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Interference with visual memory in rats following infusion of the functional NMDA receptor antagonist, HA-966, into temporal regions

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Abstract

Results from lesion studies show that selective damage to the temporal cortex or lateral entorhinal cortex impairs visual memory, whereas damage to the hippocampal region does not affect retention of a visual discrimination task. Major input pathways of the above structures use glutamate as neurotransmitter. The glutamate NMDA receptor appears to play an important role for cognitive functions. The objective of the present study was to examine whether microinjections of the functional NMDA receptor antagonist, 3-amino-1-hydroxy-2-pyrrolidinone ((+)-HA-966), might result in effects mimicking those seen in lesion studies. The results show that infusion of HA-966 into the temporal cortex or lateral entorhinal cortex 1.5–3 h after the learning criterion had been obtained led to an impeded visual memory when tested 13 days later, whereas HA-966 infused into the hippocampal region did not affect memory. A similar retention deficit with HA-966 infusions in the temporal cortex or lateral entorhinal cortex was seen when testing took place 23 days later, whereas a markedly weaker effect was observed when the retention period was reduced to 3 days. It is suggested that the hippocampal region is a temporary storing site for nonspatial memory engrams, and later posttraining memory consolidation involves the temporal and lateral entorhinal cortices. Furthermore, the degree of the effect of HA-966 is related to the length of the retention period.

Keywords: Visual discrimination; NMDA receptor antagonist, functional; Microinfusion; Cortex, temporal; Cortex, entorhinal; Memory; (Rat)

1. Introduction

Optimal function of the temporal–hippocampal region appears vital for normal learning and memory in rodents and primates (Zola-Morgan et al., 1994; Myhrer, 2000). In the rat, damage to rhinal structures (perirhinal, entorhinal, temporal cortices) results in impairment of both short-term and long-term storing of sensory information (Otto and Eichenbaum, 1992; Mumby and Pinel, 1994; Wiig and Bilkey, 1994a,b, 1995; Nagahara et al., 1995; Myhrer and Wangen, 1996a). Damage to the hippocampal region (hippocampus proper, fascia dentata, subiculum) interferes with working memory as well as with long-term storing of spatial and contextual information (Rawlins, 1985; Jarrard, 1993).

The hippocampal region receives sensory information from neocortical association areas by way of the perirhinal and entorhinal cortices. This information is transmitted from the medial and lateral entorhinal cortex via the perforant path projection system to the hippocampal region. In return, the hippocampal region is able to send information to the entire cortical mantle. Furthermore, the lateral entorhinal cortex is heavily connected with the temporal cortex, whereas the connections between the temporal and medial entorhinal cortices are relatively modest. The fiber connections of the temporal and lateral entorhinal cortices are routed in the adjacent white matter (cf. Myhrer and Wangen, 1996b).

Transections of the neural connections between the temporal and lateral entorhinal cortices cause marked retrograde amnesia in a visual discrimination task (Myhrer and Wangen, 1996a). These connections are probably predominantly glutamatergic, because the retroactive memory deficit seen in rats with temporal cortex/lateral entorhinal cortex lesions is accompanied by reduced high-affinity D-aspartate uptake in both cortical areas (Myhrer et al., 1989). The finding that systemic administration of glutamate receptor agonists can completely restore memory function in rats with temporal cortex/lateral entorhinal cortex transections suggests that the glutamatergic dys-

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function is causally related to the mnemonic deficit. Lesion-impaired memory is fully mitigated by intraperitoneal injections of *N*-methyl-D-aspartate (NMDA), glycine, D-cycloserine or DL-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA; Myhrer and Paulsen, 1992, 1995). Systemic administration of D-cycloserine has also been shown to reverse spatial working memory impairment in rats with hippocampal lesions (Schuster and Schmidt, 1992), but not in rats with entorhinal lesions (Zajaczkowski and Danysz, 1997). In a study using microinjections, it was seen that D-cycloserine infused into the temporal or lateral entorhinal cortices can fully ameliorate the impairment of fiber transections between the same structures, whereas infusion into the hippocampal region only caused a mild improvement of retention. Infusion of D-cycloserine into the frontal cortex or saline into the temporal cortex or lateral entorhinal cortex had no ameliorating effects in impaired rats (Myhrer and Paulsen, 1997). The purpose of the present study was to examine whether microinfusions of a glutamate receptor antagonist into temporal regions of intact rats could interfere with the storage of visual information and whether the degree of interference could be related to the length of the period of retention.

The excitatory responses to glutamate are mediated by several receptor subtypes, among which the NMDA receptor may be of special interest for cognitive functions. The NMDA receptor complex consists of several receptor sites, and the glycine recognition site was addressed in this study. The reason for this choice was the possibility of making comparisons with effects previously obtained with the partial agonist, D-cycloserine, which acts at the same receptor site. The functional NMDA receptor antagonist, 3-amino-1-hydroxy-2-pyrrolidinone (HA-966), reduces neural activity in the rat and is a well-explored glycine site antagonist (Singh et al., 1990). The (+)-isomer of HA-966 was used in this study, because the (+)-enantiomer has a marked antagonistic effect on the NMDA receptor responses in rat cortical slices, whereas the (−)-enantiomer has a much weaker effect (Singh et al., 1990). Furthermore, neonatal administration of the (+)-isomer disrupts cognitive behavior in adult rats, whereas the racemic mixture (±)-HA-966 does not (Wangen et al., 1997).

The hippocampal region and the entorhinal cortex seem to be involved in the process of learning in a time-dependent manner. Infusion of the NMDA receptor antagonist, aminophosphonopentanoic acid (AP5), into the hippocampal region only impairs memory when it is given immediately after learning, whereas a corresponding infusion is only effective in the entorhinal cortex when it is administered 1.5–3 h after learning (Ferreira et al., 1992; Izquierdo et al., 1992; Jerusalinsky et al., 1992). Likewise, memory enhancement is achieved on intrahippocampal infusion of platelet-activating factor (PAF), which increases glutamate release when administered immediately after training and by intraentorhinal infusion when administered 100 min after termination of training (Izquierdo et al., 1995). In the present study, HA-966 (or vehicle) was infused through implanted guide cannulas into the hippocampal region, temporal cortex, or lateral entorhinal cortex 1.5–3 h after the learning criterion had been achieved. With this design, it might be expected that HA-966 will impair the consolidation of memory engrams when infused into temporal cortex or lateral entorhinal cortex, but not when infused into the hippocampal region.

A visual discrimination task previously used in studies of pharmacological agents as well as of lesions was applied for comparisons of results. For those injection sites yielding positive effects with the standardized retention period of 13 days, the present study was expanded to test the effects of HA-966 when the retention time was 3 or 23 days, respectively. This expansion was made to examine effects of HA-966 on memory engrams of varying strength.

2. Materials and methods

2.1. Subjects

Seventy-five male Wistar rats from a commercial supplier (Møllegaard Breeding Laboratories, Denmark), weighing 280–310 g at the time of surgery, served as subjects. The experiments were approved by the National Animal Research Authority. The rats were randomly assigned to four groups, and their group assignment was not known during testing; 24 rats (control) received an infusion of saline into the temporal cortex (12 rats) or the lateral entorhinal cortex (12 rats); 21 animals received an infusion of HA-966 into the temporal cortex, 22 received an infusion of HA-966 into the lateral entorhinal cortex, and 8 received an infusion of HA-966 into the hippocampal region. All infusions were made bilaterally. The rats were housed individually and had free access to commercial rat pellets and water, except when deprived of water during the testing period. The animals were handled individually 5 days preoperatively and 2 days 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 to 1900 h.

2.2. Surgery

The rats were anesthetized i.p. with diazepam (10 mg/kg) and fentanyl fluanisone (2 mg/kg) and implanted stereotaxically with guide cannulas aimed at temporal cortex, lateral entorhinal cortex, or hippocampal region in both hemispheres. The guide cannula (25 gauge) was 0.5 mm in diameter and cut to a length of 11 mm. The upper part of the cannula was roughened in order to improve the grip of the dental cement (Durelon; ESPE, Seefeldt, Germany), which was anchored to the skull by steel screws. The site for the temporal cortex cannula was 4.0 mm

posterior to the bregma and 6.0 mm lateral to the midline. The cannula was inserted 5.0 mm from the top of the skull at an angle of 15°, with the tip of the cannula pointing laterally. The point of insertion for the lateral entorhinal cortex cannula was 7.8 mm posterior to the bregma and 6.0 mm lateral to the midline. The cannula was inserted 7.0 mm from the top of the skull at an angle of 10°, with the tip of the cannula pointing laterally. Because the hippocampal region is larger than the temporal cortex and the lateral entorhinal cortex, insertions were made at two sites in the dorsal hippocampus. The rostral insertion was made 3.5 mm posterior to the bregma and 2.0 mm lateral to the midline. The caudal insertion was made 5.0 mm posterior to the bregma and 4.0 mm lateral to the midline. In the rostral insertion, the cannula was lowered perpendicularly 2.5 mm, and in the caudal insertion, 3.8 mm from the top of the skull. The rats were infused with HA-966 or saline 1.5–3 h after the learning criterion had been achieved. A cannula 0.3 mm in diameter and 12-mm long (30 gauge) was fitted into the guide cannula and protruded 1.0 mm beyond the latter one. The infusions were made by means of a microinjection pump (Model CMA 100, Carnegie Medicin, Stockholm, Sweden). To prevent plugging of the indwelling cannulas, smaller cannulas (30 gauge) with a bent top were inserted to a depth of 10 mm.

2.3. Histology

Upon termination of testing, the animals were anesthetized as previously described. 1.0 μl of 4% methylene blue in saline was infused for 1 min 1 mm beyond the tip of the implanted cannulas. Then the animals were decapitated, and the brains were removed and frozen. The brains were sectioned horizontally on a CO_2-freezing microtome at 30 μm, every seventh section being preserved. The sections were stained with methylene blue after they had been inspected for location of cannula marks.

2.4. Drug administration

(+)-HA-966 (purchased from Research Biochemicals, Natick, MA) was dissolved in 0.9% saline. The dose was 1.0 μl (1.0 μg/μl) applied over 1 min, and the cannula remained in position for an additional min before retraction. Physiological saline (1.0 μl) was infused in the same manner. The infusions were carried out 1.5–3 h after the learning criterion had been achieved.

2.5. Apparatus

Testing of simultaneous brightness discrimination was carried out 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.

2.6. Procedure

Training of the animals started 10 days following surgery. 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 received some laps of 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 3, 13 or 23 days following the acquisition phase. Testing was terminated when the previous criterion was reached. The following behaviors were recorded: number of trials to criterion 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 top of the cylinder. Error response was scored when a negative cylinder was mounted

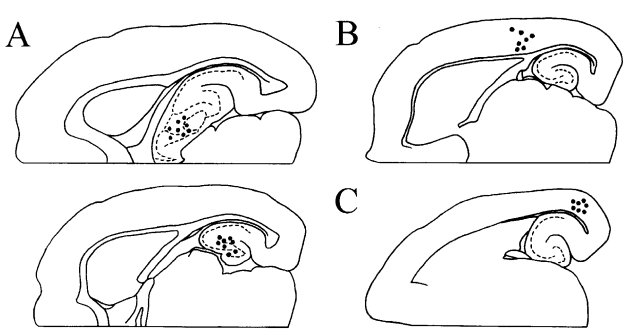

Fig. 1. Reconstruction of horizontal sections showing the localization of HA-966 infusions in the right hemisphere for each rat with a retention period of 13 days. (A) The rostral insertions (upper) and the caudal insertions (lower) in the hippocampal region. (B) Insertions in the temporal cortex. (C) Insertions in the lateral entorhinal cortex. The vertical opening of the cannula extended about 1.2 mm above the marks which show the positions of the end of cannula insertions. The localization of the infusions made in the left hemisphere appeared similar.

Table 1
Mean (±S.E.M.) measures to criterion for simultaneous brightness discrimination with retention period of 13 days

Injection site	Drug	*N*	Acquisition	Retention		Percentage saving of trials
			Trials (days 1 and 2)	Trials	Errors	
TC or LEC	saline	8	15.9 ± 0.44	6.5 ± 0.60	0.8 ± 0.25	86.2
TC	HA-966	7	17.4 ± 0.75	15.4 ± 1.41	3.9 ± 0.50	16.1
LEC	HA-966	7	16.9 ± 0.46	13.9 ± 1.2	3.7 ± 0.42	25.2
Hippocampus	HA-966	8	16.4 ± 0.60	6.3 ± 0.67	0.6 ± 0.32	88.6

Abbreviations: LEC = lateral entorhinal cortex; TC = temporal cortex.

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 one on the second day and on retention testing. A counterbalanced design was followed in which half the subjects 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 them. Because olfactory cues are of no guidance in this respect, the rats most likely respond 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 stage 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.

2.7. Statistics

Statistical overall analyses were made with 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). Percentage saving of trials was computed with the formula of Hilgard (1934).

3. Results

3.1. Histology

The dye, methylene blue, was found to have invaded an area about 1.0 mm in diameter. Fig. 1 shows the localization of the injection sites for HA-966 in the rats with retention period of 13 days. The infusions of saline into temporal cortex and lateral entorhinal cortex were all within accepted areas. Acceptable sites of infusions were also seen for all rats with retention periods of 3 and 23 days, respectively.

3.2. Behavior

For the retention period of 13 days, the rats from the various treatment categories did not differ significantly in trials to criterion during acquisition of the discrimination task ($F(3,26) = 1.1350$, $P = 0.2793$; Table 1). During retention, however, differences in performance were clearly seen. One-way ANOVA confirmed a significant treatment effect for trials to criterion ($F(3,26) = 24.72$, $P < 0.0001$). Multiple comparisons, using the Newman–Keuls post-hoc test, showed that the groups infused with HA-966 into the temporal or lateral entorhinal cortices used reliably more trials to criterion than the saline-treated control group and the group infused with HA-966 in the hippocampal region

Table 2
Mean (±S.E.M.) measures to criterion for simultaneous brightness discrimination with retention period of 3 days

Injection site	Drug	*N*	Acquisition	Retention		Percentage saving of trials
			Trials (days 1 and 2)	Trials	Errors	
TC or LEC	saline	8	16.5 ± 0.68	5.6 ± 0.37	0.4 ± 0.18	94.8
TC	HA-966	7	16.4 ± 0.65	8.3 ± 0.90	1.1 ± 0.23	71.1
LEC	HA-966	8	17.3 ± 0.89	9.9 ± 1.14	1.6 ± 0.29	60.2

Abbreviations as for Table 1.

Table 3
Mean (±S.E.M.) measures to criterion for simultaneous brightness discrimination with retention period of 23 days

Injection site	Drug	*N*	Acquisition	Retention		Percentage saving of trials
			Trials (days 1 and 2)	Trials	Errors	
TC or LEC	saline	8	17.1 ± 0.69	7.4 ± 0.88	1.0 ± 0.33	80.2
TC	HA-966	7	16.1 ± 0.55	15.6 ± 1.54	3.3 ± 0.42	4.5
LEC	HA-966	7	16.7 ± 0.71	14.1 ± 1.30	3.0 ± 0.31	22.2

Abbreviations as for Table 1.

($P < 0.001$). Neither the temporal cortex and lateral entorhinal cortex groups nor the control and hippocampal groups, respectively, deviated reliably from one another. ANOVA revealed a significant overall effect in terms of errors to criterion ($F(3,26) = 22.50$, $P < 0.0001$). Animals infused with HA-966 into the temporal or lateral entorhinal cortices made reliably more errors than did the control and hippocampus-infused rats ($P < 0.001$). The temporal cortex- and lateral entorhinal cortex-infused groups did not vary significantly and neither did the control and hippocampal groups.

For the retention period of 3 days, no significant differences were seen among the groups during acquisition of the task ($F(2,20) = 0.4281$, $P = 0.6576$; Table 2). During retention, ANOVA revealed a reliable overall effect for trials to criterion ($F(2,20) = 6.382$, $P = 0.0072$). Group comparisons showed that the animals with HA-966 infused into the temporal or later entorhinal cortices used significantly more trials to criterion than did the saline-treated rats ($P < 0.05$). A significant treatment effect was also seen for errors to criterion ($F(2,20) = 6.540$, $P = 0.0065$). Both the temporal cortex and lateral entorhinal cortex groups made more errors than did the saline group ($P < 0.05$).

For the retention period of 23 days, no reliable treatment effect was seen for the groups during acquisition ($F(2,19) = 0.5610$, $P = 0.5798$; Table 3). During retention, ANOVA showed a significant overall effect for trials to criterion ($F(2,19) = 12.94$, $P = 0.0003$). Group comparisons confirmed that the rats treated with HA-966 in the temporal or lateral entorhinal cortices used reliably more trials to criterion than did the saline-treated animals ($P < 0.001$). ANOVA also uncovered a reliable effect for errors to criterion ($F(2,19) = 12.90$, $P = 0.0003$). The rats infused with HA-966 into the temporal or lateral entorhinal cortices made significantly more errors than did the saline-treated control group ($P < 0.001$).

4. Discussion

The results from the present study showed that infusion of (+)-HA-966 into the temporal cortex or the lateral entorhinal cortex 1.5–3 h after the learning criterion had been achieved resulted in impaired retention of visual memory, whereas HA-966 infused into the hippocampal region was without effect when the retention period was 13 days. These findings suggest that both the temporal cortex and lateral entorhinal cortex are involved in late post training memory processing, and that the hippocampal region probably is not. When the retention period was 23 days, the degree of impairment was similar to that seen for 13 days following infusions of HA-966 into the temporal cortex or lateral entorhinal cortex (Fig. 2). The effects of such infusions were considerably diminished when the retention period was only 3 days. The percentage saving of trials was related to the length of time between acquisition and relearning for the groups treated with the functional NMDA receptor antagonist (Tables 1–3).

Infusion of HA-966 into the temporal or lateral entorhinal cortices resulted in a comparatively low level of saving of trials during relearning of the task 13 and 23 days following the treatment (mean of 20% and 14%, respectively). This finding suggests that the antagonist interfered specifically with the information about the principle that the black or the grey cylinder was the positive one (reference memory). The ability to relearn the task was apparently not affected. This result contrasts with effects observed after transection of the fiber connections between the temporal and lateral entorhinal cortices which also impairs the ability to relearn the present discrimination

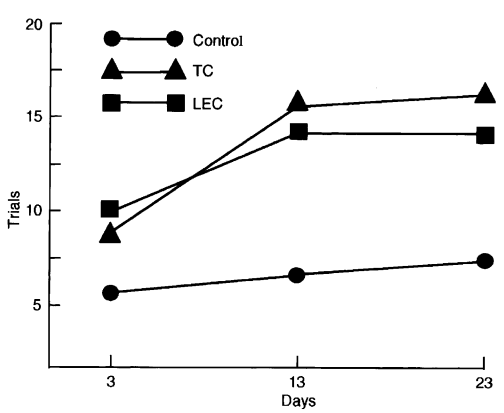

Fig. 2. Summary of retention scores presented in Tables 1–3. Trials to criterion in rats treated with HA-966 in the temporal cortex (TC) or the lateral entorhinal cortex (LEC) as a function of days of retention. Control groups received saline in the same structures.

task (saving of trials −25%; Myhrer, 1991). The differential effects seen between the short and the longer retention periods imply that HA-966 probably did not produce a retrieval deficit. The differential effects of various retention times also suggest that the behavioral changes are associated with a cognitive impairment and not with an interference with perceptual, motor, motivational, or emotional processes.

The most likely mechanism underlying the memory impairment is a block of the glycine recognition site of the NMDA receptor, resulting in reduced responding to endogenous glutamate in rats infused with HA-966 into the temporal or lateral entorhinal cortices. It has been demonstrated that systemic administration of (±)-HA-966 can effectively prevent the mitigating effect of glycine injections in rats with lesion-induced memory impairment (Myhrer, 1994). A dose of (+)-HA-966 higher than the one used in this study (1 μg/μl) may probably not produce a stronger cognitive effect, because the saving of trials was close to zero (4.5%) for the group with temporal cortex infusions when the retention period was 23 days. On the other hand, a lower dose would be expected to cause milder effects than those seen in the present study.

Suppression of neural activity in circumscribed areas by means of antagonists may mimic effects of selective lesions of the same areas. Actually, such a molecular pharmacological approach results in a temporary "functional lesion" with greater specificity than conventional lesions (Izquierdo and Medina, 1998). Subregions dominated by glutamate-mediated neurotransmission may be particularly affected by glutamate receptor antagonists. Both temporal cortex and entorhinal cortex most likely receive a number of glutamatergic nerve endings (Myhrer et al., 1989; Levisohn and Isacson, 1991; Aggleton et al., 1997). The hippocampal region also receives glutamatergic projections from the entorhinal cortices (Köhler, 1986). Results from lesion studies have shown that damage to temporal cortex or lateral entorhinal cortex following acquisition of visual discrimination results in impeded retention, whereas damage to the hippocampal region does not impair retention. However, hippocampal lesions cause an acquisition deficit, whereas such a deficiency is not seen in rats with temporal cortical or lateral entorhinal cortical lesions (cf. Myhrer, 1992). The present findings that infusion of HA-966 into the temporal or lateral entorhinal cortices, but not into the hippocampal region, impaired visual memory seem to be well consistent with earlier results from lesion studies.

The infusions of HA-966 are probably characterized by anatomical specificity. Injection of a corresponding amount of methylene blue was seen to invade an area of about 1 × 1 mm. Even if HA-966 might have spread more effectively than methylene blue, the diffusion of 1.0 μl is probably not far-reaching. The centrally positioned infusions made in the temporal cortex may not have affected neighboring areas pharmacologically, whereas the infusions into the somewhat smaller lateral entorhinal cortex may have affected the perirhinal cortex. Even if neurons in the perirhinal cortex were not pharmacologically affected by the antagonist, indirect suppression of neural activity in the perirhinal cortex may have occurred, since the connections between the temporal cortex and lateral entorhinal cortex are partly relayed in the perirhinal cortex. Selective perirhinal lesions have been shown to result in memory impairments similar to those seen following temporal cortical/lateral entorhinal cortical transection (Myhrer and Wangen, 1996a). The lack of effect seen to follow hippocampal infusions is probably not associated with the relatively large size of this structure. The double infusions made in the hippocampal region were able to affect nearly the entire dorsal hippocampus, which is usually the target area for lesions.

Systemic administration of HA-966 to rats has been shown to result in impaired delayed matching to position (Doyle et al., 1998) and reduced ability to recognize environmental changes (Myhrer, 1999). Intramuscular injection of HA-966 has been shown to result in impaired recognition memory in monkeys (Matsuoka and Aigner, 1996). Intraventricular injection of the glycine site antagonist, 7-chlorokynurenic acid (7-CK), in rats disrupts spatial memory in the Morris water maze and the working memory component in a three-panel runway task (Watanabe et al., 1992; Ohno et al., 1994). The NMDA receptor site can be affected by AP5, which causes memory deficits in habituation or avoidance tasks after microinfusions into the entorhinal cortex or hippocampal region (Ferreira et al., 1992; Izquierdo et al., 1992; Jerusalinsky et al., 1992). MK-801, which acts at the phencyclidine site of the NMDA receptor, also has been demonstrated to cause mnemonic deficits in monkeys after systemic administration (Ogura and Aigner, 1993) and in rats after intraventricular infusion (Morris, 1989). The present finding of impairment in visual memory following administration of HA-966 is in agreement with the above behavioral results obtained with agents that have blocking effects on the NMDA receptor complex.

Results from the present and previous microinfusion studies of glutamate receptor antagonists (cf. Introduction) and lesion studies using the present discrimination task (cf. Myhrer, 2000) suggest that the hippocampal region and the rhinal cortices are sequentially involved in mnemonic processing. When memory engrams have been established in rhinal cortices, the preservation process probably no longer involves the hippocampal region. When the storing process has lasted for a sufficient time, even the rhinal cortices may no longer be necessary for maintenance of the storing process. Sequential uncoupling of structures in the temporal region seems to occur when engrams grow older. The results from a study of microinfusions of the AMPA receptor antagonist, cyanonitroquinoxalinedione (CNQX), in rats support this notion. The hippocampal region is involved in nonspatial memory expression for only a few days following acquisition, and the entorhinal cortex is

involved in mnemonic processing for 1–2 months. However, the posterior parietal cortex is involved in memory expression up to at least 2 months after acquisition (Quillfeldt et al., 1996). The storing process for spatial information appears to be more dependent on the hippocampal region than that for nonspatial material. However, spatial memory traces seem to be inactive in the hippocampal region after several days, or perhaps longer (Brun et al., 2001).

Consolidation of memory occurs during the process by which memory gradually becomes more resistant to disruptive amnestic agents such as electroconvulsive shocks or drugs. Administration of HA-966 1.5–3 h following acquisition in the present study probably affected the consolidation process locally, in contrast to the more global effects following systemic administration of agents or electroconvulsive shocks. The consolidation process may go on for 1–3 weeks for a passive avoidance task in mice. However, at the same time, a gradual weakening of memory engrams starts. A passive avoidance task is forgotten within 12 weeks in mice (Squire, 1987). The complete forgetting curve has not been examined for the discrimination task used in the present study, but parts of such a curve are shown in Fig. 2. The relatively well preserved memory 3 days after treatment with HA-966 is probably associated with a recency-related strength of the memory traces.

In summary and conclusion, this study showed that infusion of HA-966 into the temporal cortex or lateral entorhinal cortex 1.5–3 h following acquisition probably disrupts consolidation of memory engrams carrying information about the present discrimination task. Corresponding infusion into the hippocampal region did not affect memory processing. These results support a prevalent opinion that the hippocampal region is involved in temporary storing of nonspatial information (Quillfeldt et al., 1996), whereas the temporal and lateral entorhinal cortices along with the perirhinal cortex can be involved in the storing of information for a longer period of time.

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