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Age- and Dose-Dependent Facilitation of Associative Eyeblink Conditioning by D-Cycloserine in Rabbits

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Normal aging selectively impairs some forms of learning. For example, aging rabbits require more than twice as many trials to acquire 500-ms trace eyeblink conditioning than do young rabbits. *N*-methyl-D-aspartate (NMDA) receptor antagonists also impair trace conditioning. The effects of daily D-cycloserine (DCS; a partial agonist of the NMDA receptor-glycine site) treatment were tested on trace conditioning of young or aging rabbits using a conservative quantitative approach. DCS dose dependently improved acquisition, maximally reducing trials to criterion by ≈50%. Dose–response curves were right-shifted by aging (twice the dose was required to achieve the same enhancement compared with controls). DCS did not affect nonassociative performance but sharpened the conditioned stimulus tone intensity discrimination. DCS thus can functionally modulate NMDA receptors in normal aging, enhance associative learning at all ages, and reduce or reverse age-dependent learning deficits.

Excitatory amino acids are ubiquitous central neurotransmitters (Meldrum, Moroni, Simon, & Woods, 1991; Watkins & Collingridge, 1989) with important roles throughout the brain. A number of major classes of receptors for glutamate have been described and characterized. Initially divided based on their pharmacological and physiological characteristics into *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate, and metabotropic receptor families (see Collingridge & Lester, 1989; Watkins & Collingridge, 1989), more recent work has resulted in the sequencing, cloning, and testing of recombinant varieties of these receptors in a range of preparations (e.g., Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994). Finally, behavioral effects of compounds acting on these receptors have been probed at the organismal level (e.g., Fanselow, Kim, Yipp, & de Oca, 1994; Laroche, Tong, & Jahr, 1993; Morris, Anderson, Lynch, & Baudry, 1986; Thompson & Disterhoft, 1997). It is with these learning-specific effects that this article is concerned.

Several forms of behavioral plasticity have been shown to be dependent on activation of NMDA receptors (see Morris, Davis, & Butcher, 1990). Selective and specific NMDA receptor antagonists block the acquisition but not expression of associative learning in a number of tasks, including spatial learning (Handelmann, Contreras, & O'Donohue, 1987; Heale & Harley, 1990; Kesner, Hardy, & Novak, 1983; McCann & Winter, 1986; McLamb, Williams, Nanry, Wilson, & Tilson, 1990; Morris et al., 1986; Shapiro & Caramanos, 1990), associative contextual learning (Laroche et al., 1989), and both trace- and delay-eyeblink conditioning (Robinson, 1993; Thompson & Disterhoft, 1997). NMDA receptor activation in situ (i.e., in conditions approximating those found in intact behaving mammals) also typically requires activation of non-NMDA glutamate receptors, to relieve voltage-dependent Mg^{2+} block of the NMDA receptor's ionophore, and is thus termed *use dependent* (Honey, Miljkovic, & MacDonald, 1985).

NMDA receptors have binding sites for glutamate and other excitatory amino acids (Meldrum et al., 1991; Watkins & Collingridge, 1989), with high selectivity for NMDA (thus giving the protein making up the multiple receptor-channel complex its name). Among other properties, NMDA receptors exhibit coagonist binding sites for what are traditionally considered inhibitory amino acids, including glycine (J. W. Johnson & Ascher, 1987; Kleckner & Dingledine, 1988; Monahan, Corpus, Hood, Thomas, & Compton, 1989), which interact with the NMDA binding sites (Grimwood, Wilde, & Foster, 1993; Kemp & Leeson, 1993; Lester, Tong, & Jahr, 1993). The functional consequences of this coagonist requirement of glycine (Thomson, 1990b) for activation of the NMDA receptor are examined behaviorally in this study.

Both NMDA receptors and NMDA receptor function are significantly altered by aging, and not through simple loss of neurons or loss of receptors. NMDA receptors are still present in roughly the same areas in aging human hippocampus as in young tissue (M. Johnson et al., 1989). Extracellular concentrations of D-serine (an endogenous glycine agonist) are relatively constant (approximately 2.18 nmol/mg) in aging, Parkinson's, and Alzheimer's tissue (Chouinard, Gaitan, & Wood, 1993). However, glycine-stimulated MK-

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801 binding is reduced in Alzheimer's disease (Procter, Stirling, Stratmann, Cross, & Bowen, 1989; Procter, Wong, Stratmann, Lowe, & Bowen, 1989). Glycine binding sites as determined by ligand binding studies are reduced in number in aging rat hippocampus and cortex but are stable in number in the cerebellar region (Miyoshi, Kito, Doudou, & Nomoto, 1990). NMDA-stimulated norepinephrine release is reduced in aging hippocampus, an effect partly restored by D-cycloserine (DCS; Pittaluga, Fidele, Risiglione, & Raiteri, 1993). NMDA-stimulated excitatory postsynaptic potentials (EPSPs) and long-term potentiation (LTP) are both reduced in aging (Baskys, Reynolds, & Carlen, 1990). Magnusson and Cotman (1993), studying aging mouse hippocampus and cortex, noted a differential reduction in binding of the noncompetitive antagonist MK-801 (which binds to a site within the ionophore of the activated receptor) and in binding of agonists to the NMDA agonist site itself, with the latter exhibiting more severe reductions. They surmised that neither the simple loss of cells nor of receptors per se could account for the differences observed but that age-dependent changes in the structure of the NMDA receptor protein might account for the data. Depending on the nature of these changes, therefore, it might be possible to restore function to states approximating that observed in young tissue. For these and related reasons, a hippocampus-dependent task, trace eyeblink conditioning (Kim, Clark, & Thompson, 1995; Moyer, Deyo, & Disterhoft, 1990; Soloman, van der Schaaf, Thompson, & Weisz, 1986), which is strongly affected by aging (Solomon & Groccia-Ellison, 1996; Thompson, Moyer, & Disterhoft, 1996a), was selected for the current behavioral study to assess this issue.

Agonists and antagonists of the glycine coagonist site on the NMDA receptor have opposing effects on associative learning. Agonists potentiate learning in a number of tasks (Baxter et al., 1994; Fishkin, Ince, Carlezon, & Dunn, 1993; Flood, Morley, & Lanthon, 1992; Hood, Compton, & Monahan, 1989; Monahan, Handelmann, Hood, & Cordi, 1989; Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994), whereas antagonists impair acquisition (Matsuoka & Aigner, 1997). Previous work from our laboratory (Thompson, Moskal, & Disterhoft, 1992) has demonstrated that daily treatment with DCS (D-4-amino-3-isoxazolidone; 6 mg/kg) facilitated the acquisition of hippocampally dependent 500-ms trace eyeblink conditioning in young rabbits. DCS readily crosses the blood–brain barrier and has a long clinical history as an antibiotic (see Mandel & Sande, 1990). Most relevant to our study, DCS is a partial agonist for the glycine site on the NMDA receptor (Monahan, Corpus, et al., 1989).

Aging rabbits exhibit severe deficits in acquiring the 500-ms trace eyeblink conditioning task compared with young controls (Thompson et al., 1996a), with an increasing severity of the deficit with increasing age. Our study extends earlier work (Thompson et al., 1992) by addressing issues of the dose dependence of DCS's behavioral effects in young animals. This study also was designed to determine whether DCS could ameliorate or even reverse age-dependent learning deficits and to determine whether the dose–response curves in aging differ from those in young rabbits. Given the known differences in acquisition between young and aging rabbits, we included a number of controls to examine potential drug- or age-dependent nonassociative effects. The effects of a range of doses of DCS was tested on acquisition of the trace conditioned eyeblink response by both young and aging rabbits.

Method

Subjects

The subjects used were 75 young (3.4 ± 0.2 months) and 75 aging (37.4 ± 0.3 months) female New Zealand White rabbits (*Oryctolagus cuniculus*) obtained from Hazelton Research Products (Denver, PA) and Kuiper Rabbitry (Gary, IN). All rabbits were experimentally naive before training. The rabbits were bred for experimental use, were housed individually in a general colony room, and were maintained on a 14:10-hr light–dark schedule. Rabbits had ad-lib access to water, with a diet of approximately 170 g/d Teklad 7 (Harlan Sprague Dawley), designed by our staff veterinarians to maintain rabbit health in our restricted colony environment. Certified birthdates were obtained for all rabbits used, and complete records were maintained indicating date of arrival, surgical preparation, and daily weight. Animal care was in accordance with guidelines established by the United States Department of Agriculture and approved and managed by Northwestern University's Animal Care and Use Committee.

Health was noted daily, and any rabbit exhibiting chronic respiratory infections, postsurgical infections, gastrointestinal distress, or other conditions that could interfere with conditioning were not used. Similarly, rabbits that failed to thrive (i.e., did not maintain or gain weight before or during training) were not used. Neither was a common occurrence (less than 5% of all rabbits received from suppliers), and no age or drug treatment relationship was noted. Auditory startle reflexes were informally assessed to ensure that data from rabbits with suspected hearing difficulties were not used. All young and old rabbits used exhibited normal auditory startle responses to novel stimuli. Post mortem examinations indicated that 2 rabbits (1 young and 1 aging) omitted before study because of the absence of startle responses suffered from inner-ear infections, a relatively isolated occurrence; none of the rabbits used in the study exhibited these inner-ear inflammations. More formal tests of auditory sensitivity and of nonassociative effects of aging on eyeblink conditioning also were made and are detailed.

Experimental Groups

All rabbits used were trace conditioned, as described next, with the exception of 10 young and 10 aging rabbits that were pseudoconditioned. Groups of aging or young rabbits each received 0.25 ml daily injections containing intramuscular doses of 0.0, 0.75, 1.5, 3.0, 6.0, or 12.0 mg/kg DCS (Searle Pharmaceuticals, Skokie, IL) in 0.9% saline (pH = 7.4) within 5 min before daily training. (Higher concentrations were not tested because of difficulty in achieving solution in the small volume used within this pH range.) Each group of rabbits received only one of this range of dose treatments. All rabbits were trained in pairs, with individuals within cohort sets counterbalanced among experimental groups. One aging and 1 young rabbit always made up a pair, with multiple pairs making up each cohort set (sets were based on date of arrival in our animal care facilities). All rabbits were trained to behavioral criterion or until they failed to reach criterion, as operationally defined later. To allow for conservative assessment of DCS-related

alterations in learning, we excluded data from both rabbits within a pair if either rabbit failed to reach criterion. Enough rabbits were trained to yield a sample size of 8 per each trace-conditioning group (see the Results section).

Trace Conditioning

Rabbit trainers never knew the experimental condition of the rabbits. Standard behavioral protocols that have been previously described were used (for more details, see Moyer et al., 1990; Thompson et al., 1992; Thompson & Disterhoft, 1997; Thompson et al., 1996a; Thompson, Moyer, & Disterhoft, 1989b). All rabbits were surgically fitted with nylon restraining headbolts, were allowed 48 hr for postsurgical recovery, and then were habituated to restraint in the training chambers. Pairs of rabbits were trained in individual sound-attenuated chambers for daily 80 trial sessions, with intertrial intervals averaging 45 s. Stimulus presentation and response measurement were performed by microcomputers using custom software (Akase, Thompson, & Disterhoft, 1994). The right eyelid was held open with stainless steel eyeclips attached to Velcro straps, and nictitating membrane extension responses (NMRs) were measured noninvasively via changes in light reflectance with an optical detector (see Thompson, Moyer, Akase, & Disterhoft, 1994). The tone conditioned stimulus (CS) used for training was an 85-dB, 100-ms long, 6-kHz pure tone presented via stereo headphones. The tone CS was followed (after a 500-ms stimulus-free trace interval) by the unconditioned stimulus (US). The corneal airpuff US used for all conditioning and pseudoconditioning was a 150-ms, 3.0-psi corneal airpuff delivered from a pipette tip placed 3 mm away from the posterior corner of the right cornea, sufficient to elicit a reliable NMR as the unconditioned response (UR). A constant US intensity was used for all rabbits to allow tests for drug-induced nonassociative differences in somatosensory sensitivity to the US. Only paired CS–US trials were used for trace conditioning to avoid confounds related to CS preexposure or to blocking consequent to unpaired stimulus presentations. In our experience, this design allows satisfactory assessment of UR performance across a variety of treatment conditions (Thompson & Disterhoft, 1997; Thompson et al., 1992, 1996a). Pseudoconditioning tests also were carried out in parallel experiments and are detailed shortly.

All trace-conditioned rabbits were trained to a behavioral criterion of 80% conditioned responses (CRs; defined in all experiments as NMRs beginning after CS onset but before US onset) within an 80-trial conditioning session. Training of rabbits continued using daily 80-trial sessions until the rabbit's performance reached 64 CRs within an 80-trial session (thus termed *80% CRs* for that session) or until a total of 2,000 training trials were received if criterion was not reached (i.e., for a maximum of 25 daily sessions). Performance was assessed in terms of the number of trials required to reach this relatively rigorous behavioral criterion (see Thompson et al., 1996a). UR characteristics were typically stable after one session of training and were averaged across all trials during the final session of training, with measures of UR amplitude and latency reported.

Postacquisition CS-Intensity Discrimination Testing

An additional 12 young and 12 aging rabbits were trained to criterion using the trace-conditioning paradigm described earlier. All received daily intramuscular injections of 0.25 ml saline during this acquisition phase. On reaching criterion, aging and young rabbits were further divided into two groups, with half continuing as saline controls and half receiving daily intramuscular doses of the maximally effective dose of DCS for their age group (6.0 mg/kg for young rabbits and 12.0 mg/kg for aging rabbits) within 5 min before subsequent daily training. On the 3 days before reaching criterion, these rabbits then received an additional three sessions of paired CS–US presentations with the following variations: CS intensity was pseudorandomly varied across blocks of 5 trials, using an attenuator, in 5-dB increments. The CS intensities tested varied from 65 to 85 dB, with the highest intensity being that previously presented paired with the US during acquisition. Performance was assessed as an average of the percentage of CRs elicited for CSs of each intensity. Age- and drug-dependent effects were assessed by comparing the number of CRs elicited at different intensities.

Pseudoconditioning

Ten young and 10 aging pseudoconditioned rabbits were used to test for nonassociative effects of DCS treatment, with each receiving the same CS and US used for trace conditioning but explicitly unpaired. Half of each age group served as saline controls, and half received daily treatment with the maximally effective dose of DCS for their age group (6.0 and 12.0 mg/kg, respectively, for young and aging rabbits). Pseudoconditioning sessions consisted of explicitly unpaired presentations of 80 CS and 80 US presentations at pseudorandom intervals (10 of each in each block of 20 trials), with the average intertrial interval half that used for conditioning. Pseudoconditioned control rabbits thus received 160 trials each per session for 10 daily sessions. This ensured that pseudoconditioned rabbits received the same number of unpaired stimuli at the same average rate as paired stimuli were received by conditioned rabbits within a session. Measures of response number, amplitude, and latency were assessed and averaged across all trials and compared with results from paired trials.

Statistical Analyses

A Drug Dosage × Age (2 × 2 design) analysis of variance (ANOVA) was carried out (Statview II, Abacus Concepts). Behavioral data were then tested for main effects of drug dosage (dose dependence) independent of age group and within both age groups using one-way ANOVAs. Posttests were carried out with a Scheffé test. Statistical significance was set at the .05 level. Behavioral data are represented as means ± standard errors of the mean.

Results

All young rabbits and most aging rabbits tested were able to successfully acquire the 500-ms trace eyeblink conditioning task as defined here. Both age groups, irrespective of drug treatment, began training with low rates of responding to the tone CS, with blinks during the trace interval increasing in frequency over training (see Figure 1A). For trace-conditioned, saline-treated controls, these associative CRs increased both in frequency and amplitude over training for all young rabbits and for 8 of 12 aging rabbits, with criterion performance of 80% CRs per session reached in less than 2,000 training trials. A subset of 4 of 12 aging saline-treated control rabbits failed to reach criterion (see Figure 1B), exhibiting fewer than 30% CRs by their 25th training session (i.e., after 2,000 training trials), and were deemed very severely impaired (see Thompson et al., 1996a). Neither young nor aging pseudoconditioned rabbits

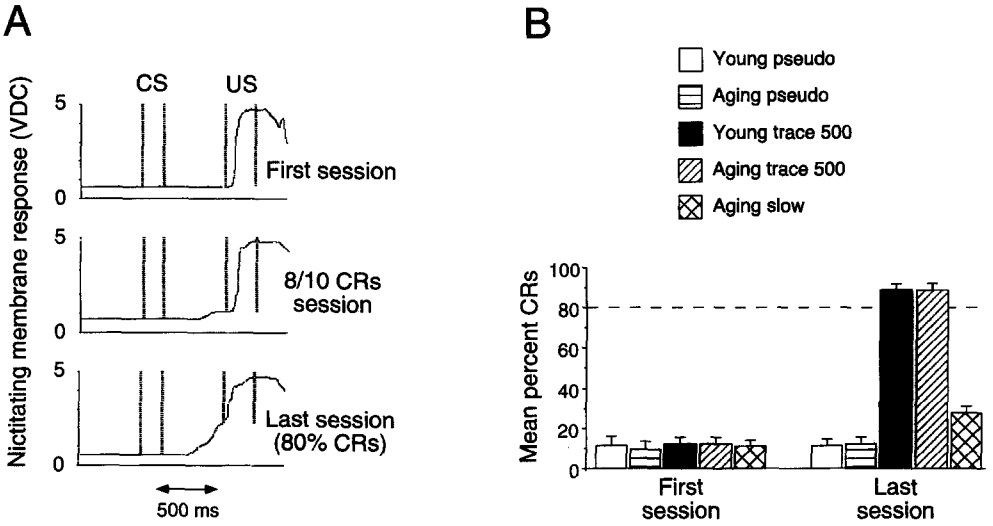

Figure 1. Trace eyeblink conditioning using a 500-ms trace interval was slowly acquired, with age-dependent deficits observed. A: Representative responses from three eyeblink conditioning sessions are shown for an aging saline-treated control rabbit. On the first session, no conditioned responses (CRs) were emitted in the 500-ms trace interval between presentation of the tone conditioned stimulus (CS) and the airpuff unconditioned stimulus (US). After five additional sessions of training (a total of 480 trials), the rabbit reached a simple criterion of 8 of 10 CRs. These CRs were of relatively short duration and low amplitude, but they met classic definitions of CRs, with a total session performance of 27.5% CRs. After a total of 960 trials (12 sessions of training), the rabbit reached behavioral criterion, emitting CRs in more than 80% of the trials. The CRs in this final session were not only more numerous but were of larger amplitude and longer duration than those observed earlier in training. B: Data are shown only for saline-treated control rabbits. Young and aging pseudoconditioned rabbits did not increase the number of blinks occurring in the 500-ms interval after unpaired CS presentation, whereas all young and most aging rabbits trained with paired CS–US presentations did so, demonstrating associative learning. However, a small subset of 5 of 53 aging rabbits tested failed to reach criterion, even after 2,000 training trials (25 daily sessions). These slow-learning rabbits failed to perform more than 30% CRs on any given session. VDC = volts DC.

increased their rates of responding to unpaired tone CSs (see Figure 1B) across trials irrespective of drug treatment ($p > .73$).

Age-dependent deficits in the acquisition of the 500-ms trace eyeblink conditioning task were found. A nonsignificant trend toward an interaction between age and dosage of DCS was observed, $F(5, 84) = 2.0$, $p > .08$. When acquisition was assessed independent of drug treatment, aging rabbits required significantly more trials to reach the behavioral criterion of 80% CRs per session than did young rabbits, $F(1, 84) = 92.9$, $p < .0001$. Aging saline-treated control rabbits required significantly more training (97.8% more trials) to acquire hippocampally dependent trace eyeblink conditioning than did young saline-treated controls ($p < .0001$). As detailed later, DCS treatment at high doses reversed the learning impairments exhibited by aging controls.

DCS, a partial agonist of the glycine coagonist binding site on the NMDA receptor, dose and age dependently improved acquisition in both young and aging rabbits (see Figure 2). Independent of age group, a dose-dependent enhancement of learning was observed, with fewer trials required to reach criterion with increasing doses, $F(5, 84) = 6.7$, $p < .001$, as can be seen in Figure 2A. When the dose–response curves were broken down by age group (see Figure 2B), an inverted U-shaped curve was observed for young rabbits and a progressive decline with increasing dosage was observed for aging rabbits. The dose-dependent effects of DCS were significant both for young, $F(5, 42) = 7.4$, $p < .0001$, and for aging, $F(5, 42) = 3.3$, $p < .01$, rabbits. In young rabbits, doses of 3.0 and 6.0 mg/kg produced significant enhancements compared with saline controls ($p < .003$). In aging rabbits, doses of 6.0 and 12.0 mg/kg produced significant enhancements compared with saline controls ($p < .01$). DCS apparently dose dependently eliminated the age-dependent behavioral heterogeneity (i.e., the severe disability of some aging rabbits that resulted in their inability to reach criterion) observed in this task. Only 1 of 9 aging rabbits tested at the lowest dose (0.75 mg/kg) of DCS failed to reach criterion, compared with 4 of 12 saline controls. All aging rabbits receiving higher doses of DCS ($\geq$1.5 mg/kg) reached criterion.

A notable rightward shift in the dose–response curves was observed with aging (see Figure 3A). Compared with saline controls, the maximal improvement in acquisition produced by DCS treatment approximated a 50% reduction in trials to criterion. However, approximately twice the dose of DCS was required to produce the same effect in aging than in

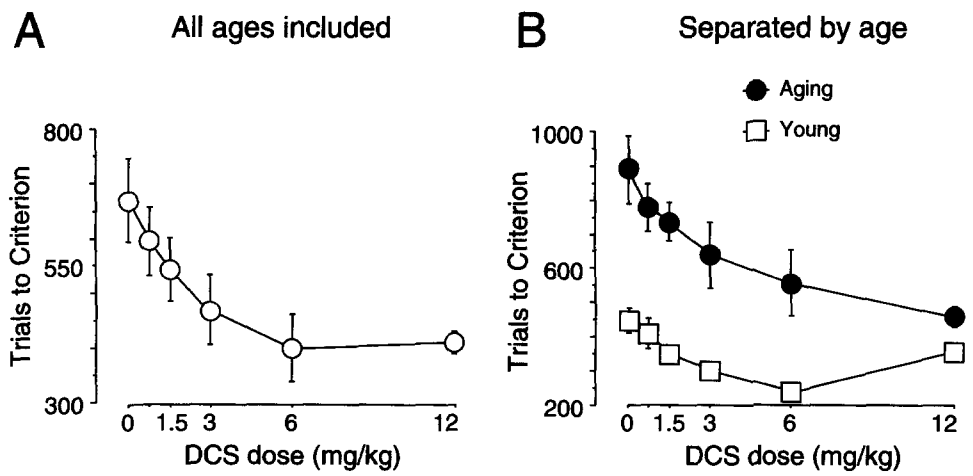

Figure 2. A: D-cycloserine (DCS) dose dependently enhanced the acquisition of trace conditioning, irrespective of age group. Although this is a statistically significant main effect, only a shallow U-shaped inflection is observable in the dose–response curve when aging and young rabbits are lumped together. B: Dose–response curves for the two different age groups tested exhibited different shapes. The U-shaped dose-dependence curve seen in testing with young rabbits may not have been apparent in aging because of technical difficulties that prevented testing of higher doses.

young rabbits. We found (see Figure 3B) that DCS treatment at the highest dose tested restored acquisition of aging rabbits to performance indistinguishable from that of young saline-treated controls ($p > .72$).

DCS treatment was without effects on the UR (see Table 1), measured both during conditioning ($p > .81$) and in pseudoconditioning controls treated daily with the maximally effective dose of DCS for each age group ($p > .91$). Both aging and young pseudoconditioned rabbits treated with DCS exhibited eyeblink responses indistinguishable from those of saline-treated controls, supporting the hypothesis that DCS specifically enhanced associative learning rather than nonassociative responses to sensory stimuli.

CS intensity discrimination studies indicated that DCS

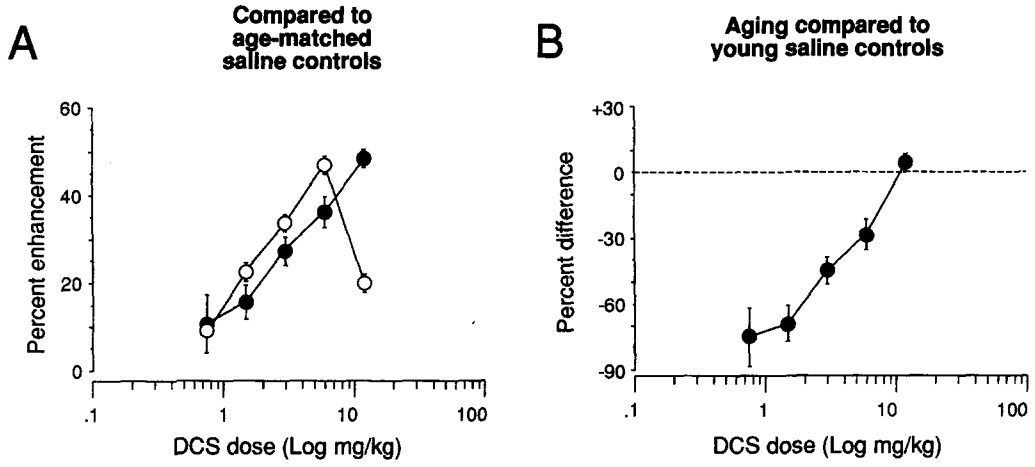

Figure 3. The dose-dependent facilitation of learning produced by daily treatment with D-cycloserine (DCS) is readily shown by comparing the performance of DCS-treated rabbits with that of saline controls. A: DCS dose dependently enhanced acquisition (when compared with age-matched saline controls) in both young and aging rabbits. However, aging rabbits required higher doses to achieve proportionately similar learning enhancements. The dose–response curve was rightward shifted by a factor of approximately 2 by aging (i.e., twice the dose was required to produce an equivalent enhancement in aging rabbits when compared with the effects observed in young rabbits). B: Treatment with DCS was effective in reducing or even eliminating age-dependent learning deficits, as seen in these comparisons with the acquisition of young control rabbits. Even low doses of DCS improved learning in aging rabbits. However, higher doses significantly improved the acquisition of aging rabbits, with a dose of 12 mg/kg producing acquisition rates that were essentially identical to those observed for young saline controls. Open circles = young; closed circles = aging.

Table 1
Response Measures Not Affected by Age or Drug Treatment

Age (months)/ Dose of DCS	UR amplitude (mV)	UR peak latency (ms)	CR frequency (% within ISI used for conditioning)
	Trace conditioned		
3.4 ± 0.2			
0.00	3,048 ± 158	204 ± 46	
0.75	2,992 ± 169	201 ± 53	
1.50	3,064 ± 147	213 ± 48	
3.00	3,027 ± 139	202 ± 43	
6.00	3,023 ± 126	209 ± 52	
12.00	3,041 ± 142	211 ± 55	
37.4 ± 0.3			
0.00	3,016 ± 157	221 ± 52	
0.75	3,002 ± 149	229 ± 46	
1.50	2,987 ± 153	218 ± 39	
3.00	3,027 ± 144	233 ± 49	
6.00	2,997 ± 147	221 ± 47	
12.00	3,005 ± 152	217 ± 51	
	Pseudoconditioned		
3.4 ± 0.2			
6.00	3,024 ± 178	215 ± 35	12.2 ± 3.5
37.4 ± 0.4			
12.00	3,031 ± 153	223 ± 41	10.1 ± 3.8

Note. Daily treatment with DCS had no effects on unconditioned responses, although acquisition of trace-conditioned eyeblinks was significantly enhanced by doses greater than 3.0 mg/kg im. Similar observations about a lack of effect on response sensitivity were obtained in pseudoconditioning control experiments with saline- and DCS-treated rabbits. DCS = D-cycloserine; UR = unconditioned response; CR = conditioned response; ISI = interstimulus interval.

did not alter auditory sensitivity to the CS intensity used for conditioning ($p > .55$) but that it did alter discrimination of lower intensity CSs, $F(1, 4) = 8.9$, $p < .03$ (see Figure 4). Equal numbers of CRs were observed for both DCS- and saline-treated controls at the 85-dB CS intensity used for training. No age-dependent differences in sensitivity to less intense CSs were noted when comparing the performance of either saline- or DCS-treated rabbits ($p > .41$), with both age groups being equally successful at discriminating different CS intensities. The magnitude of the discrimination was significantly greater in DCS-treated than control rabbits ($p < .01$), again without any age-dependent effects. At CS intensities of 65 dB (20 dB below that trained), the performance of DCS-treated rabbits was near naive levels of performance, whereas that of saline controls was less than half of criterion performance.

Discussion

Daily treatment with DCS both dose and age dependently enhanced acquisition of 500-ms trace eyeblink conditioning (see Figure 2). It did so without altering nonassociative behavioral parameters as demonstrated during conditioning and in pseudoconditioning control experiments (see Table 1). Changes were noted, however, in discrimination of auditory tone CSs of much lower intensity than those paired with the US in training (see Figure 4). Treatment with DCS led to low rates of responding to previously unpaired CS intensities, again suggesting a fairly specific enhancement of associative information processing. Furthermore, treatment with DCS was highly effective in reversing age-dependent deficits in acquisition, although a rightward shift in the dose–response curve was apparent in aging (see Figure 3).

Aging (37.4 ± 0.3 months) saline-treated control rabbits required nearly twice as many training trials to reach criterion in the 500-ms trace conditioning task as did young (3.4 ± 0.2 months) controls. As in a prior study (Thompson et al., 1992), daily treatment with 6.0 mg/kg DCS reduced the number of trials required to reach criterion by nearly half in young rabbits. In aging rabbits, however, treatment with this same dose (6.0 mg/kg) produced a reduction in trials to criterion of only one third. A dose of 12.0 mg/kg DCS daily was required in aging rabbits to produce the same enhancement in learning as 6.0 mg/kg DCS produced in young rabbits. This general twofold rightward shift in the dose–response curve for DCS was observed across the range tested in aging. In young rabbits, a fairly narrow effective range of doses was observed (3.0–6.0 mg/kg), with reduced enhancement seen at higher doses. Technical difficulties (see the Method section) precluded testing still higher doses in aging rabbits to determine whether a similar reversed inflection in the dose–response curve (i.e., an inverted U-shaped curve) occurred at higher doses. Doses of 6.0–12.0 mg/kg DCS, however, were required to significantly enhance learning in aging rabbits.

Despite the rightward age-dependent shift in the dose–response curve, daily treatment with 12.0 mg/kg DCS was able to restore learning rates in aging rabbits to those approximating young controls. Thirty-seven-month-old rab-

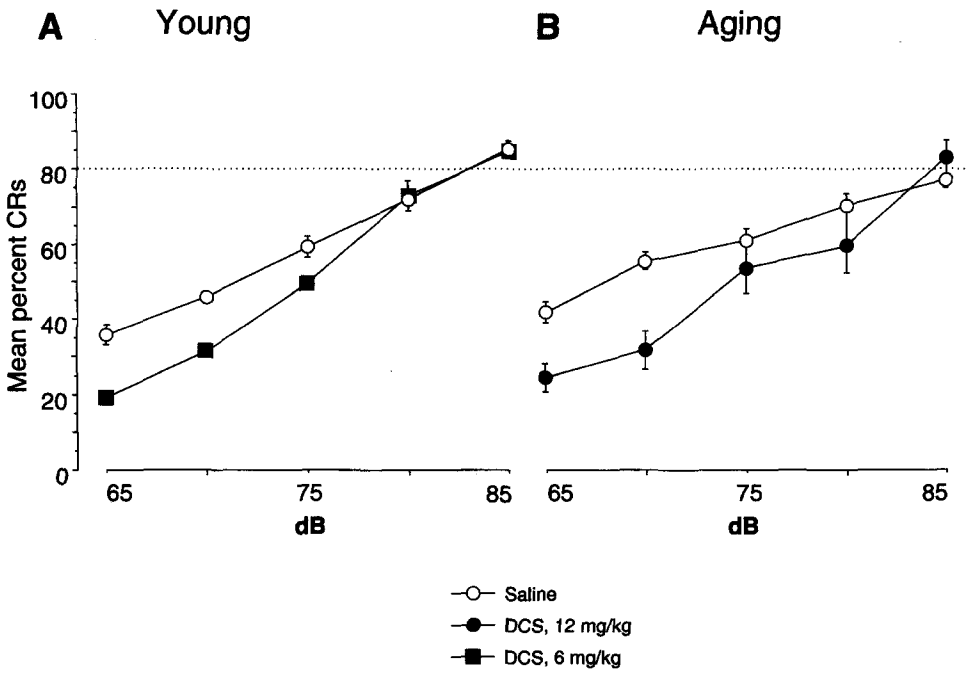

Figure 4. Conditioned stimulus (CS) intensity discrimination curves for young (A) and aging (B) rabbits. No age-dependent effects were noted in the ability of rabbits to discriminate tone stimuli of the same duration and frequency used in training but differing in intensity (SPL, measured in decibels), irrespective of drug treatment (i.e., both aging and young rabbits behaviors were similar). Saline-treated controls responded at criterion performance levels to the 85-dB CS intensity that had previously been paired with the unconditioned stimulus, but blinked less frequently to lower intensity tones. Performance to CSs of 75 dB or less was significantly lower than to 85 dB CSs ($p < .01$) for controls. Performance at the lowest two intensities tested (65 and 70 dB) was significantly reduced for DCS-treated rabbits compared with saline controls ($p < .03$) in both age groups (young or aging). Coupled with data from pseudoconditioning, it appears that DCS did not alter sensitivity to the 85-dB tone CS used in training but that it enhanced discrimination of CS intensities that differed from those associatively paired in training. CRs = conditioned responses; DCS = D-cycloserine.

bits treated with 12.0 mg/kg DCS daily acquired the 500-ms trace-conditioned eyeblink task in 460 ± 29 trials, compared with 450 ± 37 trials for young saline-treated controls. The difference in performance between these two groups was statistically nonsignificant but practically important, given that aging saline control rabbits required 890 ± 97 trials to acquire the same task. Aging control rabbits thus required significantly more training to reach criterion than young controls, an age-dependent deficit that was fully reversed by treatment with DCS. This effect was conservatively assessed. Only data from rabbits that actually acquired the task were included for analysis (i.e., no artificial assumptions were made regarding the learning curves of rabbits that failed to reach criterion within 2,000 training trials). Additionally, no comparative data from these rabbits' matched rabbits within pairs were used (see the Method section), further guarding against artifactual errors in interpretation imposed by extraneous factors (variations in ambient noise in the colony rooms, in temperature, etc.) that would tend to create cohort-specific effects.

Another serendipitious finding of our study was the apparent ability of sufficient doses of DCS to eliminate severe age-related learning disabilities (or at least those observed in the 500-ms trace conditioning task; see Thompson et al., 1996a). As noted, a small subset of aging control rabbits (4 of 12 tested here) were unable to reach criterion, exhibiting fewer than 30% CRs even after 2,000 training trials (see Figure 1). This heterogeneity was reduced even by low doses of DCS, with only 1 of 9 aging rabbits receiving 0.75 mg/kg DCS failing to reach criterion and all rabbits receiving higher doses (≥1.5 mg/kg DCS) reaching criterion. This effect deserves and will require further study. Unfortunately, we know of no methodology to preidentify (i.e., before training) individual aging rabbits that would fall into this severely impaired group. Developing such methods is a necessary requirement before conducting more rigorous analyses of this phenomenon.

As can be seen in Table 1, DCS treatment did not affect UR performance either during conditioning or during pseudoconditioning. Additionally, DCS treatment did not alter the relatively low number of nonassociative "false" CRs exhibited in response to the unpaired tone CS in pseudoconditioning, indicating that no behavioral sensitization or desensitization to the tone CS occurred. However, DCS treatment did alter CS intensity discrimination curves (see Figure 4) at intensities 15–20 dB below those paired in trace

conditioning, suggesting that a more specific enhancement of the associativity of relevant stimulus parameters occurred, inhibiting responses to inappropriate stimuli differing in salience from the paired stimulus. Again, this methodology and these analyses of the discrimination effects cannot claim to be definitive, and further work specifically focused on these issues might yield data subject to other interpretations. Additional work also is clearly needed to determine whether DCS is effective in reducing less severe age-dependent learning deficits observed in delay-eyeblink conditioning (see Solomon et al., 1986) and in other tasks.

DCS is a rigid analog of D-alanine. Unlike dietary amino acids (serine, alanine, etc.) that exhibit activity at the glycine coagonist site in vitro, DCS is excreted virtually unmetabolized, readily crosses the blood–brain barrier, and rapidly reaches concentrations in cerebral spinal fluid comparable to those in serum (Hanngren, Hansson, & Nelberg, 1961). DCS acts as a partial agonist of the glycine coagonist site on the NMDA receptor (Hood et al., 1989; McBain, Kleckner, Wyrick, & Dingledine, 1989; Watson, Bolanowski, Baganoff, Deppeler, & Lanthorn, 1990), competing with glycine for binding at the glycine site. DCS is less efficacious than the native agonist, yet it still demonstrates reasonable specificity and high affinity for the receptor. Saturating concentrations of (+)-HA-966, a specific antagonist for the glycine site on the NMDA receptor, block the learning-enhancing effects of DCS, indicating single-receptor specificity for the observed effects (see Matsuoka & Aigner, 1997), whereas DCS reverses the impairments produced by MK-801. Coupled with the lack of deleterious side effects at the relatively low doses required for behavioral effect and the lack of nonassociative behavioral effects, treatment with DCS would appear to offer considerable clinical promise.

The range of neuronal plasticity mediated via NMDA receptors is wide. Initial work with compounds acting at the glycine coagonist site often used the term modulatory because glycine alone does not activate the receptor ionophore, but it apparently is required for successful activation in conjunction with excitatory amino acids (Grimwood et al., 1993; J. W. Johnson & Ascher, 1987; Kemp & Leeson, 1993; Kleckner & Dingledine, 1988; Monahan, Corpus, et al., 1989; Yoneda, Suzuki, & Ogita, 1994). NMDA receptors and their associated glycine recognition sites are particularly enriched in the hippocampal region (McDonald, Penney, Johnston, & Young, 1990). In vitro, single NMDA-mediated synaptic EPSPs are enhanced by addition of exogenous glycine (Thomson, 1990a). Additionally, both in vivo (Mizutani, Saito, & Abe, 1991; Thiels, Weisz, & Berger, 1992) and in vitro (Izumi, Clifford, & Zorumski, 1990), glycine antagonists such as cycloleucine or 7-chlorokynurenic acid block induction of NMDA receptor-dependent LTP and glycine agonists facilitate LTP. These results indicate that extracellular glycine concentrations are likely below that required for receptor saturation, allowing for functional modulation of the NMDA receptor via addition of exogenous agonists or partial agonists such as DCS.

In vivo, as the current study and earlier studies (Baxter et al., 1994; Contreras, 1990; Herberg & Rose, 1990; Quartermain, Nuygen, Sheu, & Herting, 1991; Thompson et al., 1992) demonstrate, treatment with exogenous glycine agonists facilitate the learning of new behaviors. DCS treatment has been shown to be effective in reversing deficits in visual discrimination and in delayed non–matching-to-sample tasks in monkeys (Matsuoka & Aigner, 1997). Similarly, DCS treatment improved age-dependent deficits in place discrimination and in repeated acquisition of spatial tasks in a Morris water maze in rats (Baxter et al., 1994). Nonassociative behavioral changes have not been found in our own or others' work, but a more specific enhancement of associativity of salient stimuli has. Together, these findings suggest that the effects of DCS are not limited to a single task or to a single species but indicate a more general cognitive enhancement, with particular relevance in aging.

NMDA receptor function is notably impaired in normal aging (Barnes, 1994; Baskys et al., 1990; Gonzales et al., 1991; Magnusson & Cotman, 1993; Pittaluga et al., 1993; Tamaru, Yoneda, Ogita, Shimizu, & Nagata, 1991; Wenk, Walker, Price, & Cork, 1991) and more severely altered in age-associated clinical disorders such as Alzheimer's disease (Carlson, Penney, & Young, 1993; Procter, Stirling, et al., 1989; Procter, Wong, et al., 1989). Another major class of excitatory amino acid receptors also hypothesized to be involved in learning, AMPA receptors, are less affected by aging (Miyoshi, Kito, Doudou, & Nomoto, 1991), whereas glycine sites on the NMDA receptor are greatly reduced in the aging hippocampus (Miyoshi et al., 1990). The current results, coupled with the work of others, suggest that (at least) in normal aging NMDA receptor function, although impaired, can still be effectively restored with a compound that modulates activity of the glycine site. Whether this is also the case in Alzheimer's disease and other pathological conditions remains to be determined, although DCS is capable of altering noncompetitive NMDA receptor antagonist binding even in Alzheimer's brain tissue (Chessell, Procter, Francis, & Bowen, 1991). Treatment with DCS also may offer relief in other age-related learning disabilities. Our results also suggest that DCS treatment may be effective in improving learning disabilities occurring earlier in the life span (including possible developmentally related deficits). These and related issues offer exciting avenues for further research.

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