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Impaired visual memory in rats reared in isolation is reversed by D-cycloserine in the adult rat

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Abstract

Previous studies have shown that environmental factors can influence cholinergic and glutamatergic activity in the developing brain, and that the variations in neurochemistry are accompanied by behavioral changes in later life. Rats reared in isolated, social, or enriched environments were tested with a visual discrimination task in adulthood. The results show that saline-treated rats reared in isolation exhibited impaired retention of the discrimination task compared to rats raised in social or enriched environments. However, systemic administration of the NMDA receptor agonist, D-cycloserine (3 mg/kg), restored normal memory function in cognitively impoverished rats. Acquisition of the task was not affected by the rearing conditions. D-Cycloserine is considered to be an efficient cognitive enhancer probably able to compensate for assumed loss of NMDA receptors during isolated rearing.

Keywords: Neonatal rat; Rearing; NMDA receptor; D-cycloserine; (Adult rat); Discrimination task

1. Introduction

Differences in housing conditions have particular impact on the central nervous system of the developing rat. It was demonstrated some decades ago that rats housed in rich environments displayed increased brain weight, higher levels of acetylcholinesterase in neocortex, and increased number of dendritic spines compared to rats reared in isolation, whereas rats reared in social environments fell somewhere in between (Rosenzweig, 1984). These brain measures were found to correlate positively with cognitive measures (Rosenzweig, 1984). The antagonistic effects of enriched and isolated rearing conditions lead to different levels of high-affinity D-aspartate uptake in synaptosomes from the lateral entorhinal cortex in adult rats. The differences in this glutamatergic parameter were seen to correlate with learning and memory of a visual discrimination task (Myhrer et al., 1992). The excitatory responses to glutamate are mediated by several receptor subtypes, among which the NMDA receptor may be of particular interest for cognitive function. Chronic neonatal blocking of the NMDA receptor results in impaired spatial learning in the adult rat (Gorter and De Bruin, 1992).

The reduced glutamatergic activity observed in the lateral entorhinal cortex of rats reared in isolation (Myhrer et al., 1992) reflects loss of glutamate terminals and probably decreased number of NMDA receptors, although this was never demonstrated explicitly. It has been shown, however, that adult rats undergoing spatial training in a complex environment have increased spine density (an index of the number of excitatory synapses) in hippocampal CA1 basal dendrites compared to that of rats kept in isolation (Moser et al., 1994). Administration of an NMDA receptor agonist may be expected to compensate for a low number of NMDA receptors by producing more effective responding to endogenous glutamate. The NMDA receptor complex consists of several receptor sites, and the glycine recognition site was addressed in the present study.

The partial agonist, D-cycloserine, has been shown to be a positive modulator of the glycine site of the NMDA receptor and elicits about 60% of the maximal response to glycine (Huettner, 1991; Monahan et al., 1989). However, D-cycloserine in higher doses than 30 mg/kg appears to exhibit antagonistic properties in rats (Anthony and Nevins, 1993). Systemic administration of D-cycloserine (<30 mg/kg) has been shown to function as an effective cognitive enhancer. D-Cycloserine improves spatial memory in aged rats (Baxter et al., 1994; Aura and Riekkinen, 2000), reverses memory deficits induced by (+)-10,11-dihydro-5-methyl-5*H*-dibenzo[*a*,*d*]cycloheptene-5,10-imine (MK-801)

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or scopolamine (Andersen et al., 2002; Ohno and Watanabe, 1996; Pitkanen et al., 1995), and reverses lesion-induced memory impairment (Myhrer and Paulsen, 1995; Schuster and Schmidt, 1992).

The purpose of the present study was to examine whether administration of D-cycloserine in adulthood might compensate for the decreased glutamatergic activity and impaired cognitive function obtained by rearing in isolation. Rats reared under different conditions were tested in a three-choice simultaneous brightness discrimination task shown to be more sensitive to cognitive deficits than the conventional two-choice version of this task (Myhrer, 1992). With the three-choice test, one injection of glycine either before or after acquisition or before retention is able to restore mnemonic function in rats with glutamatergic temporal pathways disrupted (Myhrer et al., 1993). In the present study, two groups of rats reared in isolation received either one injection of D-cycloserine or saline before acquisition of the discrimination task. Two additional groups were reared in social or enriched environments, respectively.

2. Materials and methods

2.1. Animals

Fifty-one male Wistar rats from a commercial supplier (Møllegaard Breeding Laboratories, Denmark), 25-days-old (60–75 g) at the start of the study, served as subjects. The experiments were approved by the National Animal Research Authority. The rats were randomly assigned to one of three treatment categories for 56 days. Sixteen rats were reared in an enriched environment. They were placed in a Plexiglas cage ($56 \times 34 \times 20$ cm; five to six rats per cage) containing three objects that were changed three times a week. These nine objects were the same throughout the study. Fifteen rats were reared in a social environment, which was the same as described for enriched environment, but without objects present. Two groups of 10 animals each were reared in an isolated environment consisting of a smaller Plexiglas cage ($38 \times 22 \times 15$ cm) for each rat. At termination of differential rearing conditions (age 81 days), all rats were housed individually as described for isolated rats. The animals were handled individually for 5 days, being allowed to explore a table top (80×60 cm) for 3 min a day. The group assignment and drug treatment were not known during testing. The rats had free access to commercial rat pellets and water, except for water deprivation described for testing. All rats were housed in the same climatized (21 °C) vivarium that was illuminated from 0700 to 1900 h.

2.2. Drug administration

D-Cycloserine (purchased from Sigma, St. Louis) was dissolved in 0.9% physiological saline; pH 6. One dose of 3 mg/kg (Monahan et al., 1989) was administered 1.5–2 h before Day 1 of acquisition. Because neutral or acid solutions of D-cycloserine have been reported to be unstable (Merck Index, 1996), the solutions were stored for not more than 1 week at refrigerator temperature. Physiological saline was injected i.p. in a volume of 0.3 ml.

2.3. Apparatus

Simultaneous brightness discrimination was tested in a Plexiglas cage ($56 \times 34 \times 20$ cm) previously described (Myhrer and Nævdal, 1989). In brief, a Plexiglas wall with an opening (10×10 cm) in the middle divided the apparatus into two equal compartments: start compartment and goal compartment. Three interchangeable aluminium cylinders (3×7 cm) with a round well (2×2 cm) in the top served as discriminators. The cylinders were located in fixed positions (equal distance between each) along the wall opposite to the partition wall in the goal compartment. The cylinders were natural grey (aluminium) or painted black (except for the well). The well of the positive cylinder was filled with water. The only light was a 15-W bulb 60 cm above the apparatus.

2.4. Procedure

Training of the animals started from age 90 days. During acquisition and retention testing, the rats were deprived of water for 23.5 h a day. Prior to acquisition, each rat was allowed to explore the empty test apparatus for 15 min. On the first day of acquisition, the rats were trained to discriminate between the cylinders and were allowed to lap water from the well in the positive cylinder. That is, the rats were permitted to inspect cylinders until they encountered the correct one. They were given 10 trials, and the intertrial interval was 20 s during which they stayed in their home cage. On the second day, the animals were given trials until the occurrence of five correct responses in succession. Because the task is rapidly learned, the learning criterion was set low to avoid overlearning.

The animals were tested for retention of the discrimination task 13 days following the acquisition phase. Testing was terminated when the previous criterion was reached. The following behaviors were recorded: number of trials and number of errors to criterion. In order to drink or investigate whether the well in a cylinder contained water, the rats had to stand on their hind legs with at least one forepaw on the top of the cylinder. Error response was scored when a negative cylinder was mounted and found empty of water (e.g. licking the empty well). Approaching or investigating negative cylinders (except the well) was not scored as an error. The positive cylinder was either black or grey and the two cylinders of opposite color were negative. The position of the positive cylinder (left, middle, right) was changed in a prearranged randomized order. One set of randomized positions was used on the first day of training

and another on the second day and on retention testing. A counterbalanced design was followed in which half the rats in each group were trained with the black cylinder as positive and the other half with the grey cylinder as positive.

During the initial phase of learning this task, rats frequently put their snout close to negative cylinders and then left. Because olfactory cues are of no guidance in this respect, the rats most likely responded to the color. An approach to a positive cylinder is immediately followed by rearing and drinking from the well. As training proceeds, rats gradually cease approaching negative cylinders and head for the positive cylinder when entering the goal compartment. It is not likely that they change their learning strategy at this level of training by addressing the positive cylinder because of its odd appearance (one positive versus two negative cylinders) since approaching negative cylinders is seen now and then.

2.5. Statistics

Overall analyses were done with a one-way analysis of variance (ANOVA) and group comparisons with the Newman–Keuls post hoc test. Computations were carried out with the Prism system, a statistical software program (GraphPad Software, CA, USA).

3. Results

Rats from the various treatment categories did not seem to differ in acquisition of the discrimination task, whereas clear differences were seen in retention performances (Table 1). One-way ANOVA did not reveal significant differences in errors on Day 1 or errors and trials on Day 2 of acquisition ($P > 0.05$). During retention, however, ANOVA confirmed reliable differences among groups for errors to criterion ($F(3,47) = 4.327$, $P = 0.009$). Group comparisons, using the Newman–Keuls post hoc test, showed that saline-treated rats reared in isolation made significantly more errors than did the rats from an isolated environment treated with D-cycloserine ($P < 0.01$) as well as animals from enriched and social environments ($P < 0.05$). The latter three groups did not differ reliably from another. ANOVA also revealed a significant overall effect for trials to criterion ($F(3,47) = 6.370$, $P = 0.001$). Multiple comparisons showed that the group with isolated rearing treated with saline used significantly more trials than did the corresponding group treated with D-cycloserine ($P < 0.01$), the group with social rearing ($P < 0.05$), and the group with enriched rearing ($P < 0.001$). No significant differences were found among the latter three groups.

Table 1
Mean measures (± S.E.M.) to criterion for simultaneous brightness discrimination

Groups	N	Acquisition			Retention	
		Day 1 Errors	Day 2 Errors	Trials	Errors	Trials
Iso/Sal	10	2.4 ± 0.43	2.2 ± 0.80	12.4 ± 2.61	1.9 ± 0.50	10.1 ± 1.26
Iso/DCS	10	2.5 ± 0.50	1.2 ± 0.57	7.9 ± 1.46	0.3 ± 0.15[a]	6.0 ± 0.52[a]
Social	15	2.0 ± 0.32	2.0 ± 0.65	10.1 ± 1.67	0.9 ± 0.23[b]	7.5 ± 0.67[b]
Enriched	16	2.8 ± 0.31	1.1 ± 0.23	8.1 ± 0.69	0.6 ± 0.27[b]	6.0 ± 0.41[c]

Abbreviations: DCS = D-cycloserine; Iso = isolated; Sal = saline.
[a] $P < 0.01$.
[b] $P < 0.05$.
[c] $P < 0.001$.

4. Discussion

The results showed that rearing in isolation impaired retention of brightness discrimination as compared to rearing in social or enriched environments. However, administration of D-cycloserine before acquisition of the task ameliorated the retention deficit caused by isolated rearing. No deficits in acquisition of the task were seen among groups. Furthermore, rats reared in enriched or social environments and isolated rats treated with D-cycloserine did not differ as to retention performance.

The impaired retention in saline-treated animals reared in isolation may be associated with glutamatergic dysfunction, because it has previously been shown that isolated rearing results in a reduced level of high-affinity D-aspartate uptake in the synaptosome fraction from the entorhinal cortex (Myhrer et al., 1992). The temporal region appears to play a crucial role for mnemonic function. With the present visual discrimination task, it has been demonstrated that entorhinal, temporal/entorhinal, or perirhinal lesions impair retention, whereas hippocampal, perforant path, or postrhinal lesions impair acquisition (Myhrer, 2000). A developmental procedure resulting in a reduced number of NMDA receptors in the brain and in particular in the temporal region will probably have detrimental effects on cognitive functioning. It might be argued, however, that the retention deficit seen in the saline-treated rats is associated with noncognitive factors. In view of the present findings, such an argument does not appear plausible. During both Day 1 and Day 2 of acquisition, the saline group did not differ from the other groups for any of the measures (Table 1). Thus, disturbances in motivational, perceptual, emotional, or motor processes are probably not attributable to the retention deficit of the saline-treated rats reared in isolation.

In the present study, it was chosen to administer D-cycloserine prior to acquisition in order to examine whether effects of isolated rearing would also be reflected in the learning process. Glycine injected at the beginning of training for the present task was sufficient to reinstate the ability to both acquire and retain information in rats with disruption of glutamatergic temporal–entorhinal fiber connections. Furthermore, glycine injected immediately after learning or just prior to retrieval restores the retention performances of rats with lesion-induced amnesia, whereas glycine injected midway between acquisition and retention is without effect

(Myhrer et al., 1993). Results from experimentation with postoperative times of injecting D-cycloserine suggest that injections given shortly after amnesia-inducing surgery or just prior to retrieval are much more effective than injections given between these limits (Myhrer and Paulsen, 1995). Also, the dose of D-cycloserine administered is crucial for the degree of cognitive enhancing effect. The partial agonist, D-cycloserine, is a positive modulator of the NMDA receptor at low doses and the opposite effect is obtained with higher doses. In accordance with this principle, it was found that the impeded retention performance induced by scopolamine is ameliorated with a U-shaped dose–response curve by D-cycloserine in doses of 5, 15, and 50 mg/kg (Andersen et al., 2002). A corresponding dose–response profile of learning improvement in normal rats is seen with doses of 0.03–10.0 mg/kg of D-cycloserine (Monahan et al., 1989).

D-Cycloserine has been shown to improve passive avoidance performance when injected both before shock, after shock, or before retrieval. A common interpretation of such effects is that the drug has a positive effect on memory consolidation (Monahan et al., 1989). The present finding that D-cycloserine given before acquisition antagonized memory impairment during testing 15 days after the injection suggests that functional mechanisms outlast pharmacological ones. A potential candidate for involvement in such functional mechanisms may be long-term potentiation. Both NMDA and non-NMDA receptors are most likely involved in long-term potentiation (Bekkers and Stevens, 1989). However, NMDA receptors are required for the induction of long-term potentiation, whereas AMPA receptors are involved in the expression of long-term potentiation. Consistent with this notion, NMDA receptor antagonists impair acquisition, but not retrieval of previously acquired information (Morris, 1989). Furthermore, AMPA receptor antagonists have been reported to block retrieval of acquired information (Izquierdo et al., 1993).

Agonists acting at the glycine receptor site make glutamate receptors respond more effectively to endogeneous glutamate. In the case of glycine, this is seen as an increased frequency of channel openings and not an increase of current amplitude (Cotman et al., 1988). In this way, D-cycloserine may enhance synaptic efficacy and compensate for effects of isolated rearing when given to adult rats. Glycine sites at the NMDA receptors are normally below the saturation point (Dalkara et al., 1992) allowing room for modulation with agonists.

The results from the present study suggest that D-cycloserine is an effective agonist able to reinstate normal memory function in cognitively impoverished rats. This finding adds to previous results showing that D-cycloserine acts as a cognitive enhancer in aged rats, in rats treated with MK-801 or scopolamine, and in rats with lesion-induced deficits (cf. Introduction). D-Cycloserine may have clinical relevance in cases in which the glutamatergic systems are not too severely compromised. Individuals with age-associated memory impairment, Down's syndrome, or temporal lobe injuries may profit from treatments that enhance glutamatergic neurotransmission.

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