8. General discussion

The present study demonstrates the dissociative involvement of the striatal and hippocampal cholinergic systems in spatial localization using 8-arm radial maze task and the EL and AL tasks in a plus maze. The present results reassured that brain cholinergic systems play critical roles in cognitive processes and provided further evidence that the cholinergic systems in the striatum and hippocampus contribute to an efficient spatial localization in dissociable manners.

As discussed in earlier section of the present thesis, EL is comparably a more primitive memory component than AL from phylogenic aspect (Tulving & Shacter, 1991). EL can be regarded as a 'skill' in a sense that it is acquired through repetitive process and retrieved almost automatically. Skillful or automatic activities such as escaping from predators and running fast in nests have no sense of their existence if such activities do not function instantly. EL process is acquiring a series of certain body movements primarily based on organism's body position and direction, so once acquired, this 'skill' may be retrieved automatically according to Tulving's suggestion. In acquisition process, however, animals took more trials in the EL acquisition in Exp. 4 and Exp. 6 as typically seen in the comparison between Fig. 23 and Fig. 24. The phenomenon that the animals took more trials in the acquisition of the EL task than in the AL task indicates that the EL task is more difficult than the AL task for rats that carry quite evolved strategies for survival including spatial abilities typically characterized by AL. AL, which may have emerged later than EL in

phylogenic aspect, is likely to work as a primal function when animals are put in novel experimental conditions such as maze tasks. In this regard, EL is comparably more difficult than AL in acquisition process at least for rats.

There is one question that once acquired, the EL and AL retention could be performed automatically, so these retention processes are supposed to be the same in terms of retrieving previously acquired responses. This factor includes the question that the AL task could be regarded as the EL task in the point that animals may be able to learn the direction to turn (either left or right) at the choice point based on the visual stimuli seen from the start arm. Locating based on the body position and direction is undoubtedly EL ability that it may be that the AL task in the present study cannot exclude EL factor. However, the results of Exp. 4 and Exp. 5, that the EL and AL retention/acquisition were selectively impaired by striatal and hippocampal cholinergic lesion respectively, indicate that animals may have acquired the EL- and AL-strategy in the EL- and AL-task selectively. Since animals were faster learners of AL than EL in the acquisition, it is suggested that they tend to use the AL-strategy first, and shift to the EL-strategy when necessary in spatial localization. In this regard, spatial organized behavior may be accomplished by a competitive interaction between EL and AL.

The present results the hippocampal lesioned animals were severely impaired in the AL retention and acquisition throughout the trials are consistent with a report that hippocampal lesion resulted in both retention and acquisition of a place-memory task (Mumby, Astur, Weisend, &

Sutherland, 1999). Here, deficits in acquisition and retention were called as anterograde and retrograde amnesia, respectively. In this regard, the deficits following hippocampal cholinergic lesion in the present study are both anterograde and retrograde deficits in AL behavior. In addition, anterograde and retrograde amnesia here may be explained as failures to encode and retrieve the AL strategy following hippocampal lesion, respectively. Thus, it is hypothesized that the hippocampal cholinergic system is specifically involved in encoding and retrieval of the AL-strategy. Yet, there is a question whether these two amnesic factors reflect the same or different memory processes. It is also presumable that animals are in any means incapable of encoding nor retrieving the AL-strategy if they cannot process spatial cues. It is quite difficult, so far, to dissociate these spatial cue-processing and memory (encoding and retrieval) deficits since both deficits may elicit retention and acquisition impairment.

The classical notion on the role of the striatum had been the regulation of motor behavior (Yahr, 1976), and studies on the striatal cholinergic system had been limited to biochemical analysis and locomotor behavior (Dawson, Dawson, Filloux, & Wamsley, 1988; Meana, Johansson, Herrera-Marschitz, O'Connor, Goiny, Parkinson, Fredholm, & Ungerstedt, 1992; Sanberg, Hanin, Fisher, & Coyle, 1984; Sandberg, Sanberg, & Coyle, 1984; Stwertka & Olson, 1986; Zhou, Zhang, Connell, & Weiss, 1993). Some findings reported a decrease in ChAT activity and reduced cell size of the striatum in aged rats (Michalek, Fortuna, & Pintor, 1989; Waller & London, 1989), yet the involvement of the striatal cholinergic systems in memory process has not been generally investigated. The present results

suggest that the striatal cholinergic system, which contains the densest cholinergic innervation in the brain (Hoover, Muth, & Jacobowitz, 1978), plays a critical role that can be differentiated from that of the hippocampal cholinergic system.

It is suggested, from the present behavioral results, that the striatal cholinergic system plays an important role mainly in the acquisition process and less in the retention process since dysfunction in the EL retention of the striatal lesioned animals were comparably milder and saved by overtraining but severely impaired in the EL acquisition. In addition, the result that overtraining saved the retention performance of the striatal lesioned animals in the EL task indicates that the EL-strategy may be retrieved not only via the striatal cholinergic system but also via other brain systems that overtraining to striatal lesioned animals had saving effect on the EL retention. Then again, the result that the EL task was comparably more difficult than the AL task may be one of the factors that affected the worse performance of the striatal lesioned animals in the EL acquisition than in the EL retention. Therefore, whether the striatal cholinergic system play a critical role mainly in the acquisition of the EL-strategy or in both the acquisition and retention of EL-strategy, should be further investigated.

Another possibility of the dissociable functions of the striatal and hippocampal cholinergic systems could be elucidated by the functional dissociation of intrinsic and extrinsic cholinergic neurons. There are many brain regions including cholinergic local circuit neurons such as the striatum, nucleus accumbens, olfactory tubercle, amygdala, hippocampus, and

neocortex (Woolf, 1991). On the other hand, in the striatum, there are no extrinsic cholinergic fibers found. Thus, in the striatum, only intrinsic cholinergic neurons were supposed to be lesioned in the present study. As for the hippocampal systems, though the septo-hippocampal cholinergic projection has drawn major attention with regard to learning and memory functions, Van der Zee & Luiten (1999) claimed that intrinsic local cholinergic fibers also play a critical role in memory. Most of the hippocampal cholinergic terminals originate in the medial septum-diagonal band complex, but some conceivably may originate from the intrinsic cholinergic neurons (Amaral & Kurz, 1985). Van der Zee & Luiten (1999) hypothesized, according to the computational model of Hasselmo (1995), that ACh functions as a modulator regulating the level of intrinsically originating versus extrinsic originating signal transduction (i.e. signal transduction arising from local circuits versus afferent, ascending projection nuclei), and switching the hippocampus and neocortex from recall (retrieval) to learning (encoding) mode, respectively. They suggest that cholinergic neurons in the hippocampus and neocortex both contribute to spatial localization and could be differentiated with regard to learning (encoding) and recall (retrieval) processes. The hypothetical dynamics are as follows: a state in which new information is stored (high activity level of extrinsic cholinergic fibers) and a state in which this information is reactivated for recall/retrieval (high activity level of intrinsic cholinergic fibers). The way ACh exerts a shift towards learning (encoding) is by inhibiting transmitter release from intrinsic, local fibers through activation of their presynaptic muscarinic ACh receptors, and activating

postsynaptic muscarinic ACh receptors on the target neurons by which input transfer from extrinsic fibers is facilitated. It is supposed, in the present study, that AF64A injection into the striatum resulted in a selective lesion of the striatal intrinsic cholinergic neurons, whereas AF64A injection into the hippocampus affected both the intrinsic and extrinsic cholinergic fibers in the hippocampus. According to Van der Zee & Luiten (1999), the hippocampal extrinsic ACh fibers are mainly activated in learning mode, so it may play a critical role in encoding of the AL-strategy, whereas the hippocampal intrinsic fibers are mainly activated in recall/retrieval mode, so it may play a critical role in retrieving the AL-strategy. Therefore, it is presumed that the hippocampal lesioned animals in the present study were impaired both in the retention and acquisition of the AL task since both extrinsic and intrinsic fibers in the hippocampus were lesioned. On the other hand, though cognitive function of the striatal cholinergic interneurons are not discussed in the study of Van der Zee & Luiten (1999) and it is difficult to account for the impairment in EL behavior following lesion of the striatal cholinergic interneurons, it is presumed that impairment of the striatal lesioned animals was milder in the EL retention than in the EL acquisition since cholinergic neurons in the striatum are only intrinsic and neural inputs from other brain regions such as nigro-striatal pathway were spared. Furthermore, other brain systems are presumably involved in EL as described previously, so that may also be attributed to the impairment in the present study.

Recently, the striatal dopaminergic (DA) system has also been shown to play a critical role in learning and memory. Packard and White (1991)

reported the double dissociation of the striatal and hippocampal DA systems in memory consolidation processes. The infusions of the indirect DA agonist *d*-amphetamine, the direct D_2 agonist LY 171555, and the direct D_1 agonist SKF-38393 into the striatum all improved one type of memory consolidation process dissociated from the another type of consolidation process improved by the same series of DA agonists into the hippocampus. Levin, Torry, Christopher, Yu, Einstein, and Schwartz-Bloom (1997) also reported a positive correlation between T-maze accuracy and D_1 receptor binding in the frontal cortex, and between radial arm maze accuracy and D_2 receptor binding in the striatum and the dentate gyrus. These findings support the idea that identical neurotransmitter carries different functions in cognitive processes depending on the region where each neurotransmitter is located.

There is also a growing evidence suggesting the critical relationship between ACh and DA receptor systems with regard to memory function (Levin & Rose, 1992). The striatum receives dopaminergic innervation via the nigrostriatal DA pathway, originating in the substantia nigra (Moore & Bloom, 1978). Consolo, Girotti, Zambelli, Russi, Benzi, and Bertoelli (1993) reported that the striatal cholinergic activity is indirectly facilitated by stimulation of D_1 receptors and inhibited by direct stimulation of D_2 receptors. Several studies have implied the involvement of the nigrostriatal DA pathway in learning and memory processes (Carr & White, 1984; Neill, Boggan, & Grossman, 1974; Viaud & White, 1989; White, 1988; White & Major, 1978; Zis, Fibiger, & Philips, 1974). The striatal cholinergic neurons are intrinsically organized (Woolf & Butcher, 1981) and have drawn

little attention in terms of learning and memory. However, the present results showing that the decrease of only ACh level in the striatum seriously impaired EL behavior suggest that the striatal cholinergic system itself plays a critical role in a certain type of learning and memory process. It is possible that, in the previous studies, hypofunction of nigrostriatal DA system caused various cognitive deficits through hypofunction of the striatal cholinergic system. In this regard, ACh and DA may function in a complementary manner that the interaction of these two transmitter systems contributes to adaptive behaviors in certain learning situations. Yet there are few findings supporting the idea of ACh-DA interaction in the striatum with regard to the cognitive function, so more neurochemical studies are necessary for its clarification. DA systems have also been shown to be involved in the motivational systems and motoric activities, and therefore, behavioral results following the manipulation of DA systems should be assessed carefully not to confuse mnemonic, motivational, and motoric components.

The septal area receives dopaminergic, noradrenergic, serotonergic, and cholinergic afferents from several brainstem structures (Costa, Panula, Thompson, & Cheney, 1983; Lindvall & Stenevi, 1978; Mesulam, Mufson, Wainer, & Levey, 1983) and glutamate, GABA, DA, norepinephrine and a few peptides have been suggested to be involved in the septal regulation of hippocampal cholinergic activity (Costa et al., 1983; Dekker & McGaugh, 1991). Furthermore, Nilsson, Leanza, & Björklund (1992) suggested that especially catecholaminergic and serotonergic systems subserve a critical role in regulating septo-hippocampal cholinergic activity. Noteworthy

suggestion on cholinergic function is done by Krnjevic (1993) that ACh plays a critical role in the initiation of long-term potentiation (LTP). He assumes the following three different ways through which ACh affects LTP. The first is through the cholinergic suppression of K-currents that oppose cellular depolarization. The second is by the reduction of inhibitory synaptic inputs; most types of stimulation that activate excitatory input also bring into action powerful inhibitory synapses (through feedback or feedforward pathways). The third is a muscarinic facilitation of NMDA-evoked currents.

Thus, hippocampal cholinergic system may function under the interaction with other neurotransmitter systems. However, unlike the striatal cholinergic systems, there is a large body of evidence showing that the change of ACh level itself, other than DA, glutamate and so on, in the hippocampus has been regarded as a neurotransmitter selectively involved in a certain type of information processing such as discrimination learning (Yamamoto, Hori, Tanaka, Iwano, & Nomura, 1995) and acquisition of a rewarded operant responses (Orsetti, Casamenti, & Pepeu, 1996) employing in vivo microdialysis techniques, though the results of the current study and those employing microdialysis techniques cannot be discussed on the same basis since the behavioral tasks used are different in terms of what is required to perform those tasks.

As described above, striatal and hippocampal AF64A injection in the present study selectively decreased ACh levels only in the striatum and in the hippocampus, respectively. Neither striatal nor hippocampal AF64A injection affected ACh level in the cortex. Therefore, the deficits found in

the present study are not accounted for by the function mediated by the cortical cholinergic neurons. Thus, its cholinergic functions are not discussed in the present study. Yet the cortical cholinergic systems, especially the forebrain cholinergic systems have drawn attention as a major substrate in learning and memory. Since "cholinergic hypothesis of geriatric memory function" by Bartus, Dean, Beer, & Lippa (1982) emphasized the importance of the forebrain cholinergic systems in cognitive processes, a main focus on the responsible cholinergic systems for learning memory had been the forebrain systems. The cortical cholinergic neurons are undoubtedly responsible for certain learning and memory functions (Nabeshima, 1993; Rasmusson, 1993; Woody and Gruen, 1993), so it is also important to investigate the cortical cholinergic function in terms of learning and memory. Pepeu (1993) discussed that the central cholinergic systems have a widespread activatory function which prepares the neurons for information accumulation and retrieval. It is indicated by these reports that a variety of learning and memory deficits are elicited by hypofunction of initial activatory function in brain regions subserved by cholinergic systems. Thus, some learning and memory functions subserved in certain brain regions may not be activated if cholinergic activity in the regions is somewhat disturbed. It should be noted that cholinergic systems are suggested to be regulated by other neurotransmitter systems as described previously, the cholinergic systems are not the only neurotransmitter systems that is responsible for the activation. Therefore, underlying function, which makes learning and memory function impaired, should be further investigated.

In conclusion, both striatal and hippocampal injections of AF64A impaired the radial arm maze behavior. In addition, the reduction of the striatal ACh resulted in an impairment in EL behavior which appears to be due to the deficit in encoding of the EL-task-solving strategy, whereas the reduction of hippocampal ACh lead to a serious impairment of AL behavior which may result from the deficit in both retrieval and encoding of the AL-task-solving strategy. Taken together, the striatal and hippocampal cholinergic systems appear to function simultaneously that each function of them is indispensable for an efficient performance in spatial localization.