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BRIEF REPORT

D-Cycloserine Causes Transient Enhancement of Memory for a Weak Aversive Stimulus in Day-Old Chicks (*Gallus domesticus*)

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The *N*-methyl-D-aspartate (NMDA) receptor plays an important role in acquisition of memory after one-trial passive avoidance training in day-old chicks. Here, we examined the effect of D-cycloserine, a partial agonist of the glycine site on the NMDA receptor, on memory retention after training on weak aversive stimuli (10% methyl anthranilate). Bilateral, or unilateral, intracranial injections of 5 μl of 600 $\mu mol \cdot l^{-1}$ D-cycloserine (DCS) were made into the intermediate and medial hyperstriatum ventrale 5 min posttraining and chicks were tested either 30 min or 1, 6, or 24 h later. Enhanced memory retention (number of chicks avoiding) occurred after bilateral, or left hemisphere, injections of DCS (compared to saline-injected controls), when chicks were tested at 1 and 6 h, but not at 30 min or 24 h posttraining. These data suggest that activation of the glycine site on the NMDA receptor in the immediate posttraining period can transiently extend the retention period of memory for a weak aversive stimulus.

The *N*-methyl-D-aspartate (NMDA) subtype of the glutamate receptor is prominently involved in excitatory transmission in the mammalian CNS and plays a key role in synaptic plasticity, including that associated with long-term potentiation (LTP) and memory formation (Bliss & Collingridge, 1993). Binding of L-glutamate to the NMDA receptor site is enhanced by glycine (Thomson, Walker, & Flynn, 1989; Bliss & Collingridge, 1993) and glycine also appears to play an important role in LTP induction (Thiels, Weisz, & Berger, 1992). An enhancement of learning performance has been reported following modulation of the NMDA receptor by the partial glycine agonist D-cycloserine (DCS) (Monahan, Handelmann, Hood, & Cordi, 1989; Thompson, Moskai, & Disterhoft, 1992; Baxter Lanthorn, Frick, Golski, Wan, & Olton, 1994) (the partial agonist was used to avoid the possibility of seizures). Moreover, Schuster and Schmidt (1992) have demonstrated reversal of impairment of memory in hippocampal-lesioned rats following administration of D-cycloserine, and memory deficits induced by muscarinic antagonists have been attenuated by treatment with D-cycloserine (Fishkin, Ince, Carlezon, & Dunn, 1993). However, in contrast, in primates, D-cycloserine did not reverse scopolamine- or phencyclidine-induced cognitive disruption (Rupniak, Duchnowski, Tye, Cook, & Iversen, 1992).

In the domestic chick considerable progress has been made in describing the cascade of events associated with memory formation and consolidation following one-trial passive avoidance training. In this task day-old chicks are presented with a chrome bead coated with a bitter-tasting substance, methyl anthranilate (MeA) (Cherkin, 1969; Rose, 1995; Stewart, Bourne, & Steele, 1992; Stewart et al., 1992). The chicks peck once and will subsequently avoid a similar but dry bead for several days. As in mammals, NMDA receptors play an important role in memory formation, and are activated within minutes of training (Stewart et al., 1992). At 30 min after

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training, increases occur in binding to NMDA receptors in specific forebrain regions, notably the left intermediate and medial hyperstriatum ventrale (IMHV) and the left lobus parolfactorius (LPO) (Stewart et al., 1992). We have also shown that the glycine site on the NMDA receptor is important in memory formation in the chick. Blockade of this site by pretraining intracranial injection of the highly selective antagonist, 7-chlorokynurenate (7-ClK), into the left IMHV, prevented acquisition of memory in day-old chicks (Steele & Stewart, 1993). Bilateral injections did not significantly block memory over that achieved by unilateral left hemisphere injection.

This led us to ask whether a compound with glycine-like activity, such as D-cycloserine, could enhance memory for the aversive experience in the chick. Passive avoidance training is especially suitable for measurement of enhancement of learning because it is possible to train chicks using weak aversants (Crowe, Ng, & Gibbs, 1989), e.g., a dilution of full-strength methyl anthranilate (Sandi & Rose, 1994) or quinine dissolved in alcohol (Bourne, Davies, Stewart, Csillag, & Cooper, 1991). These weak aversants cause the immediate disgust reaction seen with 100% MeA, but do not produce the normal high level of memory retention thereafter (Bourne et al., 1991), in contrast to the retention for full-strength MeA at this time which, up to 24 h later, is little different from that observed shortly after training (75–90%).

In the present study we investigated the effect of intracerebrally injected D-cycloserine (DCS) on enhancement of retention of memory for a 10% methyl anthranilate solution. Our data show transient enhancements in memory up to 6 h after training, but at 24 h the DCS-injected chicks show little difference in memory recall compared to saline-injected control birds.

Training. Training was essentially as described previously (Steele & Stewart, 1993; Stewart & Rusakov, 1995). In a series of experiments, groups of 50 chicks were placed in pairs in pens (under red light). Chicks were pretrained by presenting a dry white bead, three times to each pair, to encourage pecking. On the training trial chicks were presented with a 3-mm-diameter chrome bead dipped in a 10% solution (in ethanol) of the bitter-tasting substance MeA. Control chicks were presented with a similar bead coated only with water. Chicks will normally spontaneously peck at the bead and indicate that they have tasted the aversive MeA by exhibiting the appropriate response, i.e., head shaking, beak wiping, and eye closure. Those chicks which failed to peck the bead within 30 s (usually less than 5% of the total in a one-training session), were excluded from the study.

Injections. Chicks were injected intracerebrally (ic) using Hamilton microsyringes fitted with plastic sleeves which act as stops, and a Plexiglas head holder (Burchuladze & Rose, 1992) designed to direct injection into the IMHV. In a preliminary experiment chicks were given 5-μl ic injections in both right and left hemispheres with a range of concentrations of D-cycloserine (DCS-Tocris Neuroamin) (0.1–2000 $\mu mol \cdot liter^{-1}$ in 0.9% sterile saline). A 5-μl injection of 600 $\mu mol \cdot liter^{-1}$ DCS was found to be a satisfactory dose, defined as that which did not debilitate the chicks' natural pecking behavior compared with non-injected chicks on presentation of a white or chrome bead. All subsequent injections were 5 μl of 600 $\mu mol \cdot liter^{-1}$ of DCS per hemisphere with 5 μl of 0.9% sterile saline as an injection in control chicks. Chicks were injected either bilaterally or unilaterally into the left or right hemisphere and were then tested by presenting a dry chrome bead either 30 min or 1, 6, or 24 h posttraining. To ensure that there were satisfactory numbers of control and trained chicks at each time point the experiment was repeated several times (on separate days) until there were approximately 25 DCS- and 25 saline-injected birds. All testing procedures were performed "blind" with the experimenters not knowing the chicks' experimental history. Data were analyzed using χ^2 tests, with a null hypothesis that DCS has no effect on memory formation or retention.

The results of the bilateral injections of DCS (5 min posttraining) on memory retention following training on 10% MeA are shown in Fig. 1. When DCS was injected bilaterally no significant enhancement of memory retention (i.e., of the number of chicks avoiding relative to the total number of chicks) was observed at 24 h posttraining (Fig. 1), but at 1 and 6 h after training a significant enhancement of the number of chicks avoiding was seen (at 1 h posttraining an increase of 26%, $p < .05$, and at 6 h an increase of 30%, $p < .05$) (Fig. 1). At 30 min posttraining the apparent enhancement in retention (24%) falls just below the level of significance.

When DCS injections were made unilaterally to either the right or left hemisphere (Fig. 2) at 5 min posttraining, with testing at 1 and 6 h posttraining, a significant enhancement of memory retention was observed only following injections to the left, and not the right, hemisphere. At 1 h posttraining the enhancement in memory retention is 29% in birds injected in the left hemisphere compared to controls injected with saline in the left hemisphere ($p < .05$), while at 6 h posttraining left hemisphere DCS-in-

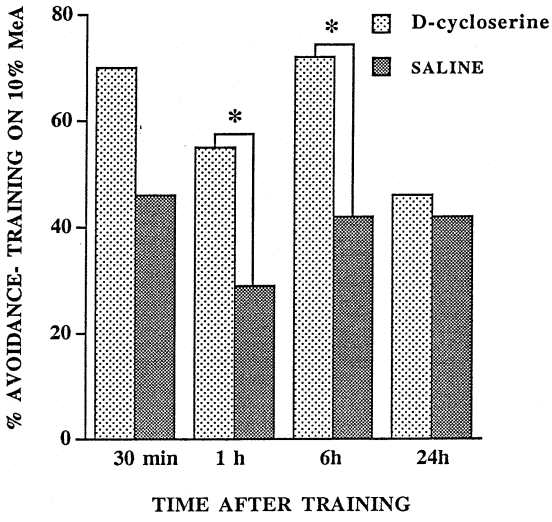

FIG. 1. Percentage avoidance of chicks injected bilaterally with 600 μmol · liter^{-1} D-cycloserine (DCS) (in 0.9% sterile saline) 5 min posttraining and tested either 30 min or 1, 6, or 24 h later. Differences between DCS-injected and saline-injected chicks are significant as indicated at $p < .05$.

jected birds show a 22% enhancement of memory over saline-injected birds ($p < .05$) (Fig. 2). This finding of a lateralized effect of DCS is supported by our previous data which demonstrated that blockade of the glycine site on the NMDA receptor by intracranial injection into the left IMHV of the highly selective antagonist 7-ClK prevented acquisition of memory in day-old chicks (Steele & Stewart, 1993). There is a difference in the time of effectiveness of the antagonist 7-ClK (only pretraining) and the agonist DCS (effective only posttraining). The most likely explanation in our view is that blockage of the site by 7-ClK pretraining impairs binding to the NMDA site. However, when injections are given posttraining, the NMDA receptors are already activated by a large response to the strong aversive stimulus of 100% MeA, which 7-ClK (at the concentration used) cannot prevent. However, in the present experiments, when DCS is given posttraining, it enhances the submaximal response to a weak stimulus (10% MeA) and thereby increases the number of birds showing retention.

It is possible that the apparent enhancement of memory results simply from short-term prevention of pecking after administration of DCS. However, this seems unlikely as the DCS-injected chicks did not show any altered motor behavior. Moreover, in preliminary experiments to establish a suitable dose of DCS, when intracerebral injections were also given pretraining, there were no qualitative difference from saline-injected chicks in terms of ability to peck at white or chrome beads, except at concentrations considerably in excess of the 600 μmol·liter^{-1} used here.

Since DCS acts as a partial agonist at the glycine site on the NMDA receptor, and blockade of this receptor causes amnesia in chicks (Steele & Stewart, 1993), it seems reasonable to propose that activation of the site by DCS has altered the functional state of the NMDA receptor, leading to increased postsynaptic activation. It also would appear likely, based on previous investigations of the diffusabilty of the glutamate receptor antagonist MK801 in the chick forebrain (Burchuladze & Rose, 1991), that a 5-μl volume of DCS injected into the IMHV would diffuse to other regions in the dorsal part of the forebrain, aided by the fact that extracellular space is quite large in the brain of the newly hatched chick, so the site of injection cannot be taken to determine the sole region in which the drug acts. Nevertheless, the finding that DCS enhances memory formation in the left, but not the right, hemisphere is in accordance with previous work in our laboratory (and that on 7-ClK described above), which suggests that the left IMHV plays a crucial role in the initial phases of memory acquisition for the avoidance response (Stewart & Rusakov, 1995; Rose, 1995). Indeed our data from a recent study using quantitative receptor autoradiography have shown a 39% increase in binding to NMDA receptors in the left IMHV and a smaller increase in the left LPO at 30 min posttraining (Stewart et al., 1992). Moreover, a significant increase in presynaptic glycine release in the left IMHV, 30 min after passive avoidance training, has

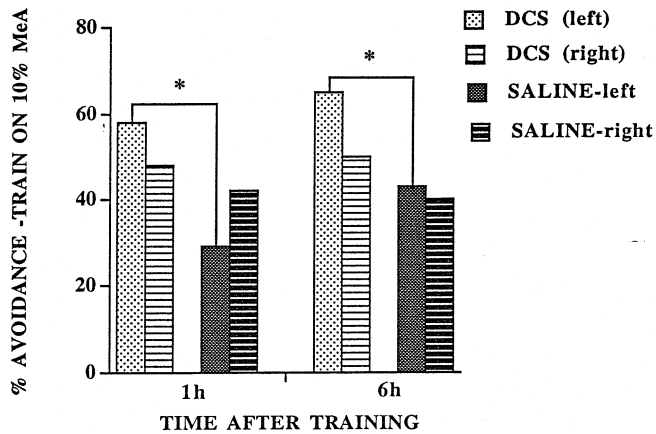

FIG. 2. Percentage avoidance of chicks injected unilaterally in either the left or right hemisphere with 600 μmol · liter^{-1} D-cycloserine (DCS) (in 0.9% sterile saline) 5 min posttraining and tested either 1 or 6 h posttraining Differences between DCS-injected and saline-injected chicks are significant as indicated at $p < .05$.

been demonstrated recently in our laboratory (Daisley, personal communication). As DCS was injected posttraining, it would appear that DCS does not enhance acquisition of memory per se, rather it affects consolidation processes but, since this is not maintained long term, it clearly falls short of initiating the cascade of events required for long-term memory in the chick.

A multistage model of memory formation in chicks has been proposed by Ng and Gibbs (1991) and Rosenzweig, Bennett, Martinez, Beniston, Colombo, Lee, Patterson, Schulteis, and Serrano (1991), comprising stages posttraining that include short (STM, up to 15 min), intermediate (ITM, up to 55 min), and long terms (LTM, 1 h up to several or more days). However, evidence from Rose (1995) indicates that at the cellular level a two-stage model of memory formation can be described. It has been known for some time that glycoproteins play a key role in long-term memory formation, due to their part in synaptic restructuring processes. The work of Rose (1994) and Crowe, Zhao, Sedman, and Ng (1994) suggests that short-term memory involves a first wave of glycoprotein production resulting not from *de novo*, but only posttranslational, modification of existing proteins. For long-term memory to be formed there is a second wave of glycoprotein synthesis (occurring approximately 6 h posttraining) which involves new protein synthesis. In our previous study, we showed that when chicks were trained on quinine, which like 10% MeA is only weakly aversive, there was no new protein synthesis in this second wave (Bourne et al., 1991). One interpretation of our present data on the enhancing effects of DCS on memory retention for the weak aversive stimulus of 10% methyl anthranilate is that it may enhance only the posttranslational modification of preexisting proteins that affect the first wave of protein synthesis, but is not sufficient to trigger the second wave and consequently long-term memory for the weak aversive stimulus. However, since our experiments have not explored the period between 6 and 24 h, we cannot rule out the possibility that retention lasts longer than 6 h.

In summary, the present data coupled with our previous report on the amnestic effect of the glycine antagonist 7-ClK on memory formation strongly suggests that in the chick, as in mammals, the allosteric glycine binding site on the NMDA receptor has a crucial role in the early phase of learning.

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