

Impact of Healthy Aging on Awareness and Fear Conditioning

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Fear conditioning has provided a useful model system for studying associative emotional learning, but the impact of healthy aging has gone relatively unexplored. The present study investigated fear conditioning across the adult life span in humans. A delay discrimination task was employed using visual conditioned stimuli and an auditory unconditioned stimulus. Awareness of the reinforcement contingencies was assessed in a postexperimental interview. Compared with young adult participants, middle-aged and older adults displayed reductions in unconditioned responding, discriminant conditioning, and contingency awareness. When awareness and overall arousability were taken into consideration, there were no residual effects of aging on conditioning. These results highlight the importance of considering the influence of declarative knowledge when interpreting age-associated changes in discriminative conditioned learning.

Classical conditioning is a form of associative learning that depends on the functional integrity of two partially independent systems in the brain. An amygdala-based system is critical for the conditioning of emotional responses, largely assessed through fear conditioning procedures (Fendt & Fanselow, 1999; LaBar & LeDoux, 2001), whereas a cerebellar-based system is critical for the conditioning of somatomotor reflexes, largely assessed through eyeblink conditioning procedures (Steinmetz, 1998; Thompson, 1990). These systems perform similar functions across species, as electrophysiological and lesion studies initially conducted in animals have been extended to human brain imaging and neurologic populations (Büchel & Dolan, 2000; Daum & Shugens, 1995).

Because simple forms of conditioning are nonverbal, relatively easy to measure, and do not tax executive resources, they have been advocated as a potentially useful probe for identifying age-related changes in learning abilities (Disterhoft et al., 2000; Powell, 1999; Solomon, Beal, & Pendlebury, 1988; Thompson, 1988; Woodruff-Pak, 1997). Several life span studies have observed impairments in eyeblink conditioning, which emerge as early as middle age even on single-cue delay paradigms (Braun & Geiselsart, 1959; Durkin, Prescott, Furchtgott, Cantor, & Powell, 1993; Kimble & Pennypacker, 1963; Knuttinen, Power, Preston, & Dis-

terhoft, 2001; Solomon, Pomerleau, Bennett, James, & Morse, 1989; Solyom & Barik, 1965; Woodruff-Pak & Finkbiner, 1995; Woodruff-Pak & Thompson, 1988). These effects are dissociable from age-associated changes in the production of voluntary eyeblinks and baseline startle responses. Aged rabbits also show decrements during conditioning of the nictitating membrane response, as characterized by a smaller percentage of conditioned responses (CRs) or a larger number of training trials required to reach a performance criterion (Coffin & Woodruff-Pak, 1993; Powell, Buchanan, & Hernandez, 1981, 1984; Solomon & Groccia-Ellison, 1996; Woodruff-Pak, Steinmetz, & Thompson, 1988). Task parameters, including conditioned stimulus–unconditioned stimulus (CS–US) interval duration and insertion of a trace interval between the onset of the CS and US, may impact aging differentially (Graves & Solomon, 1985; Harrison & Buchwald, 1983; Knuttinen et al., 2001; Woodruff-Pak, Jaeger, Gorman, & Wesnes, 1999; Woodruff-Pak, Lavond, Logan, & Thompson, 1987).

Few studies have examined conditioning of autonomic (fear) responses in aged populations. Early studies had implicated aging decrements in conditioning of skin conductance responses (SCRs) and cardiac responses (Botwinick & Kornetsky, 1960; Shmavonian, Miller, & Cohen, 1968, 1970). However, these studies failed to account for age-related changes in overall arousability and habituation across the groups, which were also reported (see Powell, 1999, for further discussion). A more recent study (Durkin et al., 1993) found age-associated impairments in discriminative heart-rate conditioning when nonassociative factors were controlled. Aging studies in nonhuman mammals have shown mixed results. Early studies showed deficits in conditioned suppression in aged rats (Pare, 1969b; Solyom & Miller, 1965), but decrements in footshock sensitivity may partly explain these effects (Pare, 1969a). Previous studies have also shown conditioned bradycardia to increase or remain stable in aged rabbits (Powell et al., 1981, 1984) but decrease in aged rats (Buchanan & Ginn, 1988). Aging studies of conditioned freezing in rats and mice have typically

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found no impairments in CS-evoked responses (Doyère, Gisquet-Verrier, de Marsanich, & Ammassari-Teule, 2000; Houston, Stevenson, McNaughton, & Barnes, 1999; Ohta et al., 2001; Oler & Markus, 1998). Contextual fear conditioning in these same animals has been variously shown to increase (Doyère et al., 2000), decrease (Stoehr & Wenk, 1995), or show sensitivity to retention interval (Houston et al., 1999; Ohta et al., 2001; Oler & Markus, 1998). Thus, in contrast to the consistent effects of aging on eyeblink conditioning across species, the impact of aging on conditioned fear is less clear.

One factor that is important to consider when interpreting associative learning changes in human aging is the influence of declarative knowledge about the reinforcement contingencies. Simple classical conditioning paradigms are typically considered examples of implicit, or nondeclarative, forms of learning (Squire & Zola, 1996). However, it is known that declarative knowledge about the experimental parameters rises in parallel with conditioned learning (Clark, Manns, & Squire, 2002; LaBar & Disterhoft, 1998). Under some circumstances, particularly for more complex designs, this knowledge can modulate task performance. The exact conditions under which contingency awareness influences conditioned learning continue to be debated by researchers (Lovibond & Shanks, 2002; Manns, Clark, & Squire, 2002; Wiens & Öhman, 2002). Clark and Squire (1998) have argued that contingency awareness is necessary for performance on trace, but not delay, eyeblink conditioning tasks. However, other studies of fear conditioning (Baer & Fuhrer, 1968, 1969, 1970; Biferno & Dawson, 1977; Dawson & Biferno, 1973; Furedy, Arabian, Thiels, & George, 1982; Furedy & Schiffman, 1973; Schiffman & Furedy, 1977) and eyeblink conditioning (Baer & Fuhrer, 1982; Benish & Grant, 1980; Carrillo, Gabrieli, & Disterhoft, 2000; Knuttnen et al., 2001; Nelson & Ross, 1974; Perry, Grant, & Schwartz, 1977; Ross & Nelson, 1973) have shown influences of awareness and attention on discriminative learning in which one CS predicts reinforcement (CS+) but another does not (CS-), even for delay tasks (but see Knight, Nguyen, & Bandettini, 2003). Moreover, awareness of reinforcement contingencies decreases with aging (Knuttnen et al., 2001). Therefore, declarative knowledge of the CS-US relationship should be assessed in aging studies of conditioning, especially studies on discriminative and/or trace conditioning paradigms.

The goals of the present study were threefold. The first aim was to characterize changes in fear conditioning across the life span in healthy adults, which has not been systematically investigated. The second aim was to determine the relationship between contingency awareness and fear conditioning using a delay discrimination paradigm. Consistent with previous reports (reviewed above), we expected that unaware participants would show less discriminative conditioning than aware participants. The third aim was to assess whether declarative influences on learning are modulated by the aging process. Consistent with Knuttnen et al. (2001), we hypothesized that a greater proportion of older adults would be unaware of the reinforcement contingencies than would younger adults. The results of the present study should thus yield insights into age-associated changes in fear conditioning and their dependence on explicit knowledge acquired during the task.

Method

Participants

A total of 87 healthy adults gave informed consent to participate in the study. Participants were divided into three age cohorts: younger adults (YC) aged 18–29 years ($n = 46$, 29 female, mean age = 20 ± 3 years), middle-aged adults (MC) aged 51–64 years ($n = 17$, 13 female, mean age = 58 ± 5 years), and older adults (OC) aged 66–80 years ($n = 24$, 16 female, mean age = 73 ± 4 years). The YC group was composed of Duke University students who were given either course credit or \$10 an hour for their participation. The MC and OC groups were recruited by one of three methods: referral from the Bryan Alzheimer's Disease Research Center at Duke University Medical Center, referral from the Center for Aging and Human Development at Duke University Medical Center, or advertisements placed in on- and off-campus publications. For their participation, the MC group was compensated \$10 an hour and the OC group was compensated \$20 an hour. These groups were matched for level of education ($M = 16$ years for both groups; MC range = 12–20 years, OC range = 13–20 years). Researchers screened all participants by phone and by written questionnaire for history of neurologic and psychiatric illness, stroke, substance abuse, and current medication use. The experiment was approved for use in human participants by the Duke University Institutional Review Board.

Neuropsychological Assessment

Participants in the MC and OC groups underwent neuropsychological examinations within several months ($M = 3.25$ months) of participation in the experiment to establish the absence of age-associated cognitive deficits. The neuropsychological battery was compiled by the Bryan Alzheimer's Disease Research Center at Duke University Medical Center and included standardized tests of memory, attention, naming, and executive function. A subset of scores from this battery is summarized in Table 1. None of the participants met diagnosis of probable Alzheimer's disease (AD) according to criteria established by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann et al., 1984).

Audiometric Screening

Immediately prior to the conditioning study, MC and OC participants were screened for age-associated hearing loss, given that an auditory US would be used. Audiometric screening was conducted using the Screening Audiometer AS208 from Interacoustics (Eden Prairie, MN) and criteria adopted from the American Speech-Language-Hearing Association (1997). The pure tone average for each ear was computed using a descend-

Table 1
A Sample of Neuropsychological Test Scores (Means \pm Standard Errors) in Control Groups

Test	Middle-aged control	Older control
Mini-Mental State Exam	29.0 \pm 0.2	28.8 \pm 0.2
Wechsler Memory I Recall: raw score	32.1 \pm 1.4	31.0 \pm 1.2
Wechsler Memory II Recall: raw score	28.9 \pm 1.5	28.3 \pm 1.4
Digit Span Forward Recall	8.9 \pm 0.5	8.9 \pm 0.5
CERAD Word List: Delayed Recall	7.7 \pm 0.5	7.9 \pm 0.3
Symbol Digit Modality Test	50.8 \pm 1.8	47.2 \pm 1.4

Note. CERAD = Consortium to Establish a Registry for Alzheimer's Disease.

ing staircase method at frequencies of 500, 1000, and 2000 Hz. Mild hearing loss was indicated by threshold decrements of 26–30 dB. Researchers characterized 11 of 24 participants in the OC group and 3 of 14 participants in the MC group as having mild hearing loss. For these participants, the intensity of the US was increased by 5 dB. Higher intensities could not be administered because of acoustic distortion.

Experimental Design

Researchers used a delay discrimination task consisting of habituation, acquisition, and extinction phases presented contiguously. Two isoluminant colored squares (red and green) were used as conditioned stimuli and were presented centrally on a computer screen in a pseudorandom sequence. During the habituation and extinction phases, the CSs were presented without reinforcement. During the acquisition phase, one CS was designated the CS+ and was reinforced by a coterminating white noise US, whereas the other CS was designated the CS– and was unreinforced. CS duration was 4 s, US duration was 1 s, and the interstimulus interval duration was 17 ± 2 s. The white noise US was presented as four sequential 125-ms bursts separated by 125-ms interburst intervals, and the intensity was adjusted between 100 dB and 105 dB on the basis of the participant's performance on the audiometric screening test. There were eight habituation trials, eight acquisition trials, and 16 extinction trials (50% CS+, 50% CS–). The number of extinction trials was doubled relative to the other phases to ensure that full extinction took place. Color assignment of the CS+ was counterbalanced across participants, and two pseudorandom trial sequences were constructed and counterbalanced across participants with the constraint that no more than two exemplars of the same CS type be presented sequentially. The experiment was presented using Superlab 2.0 software (1999).

Skin Conductance Monitoring

SCRs were used as the dependent measure of conditioning. Researchers monitored SCRs from the participant's nondominant hand using silver–silver chloride electrodes attached to the middle phalanges of the second and third digits by velcro straps (BIOPAC Systems, Goleta, CA). A saline-based gel (Signa Gel; BIOPAC Systems) was used as a conductive electrolyte. Skin conductance was monitored at 250 Hz and stored offline for analysis. Physiologic recording was synchronized to the stimulus presentation by a National Instruments DIO-24 card (Austin, TX). The physiologic data were scored according to conventional methods as previously described (LaBar, LeDoux, Spencer, & Phelps, 1995). For inclusion in the data analysis, researchers established the following criteria: SCR latency = 1–4 s, SCR duration = 0.5–5.0 s, and minimum SCR amplitude = 0.02 microSiemens (μ S). Responses that did not meet these criteria were scored as zero.

Measurement of Awareness

Declarative knowledge about the CS–US contingencies was assessed in a semistructured interview following the extinction phase of the study (Papka, Ivry, & Woodruff-Pak, 1997). Participants were asked a series of eight questions about the experimental parameters (see the Appendix). Participants were only classified as aware if they were able to report that a given colored square (the CS+) predicted presentation of the noise US. If the participant produced the correct response early during the interview, the remaining questions on the list were not asked. Awareness classifications were concordant across two raters present during the postexperimental interview (Craig A. Cook and Dana C. Torpey).

Statistical Methods

Conditioned SCRs were collapsed into “early” and “late” trial blocks of the habituation, acquisition, and extinction phases prior to statistical anal-

ysis. The early and late trial blocks constitute a mean of 2 trials per CS type for the habituation and acquisition phases and a mean of 4 trials per CS type for the extinction phase, as this phase contained double the number of total trials relative to the other phases. Learning-related changes were hypothesized to be found in the late acquisition and early extinction phases, as reported previously (LaBar et al., 1995). Therefore, these phases were used to characterize differential learning. Analyses of variance (ANOVAs) were conducted using age group as a between-subjects factor and CS type and trial block as within-subjects factors. SCRs were square-root transformed to attain normality prior to statistical analysis. An alpha level of .05 was set for all analyses. Researchers also calculated difference scores in which responses to the CS– were subtracted from those to the CS+ across trial blocks. According to this measure, difference scores of zero reflect no differential learning, difference scores above zero reflect greater responses to the CS+ relative to the CS–, and difference scores below zero reflect greater responses to the CS– relative to the CS+ (LaBar et al., 1995). Subsequent analyses were conducted on these difference scores to assess learning as a function of awareness within each age group. To characterize unconditioned responses to the US, we conducted an ANOVA using trial as a within-subjects factor and age group as a between-subjects factor. Post hoc tests were conducted using Bonferroni-corrected *t* tests and one-way ANOVAs, as appropriate.

Results

Unconditioned Responding

A two-way mixed ANOVA conducted on SCRs to the US revealed significant main effects of age group, $F(2, 84) = 7.75$, $p < .01$, and trial, $F(3, 252) = 2.64$, $p = .05$, but no Age \times Trial interaction (see Figure 1). Post hoc one-way ANOVAs indicated that the YC group had higher unconditioned responses than both the MC group, $F(1, 61) = 7.66$, $p < .02$, and the OC group, $F(1, 68) = 12.32$, $p < .01$. The MC and OC groups did not differ from each other ($F < 1$). It is important to note that these age-associated changes in unconditioned responding did not interact with trial, so any group differences in acquisition are not confounded by differential sensitivity to the US over time.

Subsequent analyses were conducted as a function of performance on the audiometric screening test. There were no significant differences in unconditioned responding between MC and OC participants who had mild hearing loss compared with those with

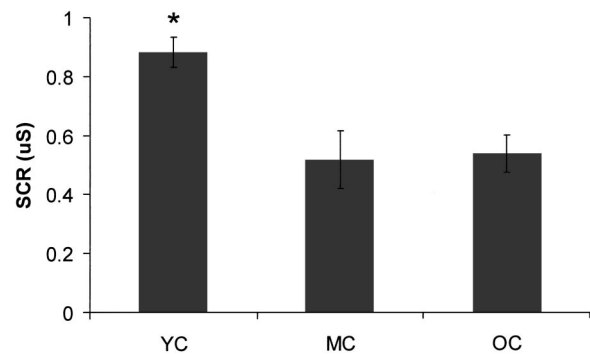


Figure 1. Mean unconditioned response to the white noise burst as a function of age group. Error bars represent standard error of the mean. YC = young cohort; MC = middle-aged cohort; OC = older cohort; SCR = skin conductance response; μ S = microsiemens (μ S). * $p < .05$.

normal hearing thresholds, $F(1, 39) = 1.81, p = .19$. This indicates that our adjustments in US intensity on the basis of the audiometric screening results were sufficient to yield equivalent unconditioned responding across hearing-impaired and normal hearing participants.

Because the unconditioned responding results indicate age-associated differences in overall arousability, we normalized conditioned SCRs prior to statistical analysis according to methods described by Lykken (1972). The normalization procedure expresses conditioned responses as a proportion of each individual participant's largest response elicited to the US, which accounts for overall arousability differences across groups. In this way, any learning-related changes are not confounded by age-associated decrements in baseline arousal levels.

Habituation

A three-way mixed ANOVA on conditioned responses elicited during the habituation phase (see Figure 2) revealed an Age \times Trial interaction, $F(2, 84) = 4.70, p < .01$, and main effects of age group, $F(2, 84) = 4.39, p < .02$, and trial block, $F(1, 84) = 8.08, p < .01$. There were no significant main effects or interactions with CS type. The main effect of trial block indicates greater responses during the early habituation phase relative to the late habituation phase. Follow-up one-way ANOVAs showed that age group differences were found only during the early habituation phase, $F(2, 84) = 6.07, p < .01$. In this phase, the YC group had higher SCRs than the MC or OC groups ($ps < .05$), which did not differ from each other. These results indicate that the YC group had larger initial orienting responses to the CS+ and CS- than the MC and OC groups, but all participants were habituated to the same level of responding prior to the start of acquisition training.

Discriminative Learning

A three-way mixed ANOVA computed on normalized conditioned SCRs during late acquisition and early extinction revealed a three-way interaction between age group, CS type, and trial block, $F(2, 84) = 3.42, p < .04$ (see Figure 2). In addition, two-way interactions were significant between age group and trial block, $F(2, 84) = 5.27, p < .01$, and between age group and CS type, $F(2, 84) = 3.18, p < .05$. Finally, main effects of age group, $F(2, 84) = 5.01, p < .01$; trial block, $F(1, 84) = 9.21, p < .01$; and CS type, $F(1, 84) = 24.00, p < .01$, were also found. The main effect of CS type indicates greater responses to the CS+ compared with the CS-, suggesting that conditioned learning took place overall. However, conditioned learning was attenuated in aging during the late acquisition trial block. Post hoc one-way ANOVAs in late acquisition revealed a main effect of age group, $F(2, 84) = 7.35, p < .01$, and an Age \times CS interaction, $F(2, 84) = 4.46, p < .03$. These age-related effects were not significant in early extinction ($ps > .05$). Follow-up t tests showed that in late acquisition, responses to the CS+ were larger in the YC group than in the MC and OC groups ($ps < .05$), which did not differ from each other. There were no significant group differences in response to the CS-. Thus, aging was associated with decrements in differential conditioning, as indicated by reduced reactivity on paired CS-US trials in late acquisition for both MC and OC groups relative to the

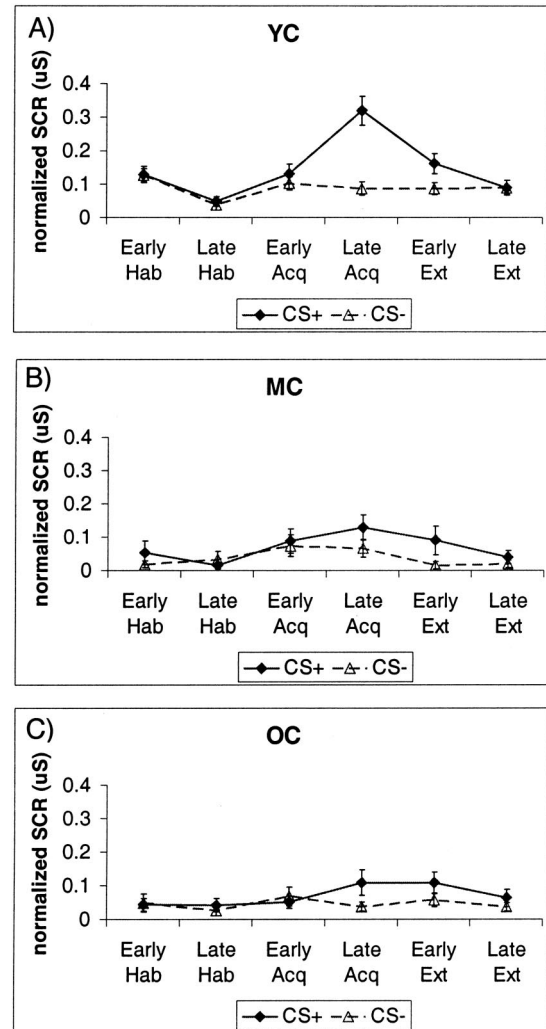


Figure 2. Mean conditioned response to the squares across all experimental phases in (A) young cohort (YC), (B) middle-aged cohort (MC), and (C) older cohort (OC). During acquisition, one conditioned stimulus (CS+) predicts reinforcement, but the other (CS-) does not. Error bars represent standard error of the mean. Hab = habituation; Acq = acquisition; Ext = extinction; SCR = skin conductance response; uS = microsiemens (μS).

YC group. This effect was found despite controlling for age-related differences in overall arousability by SCR normalization procedures.

We also conducted an exploratory analysis to examine the influence of gender on conditioned learning, although this was not a main objective of the present study. Only the YC group was included in this analysis because the sample size was large enough to make meaningful comparisons while controlling for other factors (e.g., awareness). A three-way mixed ANOVA was conducted on late acquisition and early extinction phases, with gender as a between-subjects factor and CS type and trial block as within-subjects factors. No significant differences in conditioned learning as a function of gender emerged from this analysis either as a main

effect, $F(1, 44) = 1.56, p = .22$, or as interactions with CS type and trial block ($F_s < 1$). Therefore, conditioning was equivalent in young male and female participants.

Awareness of the CS-US Contingency

Awareness of the CS-US contingency was assessed in a postexperimental interview (see the Method section). According to this measure, 85% (39 out of 46) of the YC participants were aware, 71% (12 out of 17) of the MC participants were aware, and 42% (10 out of 24) of the OC participants were aware. A chi-square test indicated that awareness significantly declined with aging, $\chi^2(2, N = 87) = 13.96, p < .01$. Follow-up tests showed that the aging effect was caused by reduced awareness in the OC group relative to the YC group ($p < .01$).

Because declarative knowledge was assessed postexperimentally, it is possible that awareness was related to the ability to retain information about the CS-US contingency over time (Lovibond & Shanks, 2002), especially for older participants. To examine whether differential forgetting contributed to the awareness results, we compared the delayed recall scores of aware and unaware OC participants on the Wechsler Memory Scale—Revised Test (Wechsler, 1987; WMS-R). This test was administered as part of the neuropsychological assessment battery (see Table 1). The Wechsler Memory II Delayed Recall subtest probes memory for complex prose passages using a retention interval similar to that interposed between the acquisition and interview portions of the conditioning session. This analysis yielded no significant effects, $t(22) = 1.47, p = .15$. Therefore, it is unlikely that forgetting accounts for the differences in awareness across elderly participants, especially because the WMS-R consists of far more difficult tests of delayed recall.

Discriminative Learning by Awareness

Because a greater proportion of OC participants were unaware of the reinforcement contingencies, it is possible that the age-associated decrement in discriminative conditioning (see Figure 2) is related to decreased declarative knowledge with aging. To determine whether this was the case, we conducted a three-way mixed ANOVA on the differential learning scores with age group and awareness as between-subjects factors and trial block as a within-subjects factor. The results revealed only a significant main effect of awareness, $F(1, 81) = 8.83, p < .01$ (see Figure 3). This finding indicates greater conditioning in aware versus unaware participants overall. It is important to note that there were no significant main effects or interactions with age group. Thus, when awareness is taken into consideration, older adults do not show significant decrements in differential conditioning relative to younger adults (see Figure 4A). To reemphasize, the apparent decline in conditioning with aging in the overall analysis (see Figure 2 and Figure 4B) is caused by the greater proportion of unaware participants in the aged samples. When only aware participants are compared, there is no residual impact of aging on conditioned learning either as a main effect ($F < 1$) or as an interaction with trial block ($F = 1$; see Figure 4A). No significant conditioning took place within unaware participants ($F < 1$).

Although the ANOVA conducted on late acquisition and early

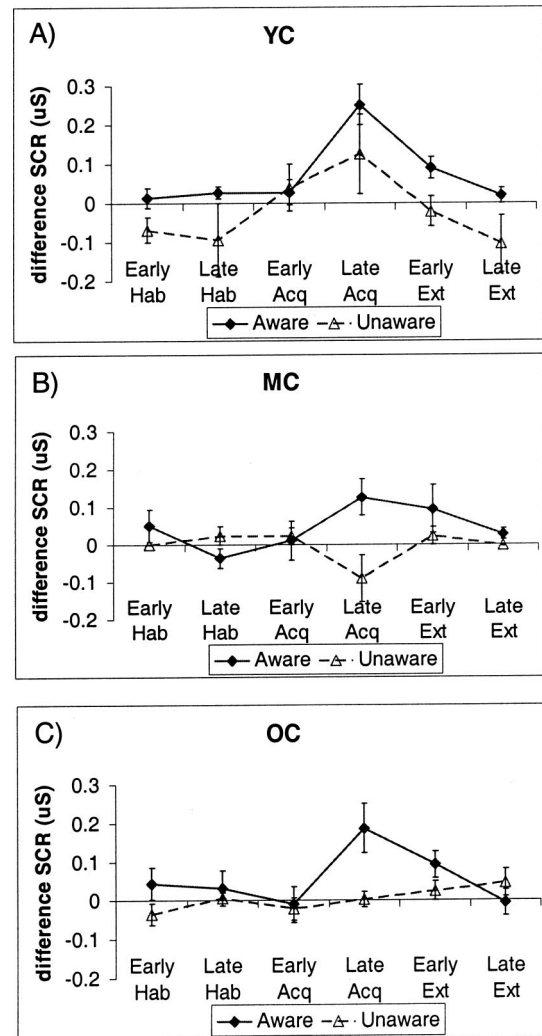


Figure 3. Mean differential learning curves as a function of awareness across all experimental phases. Participants were divided into those that were aware versus unaware of the reinforcement contingencies in the (A) young cohort (YC), (B) middle-aged cohort (MC), and (C) older cohort (OC). Skin conductance responses (SCRs) are expressed as difference scores between reinforced and unreinforced conditioned stimuli (CS+ minus CS-) within each experimental phase. Values at zero reflect no differential fear learning. Error bars represent standard error of the mean. Hab = habituation; Acq = acquisition; Ext = extinction; uS = microsiemens (μS).

extinction trial blocks did not show an interaction between aging and awareness in terms of differential conditioning, qualitative inspection of the learning curves indicates a greater detrimental impact of unawareness in the MC and OC groups (see Figure 3). To further probe this issue, we conducted two sets of analyses. First, orthogonal polynomial contrasts were set up to test for quadratic trends in the difference scores using data from all experimental phases. This analysis revealed a quadratic trend in the learning curve for the unaware YC subgroup, $F(1, 6) = 6.38, p < .05$, but not for the unaware MC and OC subgroups, whose

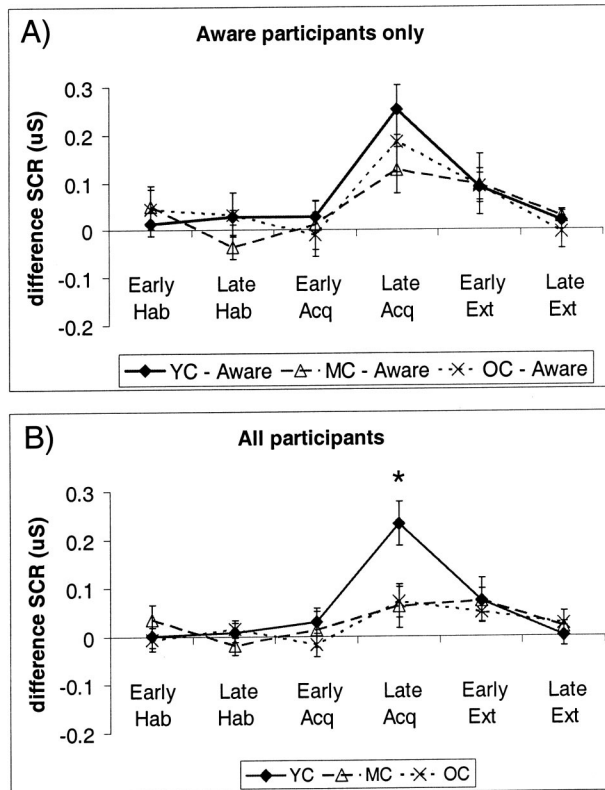


Figure 4. Mean differential learning curves across all experimental phases presented for (A) aware participants only and (B) all participants combined (YC = young cohort, MC = middle-aged cohort, OC = older cohort). No significant aging effects are found when awareness is controlled. Skin conductance responses (SCRs) are expressed as difference scores between conditioned stimuli (CS+ minus CS-) within each experimental phase. Values at zero reflect no differential fear learning. Error bars represent standard error of the mean. Hab = habituation; Acq = acquisition; Ext = extinction; uS = microsiemens (μ S). * $p < .05$.

learning curves were flat ($F_s < 1$). The second analysis computed planned contrasts on the differential learning scores during late acquisition as a function of awareness for each age group separately. This analysis revealed no significant effect of awareness in the YC group, $t(44) = 0.98$, $p > .99$, a trend toward significance in the MC group, $t(15) = 2.51$, $p = .07$, and a significant effect of awareness in the OC group, $t(22) = 2.94$, $p < .02$ (Bonferroni-corrected).

Together, these results indicate that declarative knowledge of the reinforcement contingencies is associated with the apparent age-associated decline in differential fear conditioning with aging. In addition, the relationship between awareness and discriminative conditioning shows some sensitivity to aging. Learning curves in unaware younger adults were attenuated, but not eliminated, relative to aware participants in their cohort, whereas learning curves in unaware middle-aged and older adults were completely flat. Moreover, the difference in conditioning during late acquisition between aware and unaware participants showed some augmentation with aging.

Discussion

The present study investigated fear conditioning across the adult life span using a delay discrimination task. There were three main findings relevant for understanding the relationship among awareness, fear conditioning, and aging. First, unconditioned responses to a white noise US were attenuated in both middle-aged and older adults relative to younger adults. Second, a greater proportion of elderly participants were unaware of the CS-US contingency. Third, discriminative conditioning was impaired in aging, even when group differences in baseline arousability were controlled by SCR normalization procedures. However, when awareness was taken into consideration, there were no residual effects of aging on conditioned learning. In combination, these results suggest that delay fear conditioning is preserved in healthy aging when contingency awareness and nonassociative arousability are accounted for. Implications for aging studies of classical conditioning, emotional learning, and the interdependency of explicit and implicit brain systems are discussed below.

Arousal and Aging

Relative to the YC group, the MC and OC groups showed decrements in both unconditioned responses to the white noise US during acquisition and orienting responses to the CS during habituation. These results highlight the importance of (a) controlling for overall arousability when quantifying conditioned responses (e.g., through SCR normalization procedures; Lykken, 1972) and (b) including a short habituation phase prior to learning so that acquisition is not confounded by initial orienting differences across age groups. The inclusion of the MC group was critical for revealing that unconditioned responses decline as early as middle age. These results are consistent with other research showing decrements in autonomic orienting and habituation with aging (Drory & Korczyn, 1993; Eisenstein, Bonheim, & Eisenstein, 1995; Kim, Beversdorf, & Heilman, 2000). Because an auditory US was used, it was also important to compensate for age-associated hearing deficits. Our procedures for adjusting US intensity were effective in equating arousal responses across aged participants with normal hearing and those who had mild hearing loss. Future studies are needed to determine whether arousal decrements that occur with aging and their effects on memory reflect primarily peripheral or central mechanisms.

Classical Conditioning in Aging

The impact of healthy aging on fear conditioning has not received much investigation to date. Durkin and colleagues (1993) previously reported aging impairments on discriminative heart-rate conditioning, but awareness was not measured. Studies in nonhuman animals have found some age-related changes in more complex aspects of fear conditioning, such as acquisition of contextual conditioning and its retention after a delay, but simple forms of fear learning remain relatively intact (Doyère et al., 2000; Houston et al., 1999; Ohta et al., 2001; Oler & Markus, 1998; Stoehr & Wenk, 1995). The results of the present study are consistent with the animal findings in showing no age-related reductions in fear conditioning on a simple discrimination task when arousal and

awareness are controlled. Volumetric studies of the human amygdala have shown only a 2% volume reduction in healthy aging (Herzog & Kemper, 1980) that occurs gradually in senescence (Mu, Xie, Wen, Weng, & Shuyun, 1999). Aging deficits in conditioned fear behavior on more complex tasks may be related to age-associated changes in interactions between the amygdala and the hippocampus and prefrontal cortex (e.g., Almaguer, Estupiñán, Frey, & Bergado, 2002). In humans, functional neuroimaging studies are needed to identify the neural sources of such potential changes and to quantify their relationship to conditioned fear behavior.

In contrast, many studies have shown age-associated impairments on eyeblink conditioning tasks across species, even on simple paradigms (reviewed in Disterhoft et al., 2000; Powell, Buchanan, & Hernandez, 1991; Solomon et al., 1988; Thompson, 1988; Woodruff-Pak, 1997). In humans, a relationship between cerebellar volume and simple delay eyeblink conditioning in aging has been reported (Woodruff-Pak, Golenberg, Downey-Lamb, Boyko, & Lemieux, 2000; Woodruff-Pak et al., 2001). Moreover, functional connectivity between the prefrontal cortex and the cerebellum and temporal-diencephalic structures is altered with aging during simple delay eyeblink conditioning (Schreurs, Bahro, Molchan, Sunderland, & McIntosh, 2001). In nonhuman animals, changes in hippocampal activity accompany behavioral decrements on trace conditioning tasks with aging (e.g., Deyo, Straube, & Disterhoft, 1989; Weiss et al., 2000; Woodruff-Pak et al., 1987). Age-associated alterations in hippocampal inputs, the balance between hippocampal long-term potentiation and long-term depression, and hippocampal sensitivity to stress may contribute to these phenomena (reviewed in Foster, 1999; Landfield, 1988; Powell, 1999).

Collectively, these results suggest that healthy aging has dissociable effects on two forms of classical conditioning. Whereas age-associated changes in cerebellar function and connectivity affect performance on simple conditioning of somatomotor reflexes, the relative structural integrity of the aging amygdala appears to be sufficient to mediate simple forms of fear conditioning. Thus, considering the impact of healthy aging is another way to distinguish associative learning of autonomic responses and somatomotor reflexes, which differ in their operating characteristics, learning rates, neural substrates, and, perhaps, sensitivity to awareness (Gantt, 1960; LaBar & Disterhoft, 1998; Lennartz & Weinberger, 1992; Powell et al., 1991; Prokasy, 1973). Nonetheless, age-related changes in brain regions that modify both cerebellar- and amygdala-based conditioned behavior, such as hippocampal mediation of contextual and trace conditioning tasks, would be expected to impact performance similarly across these paradigms.

Role of Awareness in Aging

The role of awareness in human classical conditioning has received renewed attention in recent years. In a series of studies, Clark and colleagues (2002) have shown that awareness of the reinforcement contingency is necessary for trace, but not delay, eyeblink conditioning (but see Lovibond & Shanks, 2002). However, other studies have shown that awareness and attentional manipulations influence discriminative (multiple-cue) eyeblink

conditioning on both delay and trace tasks (Baer & Fuhrer, 1982; Benish & Grant, 1980; Carrillo et al., 2000; Knuttinen et al., 2001; Nelson & Ross, 1974; Perry et al., 1977; Ross & Nelson, 1973). There is more consistent evidence for a role of awareness in discriminative autonomic conditioning, irrespective of whether awareness is tested postexperimentally or online during acquisition training (Baer & Fuhrer, 1968, 1969, 1970; Biferno & Dawson, 1977; Dawson & Biferno, 1973; Furedy et al., 1982; Furedy & Schiffman, 1973; Hamm & Vaitl, 1996; Lovibond, 1992; Marin-kovic, Schell, & Dawson, 1989; Purkis & Lipp, 2001; Schiffman & Furedy, 1977). However, Knight and colleagues (2003) have recently provided evidence for differential conditioning of anticipatory (second-interval) SCRs in the absence of awareness when CS intensity is adjusted on a trial-by-trial basis to be just above or below perceptual threshold. Nonetheless, the response system under investigation and the type of conditioning procedure (single cue vs. multiple cue) may be critical to consider when assessing the relationship between awareness and human classical conditioning (LaBar & Disterhoft, 1998).

Although awareness cannot be directly tested in animal models, single-cue and multiple-cue fear conditioning paradigms have shown differential sensitivity with regard to the involvement of the cortex. In rats, fear conditioning on single-cue delay tasks can be mediated by either subcortical or cortical routes of information processing (Romanski & LeDoux, 1992). However, an intact cortical pathway is necessary for some forms of discriminative fear conditioning (Jarrell, Gentile, Romanski, McCabe, & Schneiderman, 1987; Teich et al., 1989; but see Armony, Servan-Schreiber, Romanski, Cohen, & LeDoux, 1997). Discriminative delay fear conditioning paradigms therefore may require additional cortical processing even in animal models.

The mechanisms that relate explicit knowledge gained through hippocampal-cortical processing and implicit conditioned associations established through amygdalar pathways remain unclear. At least two possible sources of interaction are possible. First, cortical and hippocampal regions can influence activity in the amygdala directly through inputs that terminate in the basolateral complex (Amaral, Price, Pitkänen, & Carmichael, 1992), which can modulate conditioned fear learning. Discriminative sensory processing along cortical pathways may enhance autonomic responses to the CS+ through this mechanism. Second, explicit knowledge may contribute to autonomic differentiation via direct parallel activation of the hypothalamus (via the fornix) and brainstem structures. Perhaps this additional hippocampal-cortical input is reduced in aging or is more critical for driving impoverished expression of autonomic arousal that accompanies aging. Further discussion of these issues is elaborated in Clark et al. (2002), LaBar and Disterhoft (1998), and Lovibond and Shanks (2002).

Research in other domains of implicit memory (e.g., priming) have emphasized the importance of controlling for the influence of explicit factors on task performance to correctly interpret aging effects (Fleischman & Gabrieli, 1998). Few conditioning studies, though, have investigated the role of awareness as a function of healthy aging. Knuttinen et al. (2001) reported that contingency awareness declined in healthy aging on discriminative eyeblink conditioning tasks. They also showed significantly impaired conditioning in unaware participants on both trace and delay discrimination tasks, irrespective of age. When the analyses were com-

bined across aware and unaware participants, Knuttinen et al. found a main effect of aging only during trace conditioning, although elderly participants were slower to acquire conditioned responses on the delay tasks as well. These results are largely consistent with the present study, except that we found a main effect of aging on delay fear conditioning that was solely attributable to the greater proportion of unaware participants.

Collectively, these findings have implications for using conditioning procedures to assess implicit learning in elderly populations and in age-associated memory disorders. When discriminative paradigms are used, cognitive factors may influence conditioning and contribute to age-associated impairments. These effects are critical to consider in age-associated memory disorders characterized by deficits in explicit knowledge, such as AD. AD patients show impaired eyeblink conditioning relative to age-matched controls, even on single-cue delay tasks (reviewed in Woodruff-Pak, 2001). Only one study to date has examined fear conditioning in AD (Hamann, Monarch, & Goldstein, 2002). Hamann et al. (2002) found impaired acquisition in AD patients relative to age-matched controls, which was interpreted within the context of putative amygdala pathology associated with the disease process. This study used a differential delay paradigm similar to the one in the present study, but awareness was not assessed. The authors discounted the role of awareness in explaining the deficit in AD because "simple fear conditioning is a type of nondeclarative memory that is intact in amnesia" (Hamann et al., 2002, p. 1192). The results of the present study raise the possibility that explicit factors do contribute to differential fear conditioning in elderly populations. Future studies of AD and other age-associated memory disorders should use single-cue paradigms and assess contingency awareness to rule out potential contamination of explicit strategies on task performance.

Limitations and Future Directions

Because this is one of the first systematic studies of fear conditioning and awareness across the adult life span, there are several limitations to bear in mind for future research. First, the present study used geometric shapes as CSs, which may enable the conditioning process to be more amenable to cognitive infiltration than with fear-relevant CSs (Öhman & Mineka, 2001). The impact of aging may be different during fear conditioning to phobic stimuli. Second, awareness was tested by postexperimental interview. Lovibond and Shanks (2002) have argued that this is a conservative test of awareness, susceptible to forgetting over time. Despite its conservativeness and the remoteness of its assessment, awareness nonetheless had a clear impact on conditioning both in the overall analysis and when unaware participants were considered alone, procedures that are recommended by Lovibond and Shanks. We ruled out differential forgetting as an explanation for the difference in awareness across elderly subgroups because they showed no differences in standard neuropsychological tests of delayed recall using similar retention intervals (see the Results section). We also attempted to minimize confusion about which phase of the study was relevant to the awareness interview by focusing the questions around the presentation of the US, which occurred only during acquisition. It is important to note that the data show a correlated influence, not a causal relationship, be-

tween awareness and discriminative learning. Online measures of awareness could be used in future studies to provide a more accurate assessment of awareness during learning. In elderly participants, however, such measures are likely to tax executive resources, divide attention, cause distraction, and lower performance because they require continual manipulation of a dial or trial-by-trial interruption of the conditioning procedure to indicate US expectancy (see Knight et al., 2003).

Finally, the present study was cross-sectional. A cohort of 30–40-year-olds would help identify the earliest stage at which unconditioned arousal and orienting responses begin to decline in adulthood. In some cells, our sample sizes were small; for instance, we had to double the sample size of the young adult cohort relative to the others to obtain a sufficient number of unaware participants for statistical analysis. Future research should use longitudinal designs to characterize the stability of fear conditioning with aging and to identify other factors that potentially influence age-associated decline on complex tasks, as has been done in eyeblink conditioning (Ferrante & Woodruff-Pak, 1995). Such studies would be important for advancing knowledge about the relationship among emotional learning, explicit and implicit brain systems, and the aging process.

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Appendix

List of Questions Used to Probe Contingency Awareness

- Do you remember the sound in the first part of the experiment?
- Was it upsetting for you to hear the sound in headphones?
- Did you try to anticipate when the sound would occur so you could prepare or brace yourself?
- Were you able to do so? How?
- Did you think there was any relationship between the sound and the timing of the experiment?
- Did you think there was any relationship between the sound and the presentation of the squares?
- Did you think there was any relationship between the color of the square and the sound?
- Did you notice any relationship between the red–green square and the sound?

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