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Trace Eyeblink Conditioning in Abstinent Alcoholic Individuals: Effects of Complex Task Demands and Prior Conditioning

Regina E. McGlinchey
Veterans Affairs Boston Healthcare System, Harvard University Medical School, and Boston University School of Medicine

Catherine B. Fortier
Veterans Affairs Boston Healthcare System and Boston University School of Medicine

Stephen M. Capozzi
Boston University School of Medicine

John F. Disterhoft
Feinberg School of Medicine, Northwestern University

Chronic misuse of alcohol affects an integrated neural circuit supporting the formation of associative memories acquired during eyeblink classical conditioning (R. McGlinchey-Berroth et al., 1995). The authors of this study investigated single-cue trace conditioning in amnesic and nonamnesic abstinent alcoholic individuals who either were or were not trained in a single-cue delay conditioning task. Overall, untrained alcoholic participants were severely impaired in acquisition, and alcoholic participants previously trained in single-cue delay conditioning performed similarly to untrained control participants. Individual performance in acquisition varied significantly within task but was relatively stable between the trace and delay tasks; there were nonamnesic and amnesic alcoholic participants who acquired responses at a normal rate in both delay and trace conditioning. The similarity of performances in delay and trace conditioning suggests a common source of impairment across both tasks.

Keywords: memory, associative learning, alcoholism

Chronic misuse of alcohol leads to characteristic changes in the brain that have been revealed with postmortem analyses and in vivo neuroimaging techniques. Such changes include atrophy of the cerebral cortex, cerebellum, and brain stem, as well as changes in the white matter underlying the cerebral cortex (De la Monte, 1988).

Certain brain regions impacted by the effects of alcoholism have been identified as belonging to an integrated neural circuit that supports the formation of new memory traces in eyeblink classical conditioning of associative relationships. In classical conditioning, learning is expressed as a conditioned response (CR) to a previously neutral but predictive conditioned stimulus (CS) that is paired over a number of trials with an aversive unconditioned stimulus (US). In its most basic form, single-cue delay conditioning, the CS and US overlap in time and terminate simultaneously. Given what is known regarding the neuropathological changes resulting from chronic alcohol misuse, it follows that individuals with alcoholism may show impairments in their ability to acquire and express associative relationships using the eyeblink classical conditioning model. Indeed, as we review below, we have documented acquisition deficits and alterations in the temporal dynamics of CRs in past delay eyeblink conditioning studies conducted with abstinent chronic alcoholic individuals (McGlinchey-Berroth et al., 1995; McGlinchey-Berroth, Fortier, Cermak, & Disterhoft, 2002). Our primary aims in the current study were (a) to further define the nature of the associative learning deficit in abstinent chronic alcoholic individuals using a "nonoptimal" classical conditioning trace paradigm and (b) to determine whether prior training in classical conditioning might help to ameliorate the acquisition deficit and timing alterations, which may provide a window into the critical underlying neuronal cause of the deficits.

Regina E. McGlinchey, Geriatric, Research, Education, and Clinical Center, Veterans Affairs Boston Healthcare System, Boston; Department of Psychiatry, Harvard University Medical School; and Memory Disorders Research Center, Boston University School of Medicine. Catherine B. Fortier, Geriatric, Research, Education, and Clinical Center, Veterans Affairs Boston Healthcare System, and Memory Disorders Research Center, Boston University School of Medicine. Stephen M. Capozzi, Memory Disorders Research Center, Boston University School of Medicine. John F. Disterhoft, Department of Physiology and Institute for Neuroscience, Feinberg School of Medicine, Northwestern University.

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Correspondence concerning this article should be addressed to Regina E. McGlinchey, Geriatric, Research, Education, and Clinical Center (182), Building 9, Veterans Affairs Boston Healthcare System, 150 South Huntington Avenue, Boston, MA 02130. E-mail: rmcglin@bu.edu

Eyeblink Classical Associative Learning: Neuronal Circuit and Mechanisms

Associative learning is a form of learning in which there is a relatively permanent change in behavior as the result of a temporal conjunction of two events (Lashley, 1916). One important paradigm for studying associative learning is the Pavlovian or classical conditioning model. Pavlov viewed the CR as a tool for uncovering the neural mechanisms of associative learning, suggesting that it be used to uncover the rules that govern an animal's behavioral adaptation to environmental contingencies (Pavlov, 1927). Indeed, the power of using classical conditioning in the study of human learning and memory formation and expression is that the neural basis of such learning is extremely well mapped out in animal models that have used a variety of response systems. Evidence indicates that the circuitry defined in animals is directly applicable

to the study of learning and memory in humans (see Maren, 2001; Woodruff-Pak & Steinmetz, 2000).

Variations of two primary tasks are typically used to investigate eyeblink classical conditioning. The most basic is the single-cue delay paradigm, in which the CS (e.g., tone) is presented, followed by the US (e.g., airpuff), and both stimuli terminate simultaneously. Another is the single-cue trace paradigm, which introduces a temporal gap (silent period) between the CS and the US. The trace conditioning paradigm is considered a nonoptimal or a more complex and demanding associative task because of the temporal separation between the CS and the US. This gap requires the formation of an abstract link or a conjoined representation between the two stimuli in order for learning to occur.

Thompson and his colleagues have extensively studied the neural substrates of eyeblink conditioning in animals (rabbits) in an effort to identify the essential memory trace circuits in the mammalian brain (e.g., R. F. Thompson, 1986). The cerebellum is essential for eyeblink conditioning; the deep cerebellar nuclei and overlying cerebellar cortex mediate both the acquisition (Raymond, Lisberger, & Mauk, 1996) and storage (Kim & Thompson, 1997) of memories involving an eyeblink CR. In the context of a delay eyeblink conditioning task (for a complete discussion, see Medina, Repa, Mauk, & LeDoux, 2002), the CS (e.g., tone) activates mossy fibers originating in the pontine nuclei that then synapse onto the output cells of the deep cerebellar nuclei (interpositus nuclei in the rabbit) and the Purkinje cells of the cerebellar cortex. At the onset of the US (e.g., airpuff), activation spreads from the inferior olivary nucleus and converges on the same deep cerebellar nuclei and Purkinje cells in the cerebellar cortex as the CS signal. In fact, it has been shown that the convergence of the CS and the US, both in the deep cerebellar nuclei and in the Purkinje cells, occurs at the level of individual neurons (Berthier & Moore, 1986; Gould, Sears, & Steinmetz, 1993; McCormick & Thompson, 1984). Signals emanating from efferent pathways in the deep cerebellar nuclei to the red nucleus and facial nucleus produce the eyeblink CR.

Alcohol is known to cause structural alterations in the cerebellum. Such alterations have been documented by traditional postmortem inspection (e.g., Harper & Kril, 1989) and, more recently, by in vivo neuroimaging studies confirming significant volume shrinkage in the cerebellar hemispheres of men with alcoholism (Sullivan, Deshmukh, Desmond, Lim, & Pfefferbaum, 2000). Victor and colleagues (Victor, Adams, & Collins, 1971; Victor, Adams, & Mancell, 1959) originally noted superior cerebellar vermian atrophy as a consequence of alcoholism, specifically a loss of Purkinje neurons, especially in individuals with concomitant thiamine deficiency. In addition, animal models have recently revealed alcohol-related dendritic regression within Purkinje neurons (Dlugos & Pentney, 2002) that appears to be reversible following ethanol withdrawal (Dlugos & Pentney, 1997). The processes involved in dendritic regression and subsequent reconstruction are highly complex, and their effects on neuronal processing downstream are completely unknown (Sullivan et al., 2003).

Given the essential role of the cerebellum in eyeblink classical conditioning, together with the neuropathological effects of alcohol on this region of the brain, one would correctly expect that individuals with chronic alcoholism would show deficits in classical associative learning. In an initial investigation, we compared the performance of amnesic Korsakoff's patients and abstinent chronic alcoholic participants to that of healthy control participants in a single-cue delay eyeblink conditioning task (McGlinchey-Berroth et al., 1995). Healthy control participants exhibited acquisition of CRs to a tone CS following repeated pairings with an airpuff US and demonstrated extinction of CRs when the CS was subsequently presented alone. In contrast, both the amnesic Korsakoff's patients and abstinent alcoholic participants demonstrated an impaired ability to acquire CRs, with the Korsakoff's patients demonstrating no acquisition relative to an unpaired CS–US baseline. We concluded that abnormalities in delay eyeblink conditioning must depend on the underlying neuropathology of alcoholism, whether or not amnesia is a presenting symptom. Further, we proposed that, because patients with amnesia due to medial temporal lobe pathology have preserved single-cue delay conditioning (Gabrieli et al., 1995), the impairments of our alcoholic and Korsakoff's participants were most likely due to the specific effects of alcohol. In particular, one might speculate that the impairment in learning may be related to the extent of deep cerebellar nuclear cell loss due to chronic alcoholism. Support for this notion comes from studies by Goodlett and colleagues (Green, Johnson, Goodlett, & Steinmetz, 2002), who found that neonatal exposure to ethanol in rats resulted in reduced levels of eyeblink conditioning acquisition and delayed activation of interpositus nucleus. They suggested that the slower than normal activation of interpositus neurons was probably the result of these rats having fewer interpositus nucleus neurons available to become plastic. Specifically, this group reported that ethanol-treated neonates had approximately 50% fewer deep nuclear cerebellar cells compared with control rats and that the number of deep nuclear cells was highly correlated with acquisition of eyeblink conditioning (Green, Tran, Steinmetz, & Goodlett, 2002).

In a second study, we used a more complex two-cue delay temporal discrimination paradigm (McGlinchey-Berroth et al., 2002). In this task, two clearly distinguishable tones of different durations were randomly presented and signaled the impending onset of the coterminating US airpuff. The task was to appropriately time (i.e., discriminate) the onset of the CR as a function of tone frequency, which predicted tone duration. This study used only abstinent alcoholic and matched control participants. In addition to replicating our earlier finding that abstinent alcoholic individuals are impaired in CR acquisition in a delay eyeblink conditioning paradigm, the data from the temporal discrimination task revealed a significant alteration in the alcoholic participants' peak latency measure at the longer of the two tone durations. This indicated that the alcoholic participants produced shorter latency CRs at this interval. We speculated that the peak latency alteration may have been caused by cerebellar cortical shrinkage due to years of alcohol abuse (Ivry & Keele, 1989; Ivry, Keele, & Diener, 1988) and that pathology extending into deep cerebellar nuclei caused the overall impairment in CR acquisition.

Trace Eyeblink Conditioning in Individuals With Alcoholism

As we reviewed, the cerebellum is essential for learning in all tasks of eyeblink classical conditioning. However, the circuitry described above is only part of a more extensive integrated pathway that is involved in relatively complex forms of eyeblink

conditioning. This pathway represents the cerebellar–pontine–prefrontal system described by Schmahmann and Pandya (1997). This system contains projections that are both *feedforward* (from the cerebral cortex to the cerebellum via the pontine nuclei) and *feedback* (from the cerebellar cortex to the deep cerebellar nuclei that project to the cerebral cortex via the red nucleus and the thalamus). Neuroanatomical evidence of this circuit was originally provided by Middleton and Strick (1994), who traced connections between the cerebellum and medial prefrontal cortex via the mediodorsal nucleus of the thalamus in primates. There is now abundant evidence indicating that the pathological effects of alcoholism on the brain are far-reaching and extend into the prefrontal cortex. The impact of alcohol on the frontocerebellar system and cognition has received considerable attention in recent years and was the subject of an interdisciplinary symposium (Sullivan et al., 2003).

Early postmortem evidence from Harper, Kril, and Daly (1987) showed a 22% reduction in the number of neurons in the superior frontal cortex of individuals with alcoholism. Of particular interest was that alcoholic and control participants did not differ with respect to primary motor, cingulate, or inferior temporal brain regions. Jernigan et al. (1991), using MRI, also found greater volume losses in the frontal lobes compared with other structures. Harper and Kril (1989) suggested that much of this pathology is due to a selective loss of large pyramidal cells. Support for the suggestion was provided by further studies (Kril, Halliday, Svoboda, & Cartwright, 1997) showing selective neuronal loss in the superior frontal cortex and by white matter changes as revealed by diffusion tensor imaging measures when controlling for age and alcohol consumption (Pfefferbaum & Sullivan, 2002; Pfefferbaum et al., 2000). Such profound changes may only occur in individuals with relatively severe alcoholism who find themselves in clinical treatment settings. Fein et al. (2002) more recently suggested that the neuronal loss previously reported might reflect a sample bias, because the studies typically include alcoholic individuals in treatment. Their data with treatment-naive individuals showed reductions in cortical gray matter volume (prefrontal and parietal regions) but no white matter volume loss.

The notion of a cerebellar–thalamic–prefrontal cortex module controlling eyeblink associative learning was proposed initially by Weiss and Disterhoft (1996) and, most recently, by McLaughlin, Powell, and White (2002). Indeed, Weible, McEchron, and Disterhoft (2000) have shown that regions of the medial prefrontal cortex in rabbits (roughly analogous to the anterior cingulate in primates) are essential for acquisition in trace eyeblink conditioning. A similar finding was reported by McLaughlin, Skaggs, et al. (2002), who found that lesions of the medial prefrontal cortex in rabbits retarded acquisition in the trace but not in the delay eyeblink conditioning task. Weible et al. (2000) further reported that lesions in the medial prefrontal cortex (the rostral anterior cingulate in primates) severely impaired the rabbits' ability to extinguish CRs. In an even more challenging task, Chachich and Powell (1998) demonstrated that prefrontal lesions impaired the ability to reverse a previously learned discrimination.

Myers et al. (2001) reported that in humans, basal forebrain damage produces a deficit in delay eyeblink conditioning, a finding that may represent a departure from animal studies indicating no effect of frontal lesions on delay conditioning. However, we have reported that patients with frontal lesions that include the anterior cingulate are unable to acquire CRs in a trace conditioning task (Capozzi, Fortier, Disterhoft, & McGlinchey-Berroth, 2002) and are impaired in delay conditioning compared with healthy control participants. Ramnani, Toni, Josephs, Ashburner, and Passingham (2000) reported learning-related activation of the anterior cingulate while using functional MRI during an eyeblink delay discrimination task. Using positron emission tomography, McIntosh, Rajah, and Lobaugh (1999) found that regional cerebral blood flow in the left prefrontal cortex showed learning-dependent changes during a visual discrimination conditioning task. In another study, Schreurs, Bahro, Molchan, Sunderland, and McIntosh (2001) reported that older adult participants did not condition as well as younger ones, and older adults had attenuated regional cerebral blood flow. Also, only younger participants had strong left prefrontal connectivity with the cerebellum, the hippocampus, the thalamus, and the temporal cortex.

This leads to the question of whether individuals with chronic alcoholism can learn to associate two stimuli in the context of a nonoptimal trace eyeblink conditioning paradigm. On the basis of the neurologic evidence, one would predict impairment that is due either to cerebellar degeneration or to frontal lobe atrophy and changes in the underlying white matter. The question is how to tease apart these possible contributing factors. In the current investigation, we approached this problem through an informal comparison of the performance of abstinent alcoholic participants in trace eyeblink conditioning with the performance of abstinent alcoholic participants in delay conditioning reported in our previous investigation (McGlinchey-Berroth et al., 1995). Recall that in our earlier study, the abstinent alcoholic participants were significantly impaired, but they were able to acquire CRs in delay conditioning beyond a baseline level (i.e., the percentage of CRs during unpaired CS–US trials). We propose that any additional impairment observed in the current trace conditioning study likely arose from neuropathological changes to the forebrain regions, given the demonstrated involvement of forebrain structures in nonoptimal associative learning paradigms. Although not definitive, this informal comparison provides evidence that may justify further extensive analysis of trace conditioning in abstinent alcoholic individuals that could more directly link learning impairments to underlying brain changes (such as neuroimaging studies).

Our secondary aim in this study was to examine the effect(s) of prior training on the acquisition and expression of CRs in abstinent alcoholic individuals. Approximately half of the participants in this study were participants in our previous investigation with single-cue delay conditioning. This provided the opportunity to examine whether any observed impairments in CR acquisition and/or expression in trace conditioning (in previously untrained abstinent alcoholic participants) would be ameliorated in abstinent alcoholic participants who had prior training in delay associative learning before participating in the nonoptimal trace conditioning task.

Method

Participants

Five groups of individuals participated in this experiment. The groups were composed of 6 male Korsakoff's (KOS) patients, 12 trained abstinent alcoholic (T-AC) participants who had previously participated in a delay conditioning paradigm (McGlinchey-Berroth et al., 1995), 12 untrained abstinent alcoholic (U-AC) participants who had no prior training in

eyeblink conditioning, 12 trained healthy control (T-HC) participants who had previously participated in a delay conditioning paradigm (McGlinchey-Berroth et al., 1995), and 12 untrained healthy control (U-HC) participants who had no prior training in eyeblink conditioning. The trained participants were tested in two sessions (one delay, one trace conditioning) that were separated by at least 2 months and up to 3 years, and the untrained participants were tested in one session (trace conditioning). All of the groups were matched for age. Note that although some of the trained participants were tested several years ago, the experimental procedures, testing apparatus, and experimental testing room were the same for all study participants.

The participants were recruited from the Memory Disorders Research Center (MDRC) of the Boston University School of Medicine and the Veterans Affairs Boston Healthcare System by distribution of flyers at local institutions, by advertisements in local newspapers, and by referrals from the Harvard Cooperative Program on Aging and area hospitals. Abstinent alcoholic participants and healthy control participants were screened to be free of any neurologic disease or illness. Amnesic KOS patients were recruited from area hospitals and referred to the MDRC by a neurologist.

Amnesic KOS patients. Six amnesic male KOS patients were tested. All of the patients were diagnosed by the Neurology Service of the Veterans Affairs Boston Healthcare System or by neurologist Michael Alexander of the MDRC and Behavioral Neurology Department, Beth Israel Deaconess Hospital, Boston, Massachusetts. All patients were tested at least 1 year after detoxification and clearing of their acute encephalopathy. Structural MRI scans were available for 3 of the 6 KOS patients. Each scan indicated mild to moderate central and peripheral cortical atrophy. Cerebellar shrinkage was specifically noted in 1 patient. A computed tomography scan was available for 1 additional patient and indicated only cerebellar shrinkage.

Neuropsychological data for the patients with alcoholic KOS amnesia are presented in Table 1. The patients' intellectual levels were within normal limits, as indicated by scores that were within one standard deviation of the means for their age groups on the Wechsler Adult Intelligence Scale—Revised (WAIS–R; Wechsler, 1991) Full Scale IQ ($M = 103$, $SE = 3.34$) and the Wechsler Memory Scale—Revised (WMS–R; Wechsler, 1987) Attention indexes ($M = 94$, $SE = 7.05$). However, these patients were severely impaired in their memory function, as indicated by their performance on the WMS–R Delayed Memory index ($M = 57$, $SE = 3.26$) and the Warrington Recognition Test (Warrington, 1984; Words: $M = 32$, $SE = 1.31$; Faces: $M = 32$, $SE = 1.99$).

T-AC participants. Twelve T-AC individuals were tested, all of whom were men. All of the T-AC participants had abstained from drinking for at least 3 months prior to participating in the study. T-AC participants reported a significant history of alcohol abuse that ranged from 5 to 40 years. The mean length of abuse was 22 years ($SE = 2.88$). On the Short Michigan Alcoholism Screening Test (SMAST; Selzer, Vinokur, & van Rooijen, 1975), a self-reported measure of alcoholic behavior, T-AC participants reported scores ranging from 5.00 to 13.00, with a mean score of 9.00 ($SE = 0.77$). Selzer et al. (1975) suggested that a score of 0–1.00 on the SMAST represents a nonalcoholic profile, a score of 2.00 indicates a possible alcoholic profile, and a score of 3.00 or higher represents an alcoholic profile.

Summary demographic and neuropsychological characteristics of the T-AC participants are presented in Table 2. It is clear from these data that all of the participants had preserved intellectual function, as indicated by their Verbal IQ score ($M = 103$, $SE = 3.76$) on the WAIS–R. In addition, all participants scored within normal limits on tasks of attention and acquisition, retention, and retrieval of verbal and nonverbal material, as assessed by the WMS–R Attention ($M = 103$, $SE = 4.08$), General Memory ($M = 112$, $SE = 4.95$), and Delayed Memory ($M = 109$, $SE = 5.19$) indexes.

U-AC participants. Twelve U-AC individuals were tested, including 2 women and 10 men. All of the U-AC participants had abstained from drinking for at least 3 months prior to participating in the study. U-AC participants reported a significant history of alcohol abuse that ranged from 3 to 25 years. The mean length of abuse was 15 years ($SE = 2.02$). As assessed with the SMAST, U-AC participants reported alcoholic profiles ranging from 7.00 to 13.00, with a mean score of 9.10 ($SE = 0.67$).

Summary demographic and neuropsychological characteristics of the U-AC participants are presented in Table 2. It is clear from these data that all of the participants had preserved intellectual function, as indicated by their Verbal IQ score ($M = 103$, $SE = 3.64$) on the WAIS–R. All participants scored within normal limits on tasks of attention and acquisition, retention, and retrieval of verbal and nonverbal material, as assessed by the WMS–R Attention ($M = 100$, $SE = 5.08$), General Memory ($M = 112$, $SE = 4.69$), and Delayed Memory ($M = 106$, $SE = 5.65$) scores.

T-HC participants. Twelve T-HC participants (5 men, 7 women) were tested. Summary demographic and neuropsychological characteristics of the T-HC participants are presented in Table 2.

U-HC participants. Twelve U-HC participants (3 men, 9 women) were tested. Summary demographic and neuropsychological characteristics of the U-HC participants are presented in Table 2.

Three one-way analyses of variance (ANOVAs) were performed to examine the between-subjects effect of group for the dependent measures of age, education, and verbal intelligence. Group was not significant for age, $F(4, 49) = 0.89$. Group was significant for education, $F(4, 49) = 7.69$, $p = .01$. Means comparison analyses revealed that the U-HC participants had significantly more education than the other four groups ($ps < .05$; see Table 2). In addition, T-HC participants had significantly more education

Table 1
Demographic and Neuropsychological Characteristics of Amnesic Korsakoff's Patients

Patient	Age (years)	Education (years)	WAIS–R: Verbal IQ	WMS–R			Warrington	
				General Memory	Attention	Delayed Memory	Words	Faces
1	67	9	96	76	109	62	37	30
2	60	9	97	99	99	61	31	34
3	65	12	108	66	99	50	29	25
4	51	12	95	84	64	50	28	35
5	54	12	112	65	83	51	31	30
6	75	14	112	88	108	69	33	39
M	62.00	11.33	103.33	79.67	93.67	57.17	31.50	32.17
SE	3.61	0.80	3.34	5.41	7.05	3.26	1.31	1.99

Note. The WAIS–R and the WMS–R scaled scores yield a normalized, age-adjusted mean of 100. The WMS–R does not provide scores below 50. WAIS–R = Wechsler Adult Intelligence Scale—Revised; WMS–R = Wechsler Memory Scale—Revised; Warrington = Warrington Recognition Test.

Table 2
Demographic and Neuropsychological Characteristics for KOS Patients, Abstinent Alcoholic Participants, and Healthy Control Participants

Group	Age (years)	Education (years)	WAIS–R: Verbal IQ	WAIS–III: Verbal IQ	WMS–R			SMAST	Approximate Duration (years)	
					General Memory	Attention	Delayed Memory		Alcohol Abuse	Sobriety
KOS	62.00 (3.61)	11.33 (3.34)	103.33 (3.34)		79.67 (5.41)	93.67 (7.05)	57.17 (3.26)			
T-AC	59.50 (2.80)	12.17 (.32)	103.33 (3.76)		111.55[a] (4.95)	103.09[a] (4.08)	109.46[a] (5.19)	9.00[b] (.77)	21.92 (2.88)	8.33 (1.99)
U-AC	56.42 (2.21)	12.92 (.77)	102.50 (3.64)		111.83 (4.69)	99.92 (5.08)	105.67 (5.65)	9.10[b] (.67)	15.08 (2.02)	8.82 (1.91)
T-HC	53.92 (4.90)	14.33 (.68)	101.83 (2.41)							
U-HC	62.58 (4.66)	16.50 (.85)	119.00[c] (5.00)	125.33[c] (4.09)						

Note. The control participants were not tested on the WMS–R. Standard errors of the means are presented in parentheses. The WAIS–R, WAIS–III (Wechsler, 1997), and WMS–R scaled scores yield a normalized, age-adjusted mean of 100. The WMS–R does not provide scores below 50. KOS = Korsakoff's patients; WAIS–R = Wechsler Adult Intelligence Scale—Revised; WAIS–III = Wechsler Adult Intelligence Scale—Third edition; WMS–R = Wechsler Memory Scale—Revised; SMAST = Short Michigan Alcoholism Screening Test; T-AC = trained abstinent alcoholic; U-AC = untrained abstinent alcoholic; T-HC = trained healthy control; U-HC = untrained healthy control.
[a] WMS–R data were unavailable for 1 T-AC participant. [b] SMAST scores were unavailable for 1 T-AC and 2 U-AC participants. [c] Two of the 12 U-HC participants were administered the WAIS–R as an estimate of Verbal IQ. Nine of the 12 U-HC participants were administered the WAIS–III as an estimate of Verbal IQ. A Verbal IQ measure was unavailable for 1 U-HC participant.

than T-AC participants ($p = .03$) and KOS patients ($p = .01$). There were no other differences between groups. Group was also significant for Verbal IQ, $F(4, 48) = 8.05$, $p = .01$. Means comparison analyses revealed that the U-HC participants had significantly higher Verbal IQ scores than did the other four groups ($ps < .05$; see Table 2). There were no other differences between groups.

Apparatus

The apparatus was a modified version of that used for eyeblink conditioning in the rabbit (Akase, Thompson, & Disterhoft, 1994; L. T. Thompson, Moyer, Akase, & Disterhoft, 1994) and one that we have used in previous eyeblink conditioning studies with humans (Fortier, Disterhoft, Capozzi, & McGlinchey, 2003; Fortier, Disterhoft, & McGlinchey-Berroth, 2000; Gabrieli et al., 1995; McGlinchey-Berroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997; McGlinchey-Berroth et al., 1995, 2002). Eyeblink movements were monitored with an infrared diode/phototransistor aimed at the right eye. This device monitors and amplifies light reflectance from the cornea in a 0–5 volts direct current range, which is then digitized and stored by the computer. In this system, eyeblink amplitude is an inverse function of the amount of reflected light contacting the photo transistor aimed at the cornea. The detector was adjusted so that the baseline of 1 V occurred when the eye was open, and the highest amplitude possible of 5 V occurred when the eye was fully closed. The detector and the airpuff delivery nozzle were attached to an adjustable arm that was mounted on a headband worn by the participants.

Stimuli

The CS was an 85-dB, 1-kHz tone that was delivered binaurally over earphones for 100 ms. The US was a 100-ms corneal airpuff delivered to the right eye that followed the CS after a silent trace period of 500 ms. The magnitude of the airpuff was 3 psi for all participants.

Procedure

Participants were brought into the laboratory individually, where the examiner reviewed the informed consent form with them. After providing consent, they were seated in an upright chair, and the examiner fitted them with the eyeblink apparatus. Throughout the session, the experimenter sat in the same room (out of the direct view of the participant) and answered questions as they arose. The experimenter read the following instructions:

> Please listen carefully to the following instructions. Remain seated comfortably and look straight ahead, avoiding all eye movements such as looking around the room. Please do not touch the headband or earphones at any time during the experiment, yet if you feel uncomfortable or feel you need to adjust anything, please let me know, and I will stop the experiment to make any adjustments.
>
> You will hear and feel a series of stimuli during the session. These stimuli will consist of some tones or beeps and a light puff of air. Please feel free to blink whenever you want. All you are asked to do is to concentrate on what is going on and let your natural reactions take over.

Each conditioning session consisted of a total of 60 conditioning trials followed by 30 extinction trials. During extinction, the CS was presented alone. Prior to the onset of each trial, there was a 750-ms baseline recording period. The intertrial interval during conditioning and extinction averaged 10 s but varied randomly from 8 to 12 s.

Definitions

An eyeblink was scored as a CR only if it was four standard deviations greater than the mean baseline response amplitude. Eyeblinks with a

latency less than 100 ms following CS onset were recorded as alpha responses and were not considered CRs (Gormezano, 1966). Spontaneous eyeblink rate was measured in the time period prior to the onset of the CS.

Results

The primary dependent measures of interest were the percentage of trials on which a CR occurred, the mean CR onset latency, and the mean CR peak latency. These measures reflect the level of acquisition and the temporal dynamics of CR expression. Other measures included the amplitude of the unconditioned response (UR) to the airpuff, the number of spontaneous eyeblinks, and the number of alpha, or short latency, responses to the CS. Our measures of the CR and UR topography are based solely on paired CS–US trials. CS-alone and US-alone trials were not presented. We conducted a preliminary ANOVA to examine performance in trace conditioning comparing trained individuals who had participated in our published delay study (McGlinchey-Berroth et al., 1995) with trained individuals who underwent identical delay conditioning procedures but were tested subsequent to that original report. No significant differences were observed in acquisition, $F(1, 22) = 1.89$; CR onset latency, $F(1, 22) = 0.43$; or CR peak latency, $F(1, 22) = 2.26$, indicating that the two groups of previously trained participants were roughly equivalent in conditioning performance.

CR Acquisition

We analyzed acquisition using an ANOVA with the overall mean percentage of CRs as the dependent measure and group as a between-subjects factor. We conducted all post hoc analyses using Fisher's protected least significant difference, with alpha set at .05. The group effect was found to be significant, $F(4, 49) = 9.42$, $p < .01$. As depicted in Figure 1, KOS patients and U-AC participants produced fewer CRs than did all other groups ($ps < .01$). There was no difference in the mean percentage of CRs between the KOS patients and the U-AC participants and no differences between the U-HC, T-AC, and T-HC participants.

The overall mean percentage of CRs was broken out into six blocks of 10 trials each and analyzed using an ANOVA with block as a within-subjects factor and group as a between-subjects factor. Figure 2 displays the learning curves for each group as a function of block. In addition to the main effect of group, $F(4, 49) = 9.34$, $p < .01$, there was also a main effect of block, $F(4, 49) = 7.98$, $p < .01$, indicating a general increase in the percentage of CRs across trials. The interaction term was not significant ($p > .47$). Because acquisition levels were relatively high in the first block, in Table 3 we display the mean occurrence of CRs for each trial in the block to clearly show the gradual onset of learning.

The mean percentage of CRs for the U-AC participants in the current single-cue trace conditioning study was remarkably similar to the performance of a comparable group in a single-cue delay conditioning task. In our prior study (McGlinchey-Berroth et al., 1995), abstinent alcoholic participants (who were untrained) acquired a mean percentage of CRs of 27.50 ($SD = 20.16\%$, range = 8.30%–65.00%). In the current study, the U-AC participants acquired a mean percentage of CRs of 28.60 ($SD = 20.90\%$, range = 3.30%–61.70%).

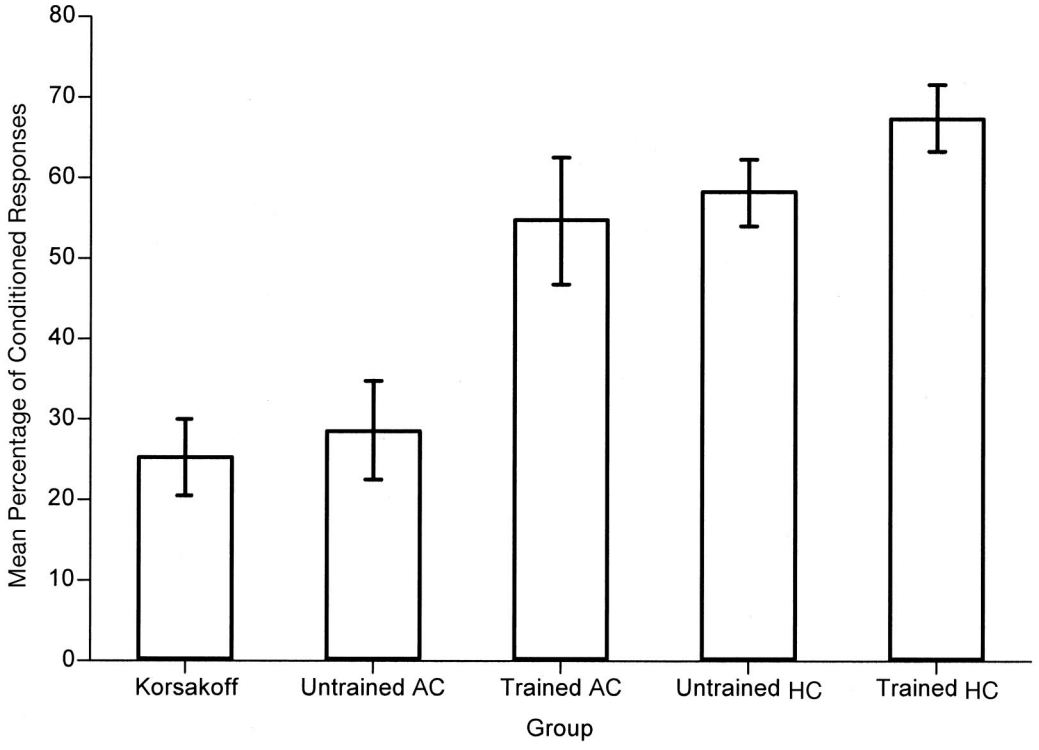

Figure 1. Mean percentage of conditioned responses acquired by each group. Korsakoff's patients and untrained AC participants acquired fewer conditioned responses than did all other groups. AC = abstinent alcoholic; HC = healthy control. Error bars represent standard error of the mean.

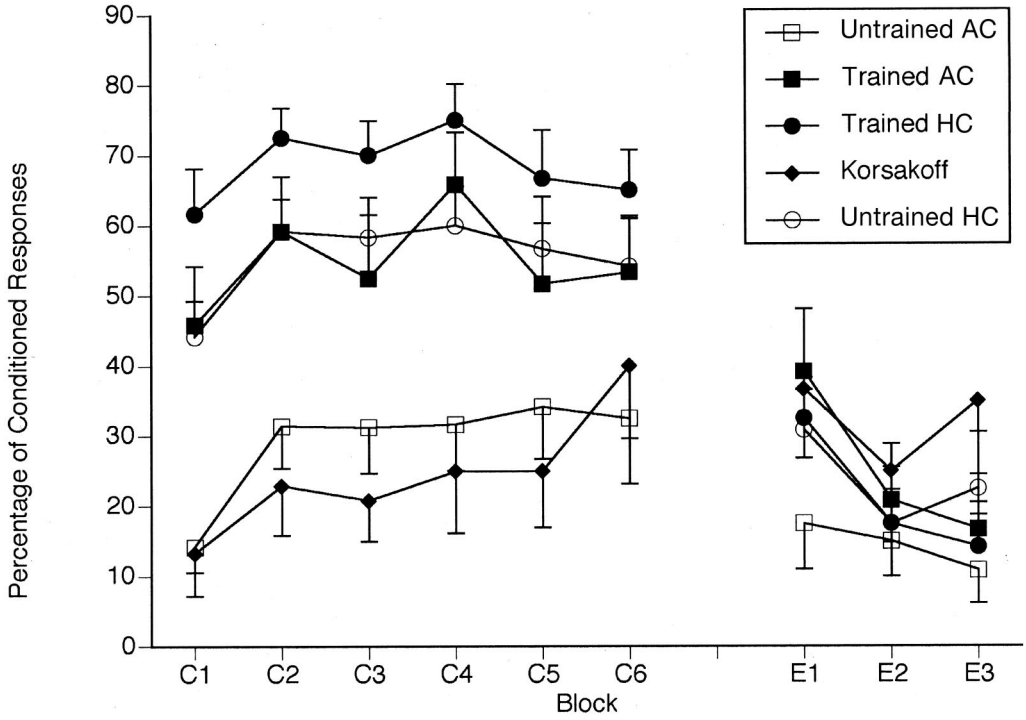

Figure 2. Mean percentage of conditioned responses for each group broken out by blocks of 10 trials each. The first six blocks refer to conditioning blocks (C); the second group of three blocks refers to the extinction blocks (E). AC = abstinent alcoholic; HC = healthy control. Error bars represent standard error of the mean.

Perhaps as important as the equivalence of the mean percentage of CRs is that the standard deviation of the mean was also remarkably similar across the two studies, and the range was extremely wide. The latter fact led us to evaluate performance on a case-by-case basis in both paradigms. A very conservative minimum of greater than 20% CRs was used as a criterion for classifying learners. Looking at the data in this way better captured the heterogeneity of the abstinent alcoholic participants' performance and revealed two distinct subgroups of individuals: one group that showed CR acquisition and one group that displayed no evidence of learning. In the trace conditioning task, 5 U-AC participants showed essentially no learning, producing a mean percentage of CRs equal to 8.00 (SD = 3.61%, range = 3.30%–8.30%), and 7 U-AC participants showed some evidence of learning, producing a mean percentage of CRs equal to 43.33 (SD = 13.54%, range = 21.70%–61.70%). The mean difference between these two subgroups was highly significant, $F(1, 10) = 38.25$, $p < .01$. Using the same criterion to define learning, we found a similar pattern in the data from our earlier delay conditioning study. In total, data were available from 11 untrained alcoholic participants (8 nonamnesic participants and 3 KOS patients). In that study, 7 participants produced 20% or fewer CRs (M = 10.48%, SD = 5.53%), and 4 produced greater than 20% (M = 50.42%, SD = 18.49%); again the difference between the subgroups was highly significant, $F(1, 9) = 30.58$, $p < .01$.

Last, we conducted a regression analysis to determine if CR acquisition in delay conditioning was predictive of acquisition in the trace conditioning task. The relationship was predictive, $F(1, 9) = 11.00$, $p < .01$, and accounted for approximately 55%

Table 3
Mean Number of Conditioned Responses During the First 10 Trials of Conditioning as a Function of Group

	Trial									
Group	1	2	3	4	5	6	7	8	9	10
KOS	.167 (.167)	.167 (.167)	0 (0)	0 (0)	.167 (.167)	.167 (.167)	0 (0)	0 (0)	.167 (.167)	.167 (.167)
T-AC	.250 (.131)	.333 (.142)	.083 (.083)	.500 (.151)	.250 (.131)	.333 (.142)	.500 (.151)	.333 (.142)	.417 (.149)	.333 (.142)
U-AC	.083 (.083)	0 (0)	0 (0)	.083 (.083)	0 (0)	.250 (.131)	.167 (.112)	.167 (.112)	.083 (.083)	.083 (.083)
T-HC	.333 (.142)	.417 (.149)	.583 (.149)	.500 (.151)	.583 (.149)	.333 (.142)	.583 (.149)	.750 (.131)	.333 (.142)	.667 (.142)
U-HC	.083 (.083)	.167 (.112)	.333 (.142)	.417 (.149)	.500 (.151)	.500 (.151)	.500 (.151)	.500 (.151)	.833 (.112)	.417 (.149)

Note. Standard errors are presented in parentheses. KOS = Korsakoff's patients; T-AC = trained abstinent alcoholic; U-AC = untrained abstinent alcoholic; T-HC = trained healthy control; U-HC = untrained healthy control.

of the variance. Thus, those who learned in delay conditioning also tended to learn in trace conditioning, and those who did not learn in delay conditioning tended not to learn in trace conditioning.

The similarity of acquisition between the trace and delay studies seems to suggest that the additional demands of the trace conditioning task did not lead to an impairment in acquisition performance over and above that which was observed in the more simple delay conditioning task. Rather, the data would seem to indicate that another common factor was at work, producing a deficit in some individuals that led to a complete inability to acquire CRs. This factor is most likely related to individual differences in the distribution of pathology secondary to individual differences in the sensitivity of alcohol's toxic effects. We will return to this in the Discussion section.

Extinction

Extinction of the CR following US termination was evaluated by comparing the final block of conditioning trials with the three blocks of extinction trials (each containing 10 trials). These data were analyzed using an ANOVA with group as a between-subjects effect and block (Block 4) as a within-subjects effect. The analysis revealed a significant effect of block, $F(3, 12) = 35.67$, $p < .01$. A series of mean comparisons indicated a significant stepwise reduction in the percentage of CRs from the last conditioning block (Block 6) to the first extinction block for all groups ($ps < .05$) except the KOS patients ($p > .7$). This indicates successful extinction of CRs after only 10 extinction trials.

The KOS patients showed a very different pattern. As a group, their mean percentage of CRs at Block 6 was 40, and it remained relatively high through the three extinction blocks. This prompted a closer examination of the individual patient data, which revealed that 2 of the KOS patients showed an increasing percentage of CRs across the six conditioning blocks, a trend that then continued into the extinction blocks. As depicted in Figure 3, the data from these 2 patients suggest significant acquisition coupled with an inability to extinguish the CR. The remaining 4 KOS patients performed at chance throughout the experiment.

Response Timing

CR onset latency was found to differ as a function of group, $F(4, 49) = 3.34$, $p < .05$. CR onset latency occurred significantly earlier for U-HC participants compared with all other groups (approaching significance for the comparison with KOS patients, $p < .07$). This finding indicates that the U-HC participants began their CRs earlier than did all other groups. Further, the CR peak latency measure, which is the more critical of the timing measures because it represents the point of maximum amplitude of the CR, revealed alcohol-related alterations, $F(4, 49) = 6.73$, $p < .01$. As depicted in Figure 4, post hoc analysis indicated that T-HC participants' peak latency occurred later than that of all other groups, including the T-AC participants. That there were no differences in peak latency between the three alcoholic groups suggests that alterations in peak latency in abstinent alcoholic individuals cannot be overcome by training. However, the difference in peak latency between U-HC and T-HC participants suggests that training can increase the precise timing of healthy individuals' CRs.

Amplitude measures are included in Table 4. The main effect of group was not significant for either the mean CR amplitude measure, $F(4, 49) = 1.46$, or the mean UR amplitude measure, $F(4, 49) = 1.43$.

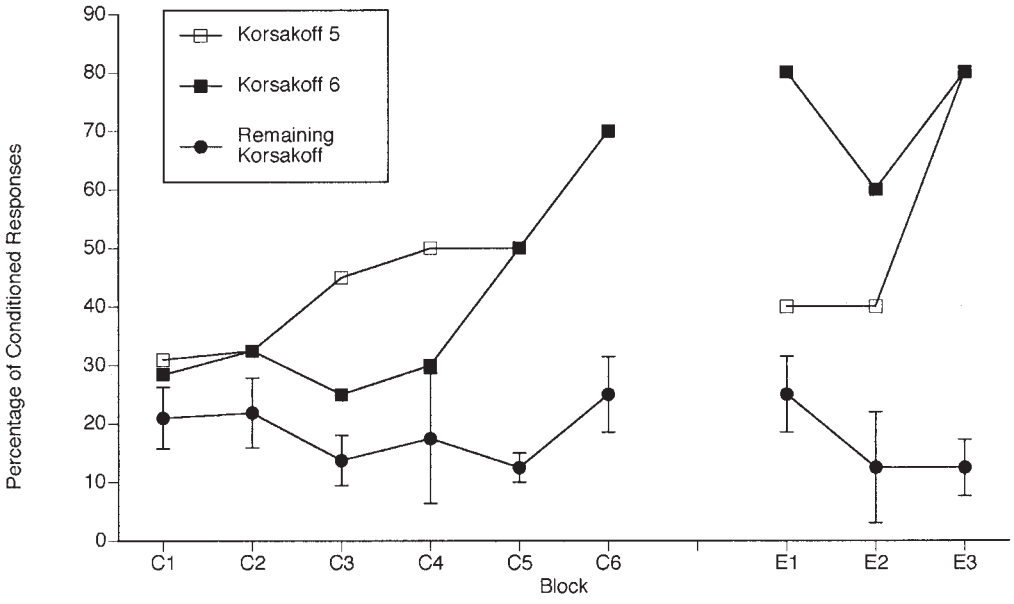

Figure 3. Mean percentage of conditioned responses for 2 Korsakoff's patients who showed an increasing percentage of conditioned responses across block and an extinction deficit, compared with the remaining 4 Korsakoff's patients who did not show evidence of acquisition. C = conditioning block; E = extinction block. Error bars represent standard error of the mean.

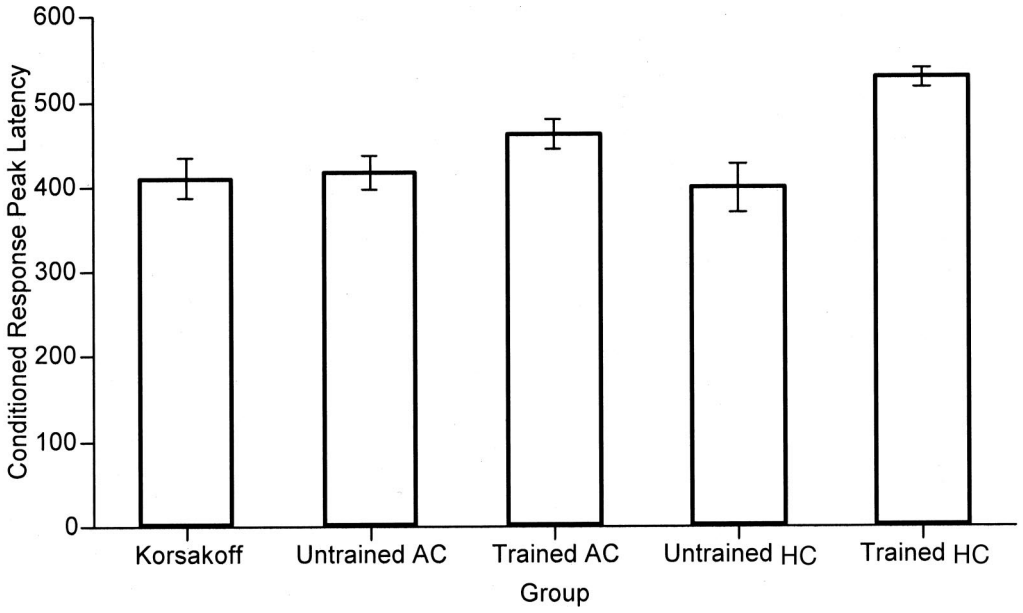

Figure 4. Mean peak latency (in milliseconds) of conditioned responses as a function of group. AC = abstinent alcoholic; HC = healthy control. Error bars represent standard error of the mean.

Spontaneous Eyeblinks and Alpha Responses

The number of spontaneous eyeblinks and alpha responses were analyzed with an ANOVA to determine the presence of group differences. These summary data can be found in Table 3. For spontaneous eyeblinks, the group effect was marginally significant, $F(4, 49) = 2.47$, $p < .06$. Post hoc tests indicated that the KOS group produced significantly more spontaneous eyeblinks than did all other groups ($ps < .05$). The group effect was not significant for the number of alpha responses, $F(4, 49) = 0.89$.

Correlations of Conditioning Performance to Measures of Alcohol Abuse

A series of correlations was performed to determine if the level of acquisition and alterations in the temporal dynamics of the CR of abstinent alcoholic participants was related to reported years of alcohol abuse or to the SMAST. Surprisingly, these correlations did not indicate any statistically reliable relationships, except for a trend between the CR peak latency measure and years of abuse ($r = .377$, $p = .09$). However, it is difficult to make conclusions on the basis of null findings. These relationships may be better evaluated in the context of a larger study.

Discussion

The primary finding from this study was that abstinent alcoholic individuals vary greatly in their ability to acquire associative relationships; some individuals with alcoholism retain the ability to acquire simple associative relationships normally, whereas others are completely unable to learn those relationships. This did not appear to depend on the complexity of the conditioning task, because there was a strong correspondence in learning abilities across the trace and delay eyeblink conditioning tasks. Learning ability was also not specifically related to the presence of an amnesic memory impairment (i.e., KOS syndrome). In particular, 5 of the 12 U-AC participants and 2 of

Table 4
Means (and Standard Errors) for Amplitude Measures, Spontaneous Eyeblinks, and Alpha Responses

Group	CR amplitude	UR amplitude	Spontaneous eyeblinks	Alpha responses
KOS	1,655.04 (452.52)	2,133.86 (316.38)	29.50 (6.62)	7.17 (3.43)
T-AC	1,642.96 (287.73)	2,499.13 (191.58)	20.08 (3.10)	3.08 (0.45)
U-AC	1,220.38 (196.69)	2,644.63 (159.28)	14.25 (2.77)	4.42 (1.64)
T-HC	1,962.02 (278.76)	2,802.08 (161.71)	15.09 (2.60)	3.58 (1.06)
U-HC	1,952.02 (164.30)	2,520.43 (174.35)	16.67 (2.40)	4.08 (0.94)

Note. CR = conditioned response; UR = unconditioned response; KOS = Korsakoff's patients; T-AC = trained abstinent alcoholic; U-AC = untrained abstinent alcoholic; T-HC = trained healthy control; U-HC = untrained healthy control.

the 6 KOS patients did show evidence of learning (although only 1 KOS patient achieved an acquisition level of 48%, a finding that suggests a more severe impairment in this group).

That the differential demands of the delay and trace conditioning paradigm did not have an impact on the pattern of learning—and that abstinent alcoholic participants appear to have an all-or-none ability to acquire CRs—suggest a common mechanism in both tasks that affects some individuals with alcoholism but not others. On this basis, we conclude that the neural structures most likely leading to the impairment in CR acquisition in these single-cue learning tasks lie primarily within the cerebellum. It is likely that the pathology inflicting individuals with alcoholism who cannot acquire CRs affects the cerebellar deep nuclei (see Green, Tran, et al., 2002) and that such pathology is not present in individuals with alcoholism who do achieve normal levels of learning.

Of import, we do not intend to imply that forebrain structures were not involved in trace acquisition. Rather, we mean only to suggest that in those alcoholic participants who could not achieve significant learning, the contribution of frontal structures was ineffective in overcoming the underlying pathology of the cerebellum, which we propose caused a very basic impairment in eyeblink acquisition. In fact, it is even possible that frontal systems were active in the nonlearners in much the same way that the hippocampus is active, but not essential, in delay eyeblink conditioning (Berger & Orr, 1983; Berger & Thompson, 1978; Berger & Weisz, 1987; Blaxton et al., 1996; Disterhoft, Coulter, & Alkon, 1986; Logan & Grafton, 1995; Weiss, Kronforst-Collins, & Disterhoft, 1996) in nonlearners.

We have hypothesized in past research that the cerebellar cortex is likely the loci of alterations in the peak latency of CRs (McGlinchey-Berroth et al., 2002), which is the best indicator of how effective the CR is in protecting the eye from the US. The data from the current study provide partial support for this supposition, evidenced by the significant difference in peak latency between the T-AC and T-HC participants. However, the peak latency between the untrained groups and the KOS patients was roughly equivalent. One might speculate that in this study, the U-HC participants may have been unable to precisely time the peak of the CR as accurately as did the healthy control group in our previous study; however, further studies are needed to fully understand the relationship between cerebellar cortical shrinkage and peak latency.

A final point of discussion is the extinction deficit seen in the 2 KOS patients who did achieve significant CR acquisition. An obvious explanation would be to attribute these apparent CRs during extinction trials as spontaneous eyeblinks that would have occurred just prior to the onset of the US during conditioning trials. Recall that the KOS patients did show a high rate of spontaneous eyeblinks (as was observed in our earlier delay conditioning study; McGlinchey-Berroth et al., 1995). However, inspection of the raw eyeblink data for these 2 patients is not consistent with this explanation. The CRs observed during the extinction trials show the normal onset and rise in amplitude just prior to the time that the US would be delivered on conditioning trials. Thus, the data are consistent with a true extinction deficit.

Extinction refers to a decrease in CRs following the termination of the US. In a recent review, Kehoe and White (2002) classified theories of extinction into four categories: unlearning theories (or error correction theories), new learning theories (focusing on the development in inhibition), generalized decrement theories (stressing the progressive dissimilarity between acquisition and extinction context leading to a retrieval failure), and nonassociative losses in responding (due to reductions in CS and US processing over trials). On the basis of data from only 2 patients, it is premature at best to offer any compelling suggestions to this debate. The deficit observed in these 2 patients may be consistent with the findings in rabbits by Weible et al. (2000), who found that the medial prefrontal cortex was critical for the acquisition and extinction of the conditioned reflex. Perhaps the extinction deficit observed in these 2 KOS patients was the result of pathology extending into these frontal areas.

The findings from this study highlight the heterogeneity of impairment found in the learning abilities of individuals with a history of chronic alcoholism. We propose that this heterogeneity is the result of individual differences in the underlying pathology caused by chronic alcohol misuse. However, this is only a hypothesis and is in no way conclusive. Further investigation using neuroimaging techniques may provide some hard evidence in support of this suggestion and may help to elucidate underlying, causative areas of pathology.

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