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Restoration of Mnemonic Function in Rats With Glutamergic Temporal Systems Disrupted: Dose and Time of Glycine Injections

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MYHRER, T., T. S. JOHANNESEN AND E. SPIKKERUD. *Restoration of mnemonic function in rats with glutamergic temporal systems disrupted: Dose and time of glycine injections.* PHARMACOL BIOCHEM BEHAV **45**(3) 519–525, 1993.—Disruption of the connections between the temporal cortex (TC) and lateral entorhinal cortex (LEC) in rats causes impaired memory accompanied by decreased levels of glutamate in these neocortical areas. Administration of glutamergic agonists to rats with TC/LEC disruptions improves retroactive memory. The purpose of this study was to test effects of glycine on proactive memory. The results show that a relatively large dose of glycine (750 mg/kg) injected just prior to training enhanced both acquisition and retention of a discrimination task in TC/LEC rats. Glycine given 1 day prior to training impaired the initial phase of acquisition but improved retention (Experiment 1). Injection of glycine immediately following training or just prior to retrieval reinstated normal mnemonic function in TC/LEC animals, whereas glycine given midway between learning and retention had no such effect (Experiment 2). A potential mnemonic model function of TC/LEC lesion is suggested, and possible beneficial effects of glycine on patients with Alzheimer's disease are discussed.

Temporal systems Glutamergic dysfunction Discrimination task Proactive memory Glycine

THE temporal region seems to be a crucial area for mnemonic functions. Parallel features are seen in the symptomatology of patients with bilateral temporal damage and patients with Alzheimer's disease (AD) (8). In the latter case, conspicuous degenerations are seen in the entorhinal cortex and the hippocampal region (6).

Disruption of the connections between the temporal cortex (TC) and lateral entorhinal cortex (LEC) in rats has been shown to produce a marked memory deficit (12). At least some of the disrupted connections probably use glutamate as neurotransmitter because reduced levels of high-affinity D-aspartate uptake in both TC and LEC are seen to accompany TC/LEC transections (11). In a subsequent study, it was found that glutamergic agonists acting selectively at the NMDA or quisqualate (AMPA) receptors produced complete amelioration of the memory deficit that follows TC/LEC lesions (13). In the latter study, effects of IP-injected glutamergic agonists (NMDA, glycine, or AMPA) were tested on retroactive memory, which is more severely affected than proactive memory by TC/LEC lesions (10). The purpose of the present study was to examine effects of the glutamergic agonist glycine on proactive memory.

Glycine is an allosteric agonist that crosses the blood-brain barrier (BBB) slightly (15). On the other hand, large doses of glycine do raise the brain levels of glycine (17). Previous positive effects obtained with glycine in attenuating lesion-induced memory deficits may also be attributable to impairment of the BBB at the lesion site. However, glycine injected in intact animals produces an immediate and temporary paralysis of the hindlimbs, indicating rapid activation of inhibitory glycinergic synapses in the spinal cord (present study).

TC/LEC lesions impair both acquisition and retention of a visual discrimination task. However, the performance on the first day of acquisition is unaffected by TC/LEC lesions, suggesting that the learning deficit seen on the second day of training may be of mnemonic nature (10). Because the TC/LEC specimen may serve as a glutamergic denervation model of AD, it appears important to study effects of glycine on both acquisition and retention in rats bearing such lesions. In this study, effects of different doses of glycine at different times of injection were examined.

The LEC is reciprocally connected with the TC, whereas the connections with the medial entorhinal cortex are more sparse in the rat (Fig. 1). The fiber connections of the TC and LEC seem to be routed in the adjacent white matter. Thus, transections through the white matter at a level corresponding

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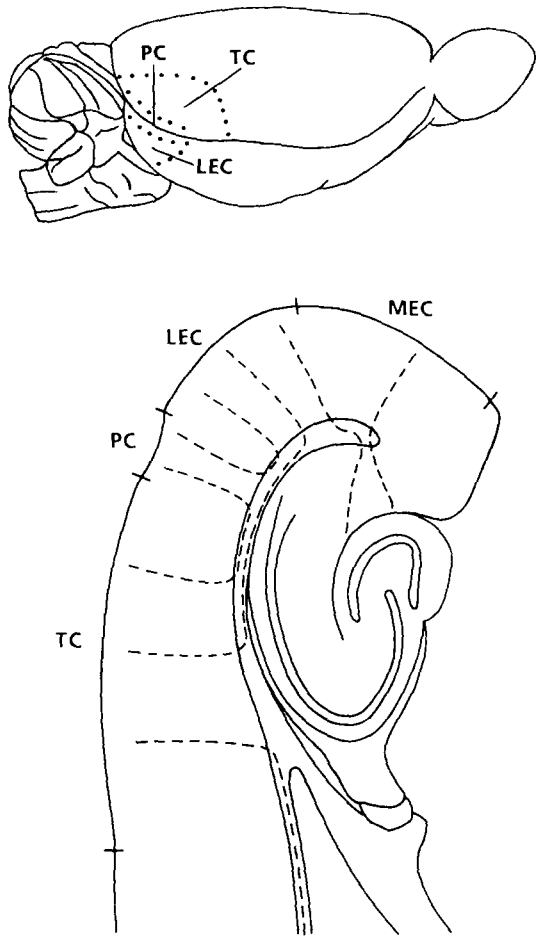

FIG. 1. Upper part: Lateral view of temporal and parahippocampal cortices. Lower part: Diagram of a horizontal section showing the various subdivisions in the temporal region, and simplified version of the connections between the subdivisions (dashed lines). Abbreviations: LEC = lateral entorhinal cortex; MEC = medial entorhinal cortex; PC = perirhinal cortex; TC = temporal cortex.

to the rhinal fissure can effectively disrupt connections between the TC and LEC [cf. (9)]. Because the transections cannot follow the exact curvature of the rhinal fissure, the lesions sever only about two thirds of the connections between the TC and LEC. In this way, about one third of these fiber systems remains intact and may be influenced by pharmacological agents along with related parts of the neural network. Previous results have shown that positive effects obtained with systemically administered glutamergic agonists are probably not attributable to peripheral adrenergic mechanisms (13).

The present study consists of two experiments. In Experiment 1, one injection of a large or medium dose of glycine was administered to rats with TC/LEC lesions at different times before the learning criterion was reached in the simultaneous brightness discrimination task used in previous studies. In Experiment 2, one injection of a large dose of glycine was administered at different times after acquisition of the discrimination task or immediately before retention performance.

EXPERIMENT 1

METHOD

Subjects

Forty-eight male Wistar rats from a commercial supplier (Møllegaard Breeding Laboratories, Denmark), weighing 290–320 g at the time of surgery, served as subjects. They were randomly assigned to six groups. The group assignment was unknown during testing. In four groups, animals received bilateral TC/LEC lesions and injections of glycine. Eight rats received one injection of 750 mg/kg on day 1 of training (the 750 day 1 group), eight rats received one injection of 375 mg/kg on day 1 of training (the 375 day 1 group), eight rats received one injection of 750 mg/kg on adaptation day (the 750 adapt group), and eight rats received one injection of 375 mg/kg on adaptation day (the 375 adapt group). Eight rats with TC/LEC lesions received one injection of saline on adaptation day. Eight rats served as control subjects and underwent sham surgery. Of these control rats, four received one injection of 750 mg/kg glycine and four received 0.5 ml saline on adaptation day. Rats were housed individually and had free access to commercial rat pellets and water. Rats were handled individually 3 days preoperatively and 1 day postoperatively, being allowed to explore a table top (80 × 60 cm) for 3 min a day. The climatized (21°C) vivarium was illuminated from 0700–1900 h.

Surgery

Rats were anesthetized IP with diazepam (10 mg/kg) and fenatyl fluanisone (2 mg/kg) and placed in a stereotaxic head holder with their skulls horizontal. The bilateral lesions were made mechanically be means of the sharp edges of cannulae (diam. 0.5 mm) provided with collars to control for insertion depth. The cannula to be used was mounted on a syringe. The point of insertion was 7.8 mm posterior to bregma and 6.7 mm lateral to midline. Each cannula was inserted into the brain in a position deviating 20° from the vertical in the sagittal plane (tip of cannula pointing rostrally). From this position, the syringe was moved 10 times back and forth in an axis deviating about 45° from the frontal plane (opening of angle pointing medially). These maneuvers were carried out in two stages with insertion depths 6 and 8 mm from the top of the skull. In this way, the distal part of the angular bundle was transected at a site corresponding approximately to the level of the rhinal fissure.

Histology

Upon termination of testing, brains were removed and frozen. Brains were sectioned horizontally on a CO_2-freezing microtome at 30 μm, every twelfth section being preserved. The sections were stained with methylene blue. The extent of fibers transected was estimated from the degree to which the white matter between the TC and LEC was damaged at the three dorsoventral levels presented in Fig. 2. The white matter (not the alveus) was divided in four equal columns, each column representing 25% of the fibers. The occurrence of damage was evaluated under relatively high magnification. The number of columns affected at each dorsoventral level were counted, and the mean percentage of damage was computed for each animal.

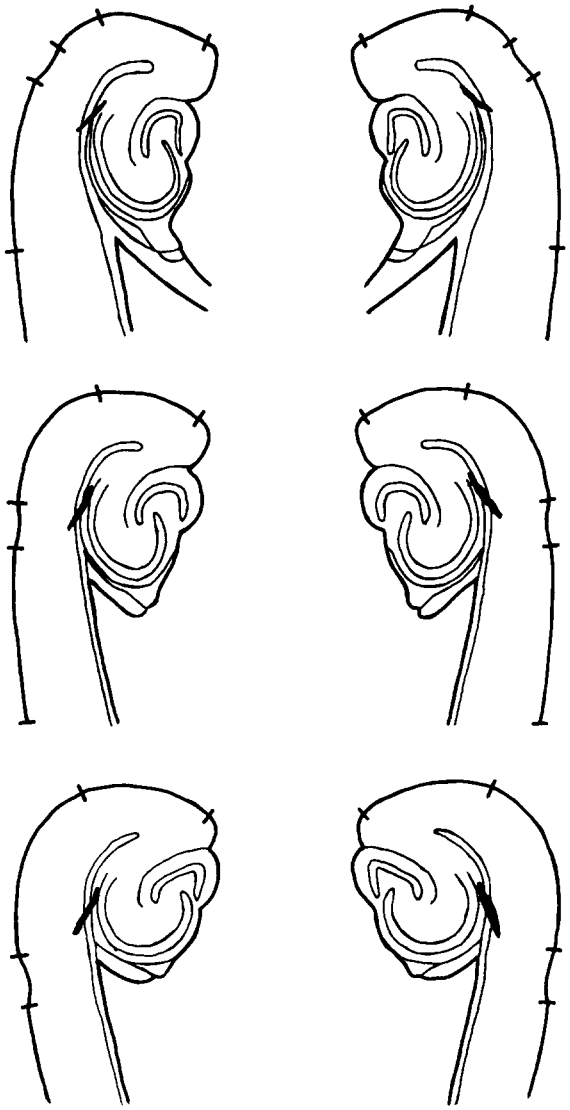

FIG. 2. Example of temporo-entorhinal lesions representative for all groups. The dark line through the white matter indicates the lesion. Distance between sections: 1.5 mm.

Administration of Injections

IP injections of glycine were given 1.5–2 h before adaptation or day 1 of training. Physiological saline (0.5 ml) was injected IP 1.5–2 h before adaptation. Glycine (purchased from Sigma Chemical Co., St. Louis, MO) was dissolved in distilled water with pH adjusted to 7.4 with NaOH.

Apparatus

Testing of simultaneous brightness discrimination was carried out in a Plexiglas cage (56 × 34 × 20 cm) previously described (12). In brief, a Plexiglas wall with an opening (10 × 10 cm) in the middle divided the apparatus in 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 discriminanda. 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.

Procedure

During acquisition and retention testing, rats were deprived of water for 23.5 h a day. On the first day (adaptation day, postoperative day 8), each rat was allowed to explore the empty test apparatus for 15 min. On the second day (day 1 of training), subjects were trained to run from the start compartment into the goal compartment, in which they were rewarded with some laps of water from the well in the positive cylinder. Rats were given 10 trials and intertrial interval was 20 s, during which they stayed in their home cage. On the third day, animals were given trials until the occurrence of five correct responses in succession. Because the task is easily learned, learning criterion was set low to avoid overlearning. Thirteen days after learning criterion had been reached, animals were tested for retention of the discrimination task. Testing was terminated when the previous criterion was reached. The following behaviors were recorded: number of trials to criterion and number and type of errors to criterion. To drink or investigate whether the well in a cylinder contained water, rats had to stand on their hindlegs with at least one forepaw on 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 day 2 of training and another on day 3 and on retention testing. A counterbalanced paradigm was followed in which half the subjects were trained with black cylinder as positive and the other half with grey cylinder as positive.

During the initial phase of learning, this task rats frequently put their snouts close to negative cylinders and then leave them. Because olfactory cues are of no guidance in this respect, they most likely respond to the color. An approach to the 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 stage of training by addressing the positive cylinder because of its odd appearance (one positive vs. two negative cylinders) because approaching negative cylinders is seen now and then.

Statistical overall analyses were made with Kruskal-Wallis one-way analysis of variance (ANOVA) and group comparisons with two-tailed Mann-Whitney *U*-test. Computations of *U*-tests were carried out with the Minitab system, a statistical software program (Minitab, Inc.).

RESULTS

Behavior

Because of uneven distribution of data, nonparametric statistics were applied. Kruskal-Wallis one-way ANOVA con-

firmed significant differences in errors on day 1 of acquisition, $H(5) = 13.97, p < 0.02$ (Table 1). The 750 adap group made significantly more errors than the sham, saline, and 750 day 1 groups ($p < 0.05$). Also, the 375 adap group made significantly more errors than the sham, saline, and 750 day 1 groups ($p < 0.05$). No other significant differences were found in this respect. ANOVA revealed reliable treatment effect in errors on day 2, $H(5) = 14.37, p < 0.02$. The saline group made significantly more errors than the sham and 750 day 1 groups ($p < 0.01$). The 375 day 1 group made reliably more errors than the sham and 750 day 1 groups ($p < 0.05$). Also, the 375 adap group made more errors than the sham and 750 day 1 groups ($p < 0.02$). ANOVA confirmed reliable differences in trials on day 2, $H(5) = 15.40, p < 0.01$. The saline group used more trials than the sham and 750 day 1 groups ($p < 0.02$). The 375 day 1 group required more trials to criterion than the sham and 750 day 1 groups ($p < 0.05$). Also, the 375 adap group used more trials than the sham and 750 day 1 groups ($p < 0.01$). The 750 adap group was not reliably different from other groups.

ANOVA confirmed a reliable effect in errors to criterion during retention performance, $H(5) = 14.17, p < 0.02$. The saline group made significantly more errors than all the other groups ($p < 0.05$). The latter groups were not reliably at variance with one another. ANOVA revealed a significant treatment effect in trials to criterion, $H(5) = 13.74, p < 0.02$. The saline group used reliably more trials than all the other groups ($p < 0.05$). No other significant differences were seen.

Histology

The TC/LEC lesions appeared as a section through the white matter at a site between the TC and LEC (Fig. 2). The transections, which often affected the alveus of the hippocampal formation, were 0.5–1.0 mm long in rostro-caudal extent and 3–4 mm long in dorsoventral extent. Because the cannula transections could not follow the exact curvature of the rhinal fissure, TC/LEC connections between a relatively small part in the caudal end of the TC and in the rostral end of the LEC were probably not accessible for denervation (in total about one third of the fibers). The mean percentage of fiber lesion for all groups was 90.8 (range 88–94), indicating that a total of about 60% of the fibers between the TC and LEC were disconnected (Fig. 2).

In the saline group, the mean percentage of fiber lesion was 89 (range 75–100). Additional damage to the subicular area in the ventralmost part of the hippocampal formation was seen unilaterally in three rats. In the 750 day 1 group, the mean percentage of fiber lesion was 92 (range 71–100). Additional damage to the ventral subiculum was seen unilaterally in two rats. In the 375 day 1 group, the mean percentage of fiber lesion was 94 (range 75–100). Additional damage to the subiculum was seen in three rats. In the 750 adap group, the mean percentage of fiber lesion was 91 (range 80–100). Additional damage to the ventral subiculum was seen in four rats. In the 375 adap group, the mean percentage of fiber damage was 88 (range 71–100). Additional damage to the ventral subiculum was seen unilaterally in three rats.

Reactions to Injections

Both large and medium doses of glycine resulted in an immediate, but temporary (1–2 min), paralysis of the hindlegs. This reaction was seen in both sham- and lesion-operated rats.

EXPERIMENT 2

METHOD

Subjects

Forty rats as described for Experiment 1 were used as subjects. In three groups, animals received bilateral TC/LEC lesions and injections of glycine. Eight rats received one injection of 750 mg/kg when reaching the learning criterion (the 750 crit group). Eight rats received one injection of 750 mg/kg immediately before retention (the 750 ret group) and eight rats received one injection of 750 mg/kg 6 days after learning criterion had been achieved (the 750 6 days group). Eight rats with TC/LEC lesions received one injection of saline when reaching the learning criterion. Eight sham-operated rats served as controls. Two of these rats received 750 mg/kg glycine upon learning criterion, two received glycine before retention, two received glycine 6 days after learning, and two received saline upon learning criterion.

TABLE 1

PERFORMANCE OF SIMULTANEOUS BRIGHTNESS DISCRIMINATION IN EXPERIMENT 1

			Acquisition						Retention			
			Day 1		Day 2							
	Dose (mg/kg)		Errors		Errors		Trials (total)		Errors		Trials	
Lesion	Time of gly	*n*	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Sham	Sal/750	8	2.0	1–3	0.0	0–1	15.0	15–18	0.0	0–1	5.0	5–8
TC/LEC	Saline	8	2.0	1–3	2.0	0–4	21.0	15–25	2.0	1–4	11.0	7–15
TC/LEC	750 day 1	8	2.0	1–3	0.0	0–1	15.0	15–17	0.0	0–2	5.0	5–11
TC/LEC	375 day 1	8	2.5	2–4	2.0	0–4	20.5	15–27	0.5	0–2	6.0	5–11
TC/LEC	750 adap	8	3.0	2–5	1.5	0–3	17.0	15–28	0.5	0–2	6.0	5–10
TC/LEC	375 adap	8	3.5	2–6	1.5	0–4	20.0	15–25	0.0	0–2	5.0	5–11

Adap, adaptation; Gly, glycine; TC/LEC, temporal cortex/lateral entorhinal cortex.

Administration of Injections

Injections of glycine (IP) were given immediately upon completion of acquisition, 6 days after acquisition, or 1.5-2 h before retention.

Surgery, histology, apparatus, and procedure were the same as described in Experiment 1.

RESULTS

Behavior

No significant differences were seen in the number of errors on day 1 (Table 2). ANOVA confirmed significant differences in errors on day 2, $H(4) = 14.33$, $p < 0.01$. The sham group made reliably fewer errors than all other groups ($p < 0.02$). ANOVA also revealed a reliable treatment effect in number of trials to criterion, $H(4) = 13.55$, $p < 0.02$. The sham group used significantly fewer trials than all other groups ($p < 0.02$).

ANOVA confirmed a significant treatment effect in number of errors during retention, $H(4) = 20.04$, $p < 0.01$. The saline group made reliably more errors than the sham, 750 crit, and 750 ret groups ($p < 0.01$). Also, the 750 6 days group made more errors than the sham, 750 crit, and 750 ret groups ($p < 0.05$). ANOVA revealed reliable differences in number of trials, $H(4) = 18.48$, $p < 0.01$. The saline group used significantly more trials than the sham, 750 crit, and 750 ret groups ($p < 0.01$). The 750 6 days group used more trials than the sham and 750 ret groups ($p < 0.01$). No other significant differences were observed.

Histology

The lesions appeared as described for Experiment 1 (Fig. 2). In the saline group, the mean percentage of fiber lesion was 89 (range 71-100). Additional damage to the ventral subiculum was seen unilaterally in four rats. In the 750 crit group, the mean percentage of fiber lesion was 92 (range 75-100). Additional damage was seen unilaterally in three rats. In the 750 ret group, the mean percentage of fiber lesion was 87 (range 75-100). Additional damage was seen unilaterally in three rats. In the 750 6 days group, the mean percentage of fiber lesion was 91 (range 75-100). Additional damage was seen unilaterally in four rats.

In both Experiments 1 and 2, enlargement of the lesion site was often observed among glycine-treated rats. Such enlargement was more dominant in the left hemisphere than in the right. Occasionally, also the left lateral ventricle was enlarged.

GENERAL DISCUSSION

Saline-treated rats with TC/LEC lesions displayed both impaired acquisition and retention relative to sham-operated control rats. However, both prelearning and postlearning injection of glycine improved the performances of rats with TC/LEC lesions. The results from Experiment 1 show that one injection of a relatively large dose of glycine (750 mg/kg) on day 1 of acquisition made TC/LEC rats both acquire and retain the discrimination task like control rats. A large dose injected on adaptation resulted in only a slight improvement of both acquisition and retention. A medium dose of glycine (375 mg/kg) on day 1 or adaptation did not improve acquisition but improved retention. However, both large and medium doses given on adaptation resulted in more errors on day 1 of training. The results from Experiment 2 show that a large dose of glycine given either immediately after training or about 2 h before retrieval fully restored the mnemonic function. A large dose given midway between learning and retention (6 days after acquisition) did not improve retention. In summary, a large dose of glycine administered at the beginning of training is sufficient to reinstate the ability to both acquire and retain new information in TC/LEC animals. Further, a large dose injected immediately after learning or just prior to retrieval is sufficient to restore the retention performance in TC/LEC rats.

The present findings of mitigating effects of glycine on proactive memory are not unexpected in view of previous results with positive effects of glutamergic agonists on retroactive memory in TC/LEC rats (13). The present results are also in agreement with those seen after administration of the glycine prodrug milacemide (an acylated glycine derivative), which readily crosses the BBB and is transformed into glycine in the brain. An optimal dose of milacemide (10 mg/kg) administered IP before shock, after shock, or before retrieval in a passive avoidance task enhances retention performance in intact rats. Lower or higher doses of milacemide did not have similar ameliorating effects (5).

Provided a large dose of glycine is administered, sufficient

TABLE 2

PERFORMANCE OF SIMULTANEOUS BRIGHTNESS DISCRIMINATION IN EXPERIMENT 2

			Acquisition						Retention			
			Day 1		Day 2							
	Dose (mg/kg)		Errors		Errors		Trials (total)		Errors		Trials	
Lesion	Time of gly	*n*	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Sham	750/sal	8	2.0	0-3	0.0	0-1	15.0	15-18	0.0	0-1	5.0	5-7
TC/LEC	Saline	8	2.5	1-3	2.0	0-3	20.5	15-25	2.5	0-4	12.5	5-17
TC/LEC	750 crit	8	2.0	0-4	2.0	0-3	19.5	15-25	0.0	0-2	5.0	5-10
TC/LEC	750 ret	8	2.5	1-3	2.0	0-4	21.0	15-25	0.0	0-1	5.0	5-8
TC/LEC	750 6 days	8	2.0	1-3	2.0	0-5	20.5	15-25	1.5	0-4	8.5	5-16

Crit, criterion; Gly, glycine; Ret, retention; TC/LEC, temporal cortex/lateral entorhinal cortex.

amounts seem to cross the BBB to have beneficial effects on mnemonic function. However, even a medium dose improved retention, even if this dose was not sufficient to enhance acquisition in TC/LEC rats. Thus, a large dose is required to obtain reliable effects of glycine. But, a large dose given 6 days after learning did not yield a positive effect on retention. The finding that glycine antagonized memory impairment may suggest that this agent interferes more with functional mechanisms than pharmacological ones.

Glycine is able to act on two distinct populations of receptors: the strychnine-sensitive receptor, mostly found in the brain stem and spinal cord, and the strychnine-insensitive NMDA receptor complex in the forebrain. The effects on memory obtained in this study most likely involved the strychnine-insensitive glycine binding site of the NMDA receptor. Glycine has been shown to potentiate NMDA responses enormously in cultured mouse brain neurons (7). It is further reported that glycine sites at the NMDA receptors in the hippocampus are normally below the saturation point in rats. Thus, modulation of NMDA receptor activity is possible by means of glycine. It is also found that endogenous glycine is required for NMDA receptors to be activated (2). The latter finding is in agreement with behavioral data providing evidence that endogenous glycine supports the process of learning and memory (18).

The TC-LEC area is evidently critically involved in preserving information from the discrimination task. Amelioration of the memory deficit may have emerged in several ways. The agonist may have triggered compensatory activity in NMDA receptors of residual synapses in the TC-LEC area or induced corresponding activity in glutamergic systems outside the TC-LEC area. Both NMDA and non-NMDA receptors seem to be involved in long-term potentiation. The NMDA class is assumed to provide the trigger for induction, and the non-NMDA class is thought to provide the enhancement of synaptic efficacy (1).

Glycine given midway between acquisition and retention did not improve memory. This lack of mnemonic effect may be related to the circumstance that memory engrams associated with the discrimination task were not particularly activated during the influence of the agonist. Glycine has probably not a general enhancing effect of memory. The agent is presumably useful for neural activity during specific encoding and retrieval of sensory information.

The finding that glycine administered on adaptation resulted in more errors on day 1 of training appears somewhat intriguing. It can only be assumed that declining pharmacological effects of glycine activate some other mechanisms as well. Glycine administered 1 day prior to adaptation did not result in more errors on day 1 (Myhrer, unpublished results). It was noticed, however, that rats given the latter treatment displayed signs of nervousness during acquisition training. Glycinergic inhibitory synapses have been found in neocortical areas of the rat and man (14). The behavioral significance of such glycinergic neurons appears to be unknown.

A tendency to enlargement of the lesion site was seen in glycine-treated rats. This enlargement was more dominant in the left hemisphere than in the right. These enlargements may be related to excitotoxic effects of glycine. Such effects may have been more prominent in the left side because the left LEC has been shown to contain higher concentration of glutamate than the right LEC in rats from our dealer (11). An alternative, but less plausible, explanation might have been a systematic bias during surgery, resulting in disruption of more blood vessels in the left than the right hemisphere. Corresponding enlargement of the lesion site was not seen when glycine was injected immediately after termination of the surgery (13).

In AD, conspicuous degeneration and marked loss of NMDA receptors are seen in the temporal region (3,4,6). Thus, the TC/LEC specimen may serve as a glutamergic denervation model of AD. According to the results of this study, one injection of 750 mg/kg glycine each day would probably enable TC/LEC rats to acquire and retain information in a way similar to intact rats if continuously encountering new tasks.

Administration of milacemide to healthy humans (1.2 g) facilitates memory (16). Large doses of glycine (5–25 g/day) administered for months to schizophrenic patients have been tried with some beneficial results (19). Thus, clinical trials with glycine to AD patients would not represent a totally untried field of experimentation. Excitotoxic enlargement of the lesion site may not be expected to occur in AD patients. The disease causes only sporadic loss of pyramidal cells, a different result from the unitary brain lesion induced in rats.

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