task interference.

Estimates of the mean number of responses emitted by subjects in each group were calculated to determine whether these differences could account for the results. Subjects in the tapping group responded the most (1720 taps), followed by the recognition group (440 responses), the choice reaction time group (420 responses), and the video group (no manual responding required). If the number of manual responses could account for the present results, then subjects in the video group should have shown optimal performance, followed by the choice reaction time and recognition groups. However, these three groups did not perform differentially.

The results obtained cannot be attributed to factors of awareness, nor can they be explained by a resource allocation model. Comments made during a post-procedural interview suggested that the tasks were not equally difficult or engaging. The recognition task seemed to be most challenging and interesting, followed by the video, which most subjects described as enjoyable and engaging. Subjects generally got bored with tapping and choice reaction time by the end of the session. If a general resource model could account for these data, subjects in the recognition group should have been most impaired in EBCC since the recognition test presumably required the most attentional and effortful resources.

The sometimes slowed choice reaction times of subjects simultaneously engaged in EBCC suggests that the cerebellum may also have been involved in this task. A few researchers have suggested that the cerebellum may be involved in the cognitive component of reaction time performance.\(^{23}\) One interpretation of the present results is that the cerebellum may have been engaged during initial choice reaction time engagement, when performance may have required greater attention or effort.

This unexpected result of cerebellar involvement in choice reaction time coupled with the expected selective disruption of EBCC during concurrent tapping implicate the cerebellum as comprising a brain memory system. Thompson has proposed that the circuitry for discrete, adaptive learned motor responses resides in the cerebellum.\(^{22}\) Studies utilizing positron emission topography revealed significant activation in the inferior cerebellar cortex and deep nuclei of adults engaged in EBCC\(^{24}\) and deactivation in ipsilateral cerebellum attributed to decreases in Purkinje cell activity.\(^{25}\) It is, therefore, possible that engaging some of the cerebellar circuitry in the tapping task resulted in impaired EBCC.

EBCC not only requires that an association be formed, but also that the conditioned response be executed at a precise point in time in order to be adaptive. Both the perception of timed stimuli and the execution of timed movements have been linked to the cerebellum.\(^{14-16}\) The need for precise timing (and, hence, the cerebellum) on the repetitive tapping task may have interfered with the establishment of the temporal relationship between the tone and airpuff. It is unclear why interference was not found on the
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tapping task. One possible explanation is that the tapping task was treated as primary, and the effects of resource sharing of a timing mechanism were restricted to ‘secondary’ EBCC.

Conclusion

Presumed simultaneous activation of different brain memory systems did not result in respective task interference whereas simultaneous activation of similar brain regions did result in task interference. The results implicate the existence of distinct neurobiologically memory systems and support the critical role of the cerebellum in EBCC and other functions of timing.

ACKNOWLEDGEMENTS: The authors gratefully acknowledge Keith Wescott and Pauline Simpson of Cognitive Drug Research, Ltd, for providing the recognition and choice reaction time tasks. We especially thank Claire Pincock for assisting in both the management and interpretation of these data. Supported by grant 1 RO1 AG03732 awarded to D.S.W. from the National Institute of Aging and grant 1R29 NS20256 awarded to R.B. from the National Institute of Neurological Disorders and Stroke.

Received 26 April 1995; accepted 3 May 1995

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