Introduction

Studying is an important daily lesson to children and the memory deficit in childhood may cause a poor record at school and may affect even a psychological and characteristic disorder afterward1). Due to fact that the average life expectancy has been extended, senile dementia, one of the diseases which has memory disorder as a presenting symptom, has risen as a new social problem2). With such a social current, the studies of learning and memory are performed vigorously.

Learning is a continuous transition of behavior through indirect-direct experience or training. Conditioning is a way to explain the learning as a connection, for example, stimulation-stimulation or response-stimulation, etc3).

Conditioning can be classified by two categories; classic conditioning, and operant (instrumental) conditioning. In classic conditioning, the learner’s
behavior has nothing to do with the accident. In operant conditioning, a reward or punishment, dependent on learner's specific behavior, is given and it reinforces the learners reaction.

Avoidance performance, one of the operant conditioning, is a learning that ceases unpleasant or harmful stimulation. Avoidance performance can be classified as two groups: active avoidance performance and passive avoidance performance. Provided the former is a learning which can avoid the punishment by a specific performance, the latter is a learning that a specific reaction can bring a punishment. This is a common method to study the learning and memory ability of the lower animals.

Recently, the NMDA (N-methyl-D-aspartase) receptor has been focused on in the studies of learning and memory. The NMDA receptor serves as a graded molecular switch for gating the age-dependent threshold for synaptic plasticity and memory formation. NMDA-dependent modifications of synaptic efficacy are the underlying mechanism of various associative learning and memory.

In oriental medicine, Xin (心), Yi (意) and Zhi (志), the part of Qi-Shen (七神) considered as recognition process, are similar to memory. Xin (心) is the process of receiving and understanding the external stimulus, and attached to the heart (心). Yi (意) is the process of memory and retrieval, and attached to the spleen (脾). Zhi (志) is the process of continuing status of Yi (意) and attached to the kidney (腎). Compared with psychology, Xin (心) can be regarded as sensory memory, Yi (意) as short-term memory, Zhi (志) as long-term memory. Therefore, the heart, spleen and kidney act as important factors in the memory process and their dysfunction may cause one of the representative symptoms of memory disorder, that is, amnesia.

In understanding the mechanism of memory disorder, it has been considered that poor memory of children is caused by not-filled or immature Nao-Sui (腦髓, brain and spinal cord), on the other hand, amnesia of the old is viewed as gradual vacancy of Nao-Sui.

Consequently, the fundamental cause of memory disorder in children and the old results from a deficiency of Nao-Sui (腦髓) where treatment of the memory disorder of both does not make a great difference.

The elements of Saenggitang (SGT), Rehmanniae Radix Preparat (熟地黃) 20 g, Corni Fructus (山茱萸) 4 g, Polygalae Radix (遠志) 3.2 g, Zizyphi Spinosae Semen (酸棗仁) 8 g, Poria (茯苓) 12 g, Ginseng Radix (人蔘) 4 g, Acori graminei Rhizoma (炒酸草) 1.2 g, Atractylodis macrocephalae Rhizoma (白朮) 4 g, Glycyrrhizae Radix (甘草) 1.2 g, Massa medicata Fermentata (麥芽) 1.2 g, Pinelliae Rhizoma (半夏) 1.2 g, Liriopis Tuber (半夏) 4 g, Cinnamomi Cortex (肉桂) 1.2 g, Euryales Semen (阿森) 12 g, and Aucklandiae Radix (鳥腳香) 0.4 g, can be founded in Bian-Zheng-Qi-Wen (辨證奇聞). SGT has been used for amnesia due to consumptive disease or deficiency of Jing (精, essence of life). This prescription is characterized by tonifying the spleen and stomach as well as reinforcing heart and kidney in treatment method. SGT has also been considered to improve memory and regulate physiological activities.

Thus, as SGT acts on all three Jang-Fu (臟腑), heart, spleen and kidney, it is supposed to have a good effect on the treatment of memory disorder and enhancement of learning and memory ability.

But scarcely any paper describes the experiments on the effects of SGT on memory processes until now. This paper describes the effects of a hot water extract (HWE) of SGT on the memory and learning process, by using the passive and active avoidance performances and a fear conditioning task, which requires the activation of the NMDA receptor.
This experiment may provide clues for the clinical application of this drug to amnesia, dementia and the other memory decreased performances.

Materials and Methods

1. Materials
   1) Animals
   Adult mice (3-6-month old littermates) were used throughout all behavior tests. Ten mice were kept in one cage in a temperature and humidity controlled room for 1 week before the experiment.

   2) Isolation of SGT-HWE
   Voucher specimens and amounts of the SGT medicinal drugs studied in this work were as follows: a total of 20 g of Rehmanniae Radix Preparat (熟地黄), 4 g of Corni Fructus (山茱萸), 3.2 g of Polygalae Radix (遠志), 8 g of Zizyphi Spinosae Semen (蒺藜仁), 12 g of Poria (白茯苓), 4 g of Ginseng Radix (人参), 1.2 g of Acori Graminei Rhizoma (菖蒲), 4 g of Atractylodis Macrocephalae Rhizoma (白朮), 1.2 g of Glycyrrhizae Radix (甘草), 1.2 g of Massa Medicata Fermentata (甘朮), 1.2 g of Pinelliae Rhizoma (半夏), 4 g of Liriopis Tuber (麥門冬), 1.2 g of Cinnamomi Cortex (肉桂), 12 g of Euryales Semen (芡 仁), 0.4 g of Aristolochiae Radix (唐木香). These 15 herbs were added to 500 ml of water and boiled for 2 hr and filtered and then concentrated to 10 ml. The extracts were lyophilized and aliquots (50 mg) were separately stored at -20°C for next experiments.
   The aqueous extracts of SGT and its 15 composed Korean herbs, which was massproduced for clinical use, were kindly supplied by the Oriental Medical Hospital of Dongguk University.

2. Methods
   1) Passive avoidance performances
   \[(1)\text{ Step through test}\]
   The chamber apparatus had a partition wall with a hole, which divided the chamber into two compartments, one bright and the other dark. As soon as a mouse entered the dark compartment from the bright one, a punishing electric shock was given through the foot grids. The time needed for the mouse to enter the dark compartment was recorded. On the first day, each mouse received a learning trial, by which it was taught that if it entered the dark compartment, it was to be punished. Twenty-four hours later, 10 mice were placed again in the bright compartment, and were left there for 300 sec. The latency and the number of mice which did not enter the dark compartment were recorded.\[\\]
   \[(2)\text{ Experimental procedures}\]
   ① Effects of the SGT-HWE on memory process in normal mice
   - The SGT-HWE was orally administrated 30 min prior to the learning trial, immediately after the learning trial or 30 min before the testing trial to demonstrate the effects of the SGT-HWE on memory registration, consolidation or retrieval process respectively. 12 mice were randomly used in each group.
   ② Effects of the SGT-HWE on memory impaired mice
   - Mice were randomly divided into control group, and two experiment groups, one being treated with 20 mg/100 g SGT-HWE and the other with 50 mg/100 g SGT-HWE. 12 mice were used in each group.
   a) Memory registration impairment
   10 min after the administration of the SGT-HWE, 30% ethanol (10 ml/kg, p.o.) or scopolamine (0.5 mg/kg, i.p.) was given to the mice to interfere with memory registration process. 20 min later, the learning trial was
given to them.

b) Memory consolidation impairment
The SGT-HWE was given 30 min before the learning trial. Electric convulsive shock (ECS) (0.4 m/s in width, 100 Hz, 94 mA for 0.2 sec) was given to the mice through the ears immediately after the learning trial in order to impair the memory consolidation process.

c) Memory retrieval impairment
On the first day, the learning trial was given to the mice. On the second day, 10 min after the treatment with the SGT-HWE, 40% ethanol (10 ml/kg, p.o.) ECS (0.4 m/s in width, 100 Hz, 80 mA for 0.2 sec) was given to the mice to impair the memory retrieval process. 20 min later, the testing trial was given.

2) Active avoidance performances

(1) Lever press test
A stainless steel lever was vertically set at one side of the lever press test chamber. A buzzer and lamp were set on the ceiling and the floor was connected to an electric stimulator. The programme of one trial was as follows: ① 40 sec interval; ② 10 sec for conditional stimulation (CS) (the warning buzzer and the lamp were on during this CS period); ③ 10 sec for unconditioned stimulation (US) (electric stimulation, an intensity of 36 V, AC) as well as the buzzer and lamp were on during the US period.

If a mouse pressed the lever during the CS or US period, the electric shock was canceled immediately. Pressing the lever during the CS period was considered as a conditioned avoidance response (CAR) and in the US period as an unconditioned avoidance response (UAR). The trial without pressing lever in CS and US period was considered as a failure. The movement of the mouse that was irrelevant to CAR or UAR was counted as spontaneous motor activity (SMA)\(^{14}\).

(2) Experimental procedures
: Effects of the SGT-HWE on memory registration
The SGT-HWE was orally administrated 15 min prior to the test. Lever press performances were tested for 7 days, once daily, at the same time of the day. The number of animals in each group was 8.

3) Motor activity
Motor activity was measured by using a tilting-type round activity cage of diameter 18 cm and height 18 cm (AMB-10; Tokyo, Japan). Measurement of 30 min motor activity was conducted immediately after and 24 hr after the SGT-HWE administration. The number of animals in each group was 9.

4) Pentobarbital-induced sleep
Fifteen min after a saline, chlorpromazine (2 mg/kg, i.p.) or the SGT-HWE administration, pentobarbital (50 mg/kg, i.p.) was given to the mice. The sleep duration (the time from the disappearing of the light reflex to the recovering of the reflex) was measured.

5) Fear conditioning task
We used a fear conditioning shock chamber (10 × 10 × 15 inches high) and TruScan multi-parameter activity monitors (Coulbourn Instruments). The conditioned stimulus (CS) was an 85 dB sound at 2,800 Hz, and the unconditioned stimulus (US) was a continuous scrambled foot shock at 0.75 mA. After the CS/US pairing, the mice were allowed to stay in the chamber for another 30 sec for measurement of immediate freezing. During the retention test, each mouse was placed back into the shock chamber and the freezing response was recorded for 3 min (contextual conditioning).

Subsequently, the mice were put into a novel chamber and monitored for 3 min before the onset of the tone (pre-CS). Immediately after that, a tone identical to the CS was delivered for 3 min and freezing responses were recorded (cued conditioning).
The retention test evaluate the contextual fear memory and the cued fear memory is conducted at 1 hr, 1 day and 10 days after training.

6) Statistics
The results of the rate of the successful mice in passive avoidance performances were analyzed by the chi-square test and the sleep prolongation test result by a student’s t-test. The rate of mice exhibit freezing response were analyzed by ANOVA. All the other data were analyzed by the Mann-Whitney’s U-test.

Results

1. Effects of the SGT-HWE on passive avoidance performances : Step through test
1) In normal mice
In normal mice, the SGT-HWE did not affect the latency and the percentage of failure mice in the step through test, which suggested that the SGT-HWE has no effect on passive avoidance performances in normal mice.

2) In memory impaired mice
The SGT-HWE neither ameliorated the memory registration impairment caused by 30% ethanol or scopolamine, nor improved the memory consolidation deficit and memory retrieval disturbance induced by ECS. In the case of mice having a memory retrieval impairment induced by 40% ethanol, 20 mg/100 g of SGT-HWE decreased the latency to enter the dark shock compartment significantly (Table 1); however, it increased the percentage of successful mice (Table 2). And then 50 mg/100 g of SGT-HWE increased the latency to enter the dark shock compartment (Table 1) and the percentage of successful mice significantly (Table 2).

2. Effects of the SGT-HWE on active avoidance performances : lever press test
In the memory registration experiment, 20 and 50 mg/100 g of SGT-HWE treated mice always showed lower CAR and SMA values than the control mice concentration-dependently (Table 3, 4) and the same UAR as the control mice. CAR of SGT-HWE treated

Table 1. Effects (time elapse) of the SGT-HWE on Memory Retrieval Impairment Induced by 40% Ethanol in the Step Through Test.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>ethanol 20</th>
<th>SGT20</th>
<th>control</th>
<th>ethanol 50</th>
<th>SGT50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latency (s)</td>
<td>229.7±5.5</td>
<td>208.0±3.6*</td>
<td>200.0±14.4*</td>
<td>229.7±5.5</td>
<td>207.7±13.4*</td>
<td>213.3±0.6</td>
</tr>
</tbody>
</table>

Ethanol : mice were orally treated with 40% ethanol 20 min before the testing trial. SGT 20 and 50 : 20 mg/100 g or 50 mg/100 g SGT-HWE was orally given 30 min before the testing trial. Time elapsed before the mice entered the dark compartment (latency) is shown. Data expressed as mean ± S.E. * : p<0.05 vs. control, Mann-Whitney’s U-test, n=12

Table 2. Effects (Percentage of Mice not Entering the Dark Compartment in the Testing Trial) of the SGT-HWE on Memory Retrieval Impairment Induced by 40% Ethanol in the Step Through Test.

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>ethanol 20</th>
<th>SGT20</th>
<th>control</th>
<th>ethanol 50</th>
<th>SGT50</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of successed mice(%)</td>
<td>70</td>
<td>45*</td>
<td>54</td>
<td>70</td>
<td>44*</td>
<td>76**</td>
</tr>
</tbody>
</table>

Ethanol : mice were orally treated with 40% ethanol 20 min before the testing trial. SGT 20 and 50 : 20 mg/100 g or 50 mg/100 g SGT-HWE was orally given 30 min before the testing trial. * : p<0.05 vs. control, ** : p<0.01 vs. ethanol, chi-square test, n=12
mice increased only up to 5 day and decreased after 5 day. On the other hand, SMA of SGT-HWE treated mice increased gradually through the whole period (Table 3, 4).

3. Effects of the SGT-HWE on motor activity

20 and 50 mg/100 g of SGT-HWE did not affect the motor activity measured by the tilting type ambulometer test performed immediately and 24 hr after the administration.

4. Effects of the SGT-HWE on pentobarbital-induced sleep

The pentobarbital-induced sleeping time was significantly extended by 50 mg/100 g of SGT-HWE. 20 mg/100 g of SGT-HWE also increased the sleeping time but had no significant difference as the control group.

Chlorpromazine (2 mg/kg, i.p.) as an active control, markedly extended the sleeping time, as well (Table 5).
5. Enhancement of contextual fear memory after training in SGT-HWE treated mice. (Contextual conditioning 1 hr, 1 day and 10 days after training).

Both contextual and cued conditioning were measured at 1 hr, 1 day and 10 days after training using separate groups of animals. We first analyzed contextual fear memory by measuring the immediate freezing response and freezing response of mice when placed back into the same shock chamber in the retention test at 1 hr, 1 day, 10 days after training. 20 and 50 mg/100 g of SGT-HWE treated mice consistently exhibited a much stronger freezing response than the control (Table 6, 7, 8). One-way analysis of variance (ANOVA) indicated no significant difference in immediate freezing between SGT-HWE treated and control mice, but a significant difference in contextual freezing when tested at 1 hr \( (p<0.05) \), 1 day \( (p<0.05) \), and 10 days \( (p<0.05) \). No significant difference in contextual freezing was noted between the 20 and 50 mg/100 g of SGT-HWE treated mice.

On the other hand, the SGT-HWE was not effective for the cued fear conditioning.

### Table 6. Enhancement of Contextual Fear Memory in the Retention Test at 1 hr after Training in SGT-HWE Treated Mice. (Contextual Conditioning at 1 hr after Training).

<table>
<thead>
<tr>
<th></th>
<th>Immediate freezing (%)</th>
<th>1 hr retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>SGT20</td>
</tr>
<tr>
<td>contextual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>freezing</td>
<td>12.3±0.6</td>
<td>9.3±1.5</td>
</tr>
</tbody>
</table>

SGT-HWE 20 and 50 : 20 mg/100 g or 50 mg/100 g SGT-HWE was orally given, Data expressed as mean± S.E. * : \( p<0.05 \) vs. control, ANOVA, n=8

### Table 7. Enhancement of Contextual Fear Memory in the Retention Test at 1 day after Training in SGT-HWE Treated Mice. (Contextual Conditioning at 1 day after Training).

<table>
<thead>
<tr>
<th></th>
<th>Immediate freezing (%)</th>
<th>1 day retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>SGT20</td>
</tr>
<tr>
<td>contextual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>freezing</td>
<td>13.3±0.6</td>
<td>12.3±0.6</td>
</tr>
</tbody>
</table>

SGT-HWE 20 and 50 : 20 mg/100 g or 50 mg/100 g SGT-HWE was orally given, Data expressed as mean± S.E. * : \( p<0.05 \) vs. control, ANOVA, n=8

### Table 8. Enhancement of Contextual Fear Memory in the Retention Test at 10 days after Training in SGT-HWE Treated Mice. (Contextual Conditioning at 10 days after Training).

<table>
<thead>
<tr>
<th></th>
<th>Immediate freezing (%)</th>
<th>10 days retention (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>SGT20</td>
</tr>
<tr>
<td>contextual</td>
<td></td>
<td></td>
</tr>
<tr>
<td>freezing</td>
<td>15.3±0.6</td>
<td>17.3±0.6</td>
</tr>
</tbody>
</table>

SGT-HWE 20 and 50 : 20 mg/100 g or 50 mg/100 g SGT-HWE was orally given, Data expressed as mean± S.E. * : \( p<0.05 \) vs. control, ANOVA, n=8
Discussion

As the memory promotion of children and senile dementia, the representative memory dysfunction disease are noticed\(^1\)\(^-\)\(^2\), studies of learning and memory ability are performed vigorously in many countries.

In oriental medicine, Xin (心), Yi (意), Zhi (志), the part of Qi-Shen (七神), that corresponds to the memory in psychology. The heart, spleen and kidney, which control Xin (心), Yi (意), Zhi (志), respectively, have a great influence on the memory process so that their dysfunction may cause a memory disorder like amnesia\(^8\)\(^-\)\(^9\).

Saenggitang recorded in Bian-Zheng-Qi-Wen (辨證奇聞) has been used for amnesia due to consumptive disease or deficiency of Jing (精, essence of life) and is characterized by tonifying the heart, spleen and kidney, which have a great influence on the memory process\(^11\). It is also considered to improve memory and regulate physiological activities\(^12\). This study describes the effects of SGT-HWE on memory and the learning process, by using the passive and active avoidance performances and fear conditioning task which requires the activation of NMDA receptor.

The NMDA receptor serves as a graded molecular switch for gating the age-dependent threshold for synaptic plasticity and memory formation. NMDA-dependent modifications of synaptic efficacy represent the basic mechanism of a various associative learning and memory\(^5\)\(^-\)\(^7\).

We assessed two forms of associative emotional memory in mice: contextual and cued fear conditioning. Animals learn to fear either a neutral conditioned stimulus (such as tone) paired with an aversive unconditioned stimulus (such as a foot shock) or a context conditioned by pairing conditioned and unconditioned stimuli. Contextual fear conditioning is hippocampus-dependent, whereas cued fear conditioning is hippocampus-independent\(^10\). Both these types of fear conditioning require the activation of NMDA receptors\(^16\)\(^-\)\(^17\).

In the contextual fear memory test, 20 and 50 mg/100 g of SGT-HWE treated mice exhibited a much stronger freezing response than the control. This suggests that the SGT-HWE improves contextual fear memory and acts as a stimulating factor for increasing the expression of the NMDA receptor, which is localized in around hippocampus of the brain tissue.

In the active avoidance performances (lever press test), which are based on the conditioned reflex theory, 20 and 50 mg/100 g of SGT-HWE concentration-dependently decreased CAR, the reaction guided by the active memory. This fact indicates that the SGT-HWE has no direct memory improving effects in normal animals.

The memory-impairment by ethanol in man has been attributed to the deficiencies in the subcortical noradrenergic and cholinergic systems\(^18\). Recently, electrophysiological experiments using the slices or isolated neurons of the hippocampus showed that ethanol inhibited the NMDA receptor (one of the glutamate receptor agonists) which activated ion current and the formation of long term potentiation, one of the basic phenomena associated with memory\(^19\).

Many memory impairment models have been established in studies of the memory and learning process\(^18\)\(^-\)\(^20\). Five of these models were used in this work: 30% ethanol-induced and scopolamine-induced memory registration deficit mice, 40% ethanol-induced memory retrieval impairment mice, ECS-induced memory consolidation deficit mice and ECS-induced memory retrieval deficit mice.

In passive avoidance performances (step through test), the SGT-HWE influenced only the memory retrieval deficit induced by 40% ethanol. 20 mg/100 g
of SGT-HWE decreased the latency to enter the dark shock compartment significantly, but increased the percentage of successful mice. As the result of 20 mg/100 g of SGT-HWE has no consistency, it is considered that this result cannot be taken to have any significance. On the other hand, 50 mg/100 g of SGT-HWE increased the latency to enter the dark shock compartment and the percentage of successful mice significantly. This may indicate that the SGT-HWE improved the memory retrieval deficit induced by 40% ethanol and interfered with the action of ethanol or mainly acted on the memory retrieval process. It is likely to be caused by either the acceleration of the alcohol metabolism or activation of neural transmissions by SGT-HWE. However, further investigations are required to elucidate the mechanism of the ameliorating effects of SGT-HWE on the ethanol induced memory impairment and the relationships between the SGT-HWE and memory process.

20 and 50 mg/100 g SGT-HWE has decreased SMA concentration-dependently in the lever press test. However, the SGT-HWE did not change the motor activity of normal mice in normal conditions immediately and 24 hr after its oral administration.

Thus, it is suggested that SGT-HWE may reduce the increased motor activity level in an excited state by environmental stimulations, which indicate the SGT-HWE had a tranquilizing and anti-anxiety action.

Although the SGT-HWE had no effect on the pain sensitivity level of mice, the SGT-HWE had no effect on the UAR and decreased CAR values concentration-dependently. Therefore it is suggested that the lower CAR values of SGT-HWE treated mice might be partly due to this tranquilizing and anti-anxiety effects. Decreased value of CAR after 5 day is suggested to be a result from experimental environment factor because all group of control, SGT-HWE at dose of 20, 50 mg/100 g have same aspect. 20 and 50 mg/100 g of SGT-HWE prolonged the sleeping time induced by pentobarbital concentration-dependently, which also indicates its tranquilizing or anti-anxiety action.

Further research is required to elucidate effects of the SGT-HWE on memory and learning process, and also expression of NMDA receptor in the brain. However, the results presented in this paper may provide fundamental data for the study about the effects of the SGT-HWE on the central nervous system and may help to reveal a strategy for other oriental medically treated animals with enhanced intelligence and memory.

References