Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.2, No.6, 249-258, 2015

Original Article

Developmental changes in associative learning behavior in male rats

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(Received December 3, 2015; Accepted December 10, 2015)

ABSTRACT — To identify the developmental point of onset of learning and memory function under an operant task, performance of young rats was examined on postnatal days (PDs) 16, 19, 22, 25 and 28, and then compared with that of adult rats (PD60). Each group of Long-Evans male rats with difference days of age was assigned to a series of fixed ratio (FR) operant tasks, in which the number of lever-pressing was required for acquisition of food reward. FR rate started from 1, changed into 2, and finished with 20. Each session conducted once a day, 7 sessions per week, lasted 30 min. Response rate (the number of lever-pressing per minute) and the number of session until animal acquired the learning acquisition criterion were measured. In FR20 learning task, performance of animals at PD32 showed the same shape as those of adult rats, showing an increase of response rate and a decrease of the number of sessions. The results indicated that memory and learning function of rats required for FR20 task might be matured at PD32. Results in the present study implied that the higher brain function used for the operant FR tasks mainly depended on the hippocampal development.

Key words: Operant task, Learning, Rat, Fixed-ratio (FR), Higher brain function

INTRODUCTION

The ontogeny of learning and memory function in rats has been described in great detail. We know that their sensory systems mature in increasing complexity with the following order: detection, recognition, conditioning, and retention (Vogt and Rudy, 1984). However, a higher order of memory and learning and cognitive functions, especially developmental process of memory and learning function for operant behavioral task has not been studied. Animals sometimes show different performance even if experiments follow the same procedure and apparatus during developmental period (Dumas, 2005). For instance, Rudy and Paylor (1988) tested young rats on postnatal days (PDs) 16, 19 and 20 with Morris Water Maze (MWM) task, a behavioral test for examining a spatial memory function of animals. Young rats on postnatal day (PD) 16 proved capable of finding a platform only when it was visible or near proximal cues. Yet rats older than PD20 could reach the platform hidden under water with a distal cue under MWM (Rudy *et al*., 1987; Carman *et al*., 2001). These results indicate that performances of animals on PDs 17 and 20 depend upon different neural substrates that develop at different periods, and that information generated by brain structure when it has matured at the period may be available (Rudy and Paylor, 1988; Rudy *et al*., 1987; Schenk, 1985). Likewise, classical conditioning experiments show age-dependent performance during developmental period in rats. A previously neutral conditioning stimulus (CS) paired with an unconditioned stimulus (US) produces a conditioned response (CR) before pairing and mimicking the unconditioned response (UR). On PD12, classical conditioning involving an olfactory, gustatory, or somatosensory CS and an administration of, is established if the conditioning test was conducted immediately after training (Stehouwer and Campbell, 1978; Gemberling *et al*., 1980; Hoffman and Spear, 1988; Miller *et al*., 1989; Haroutunian and Campbell, 1979; Kucharski and Spear, 1984a, 1984b; Markiewicz *et al*., 1986). If there is no delay between training and testing, elemental conditioning involving sensory systems used for CSs are completed by a few days more than 2 weeks of age. In rats, elemental conditioning with auditory and visual stimuli is established on PDs12-13 (Brunjes and Alberts, 1981; Hyson and Rudy, 1984; Rudy and Hyson, 1984) and PDs14-15 (Moye and Rudy, 1985; Paczkowski *et al*., 1999), respectively. The development of cognitive processes related to short- and

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long term memory retention follows that of elemental conditioning, suggesting continued development of brain structures involved in short- and long-term memory formation and/or recall. Following elementary conditioning, trace condition in which subject must retain a representation of the CS in order to make an association with the US across a stimulus-free interval (Kamin, 1965) occurs.

Although it has been already proved that some procedural changes improved performance of animals at younger than PD21 (Brown and Whishaw, 2000; Carman and Mactutus, 2001), motivational level and reinforcement magnitude in Morris Water Maze task cannot be varied (Moser *et al*., 2006), and procedural change sometimes disturbs a direct comparison with adult data (Dumas, 2005). The developmental process of associative learning abilities, involving different sensory modalities, emerges sequentially in accordance with synaptic maturation in related cortical and underlying brain structures (Dumas, 2005). If experimental variables can be manipulated to fit the developing ability of animal, it would be possible to detect behavioral changes more precisely. Likewise, the age of onset of higher-order learning and memory processes involving greater associative demand or long-term retention is delayed relative to maturation of elemental conditioning and effector systems (Stanton, 2000). However, data relating specific developmental events to behavioral alterations that demarcate maturation of complex cognitive abilities are scarce.

Operant conditioning is also a procedure to examine the ability of associative learning. Operant conditioning has been used in the psychological, pharmacological and toxicological research fields for nearly 40 years (Wenger, 1990; Poremba and Gabriel, 1997; Tremblay and Schultz, 2000) therefore, numerous data from experiments in human (Rice and Barone, 2000; Ferster and Skinner, 1957; O'Brien, 1968), primates (Rice, 1996), rodents (Lejeune and Jasselette, 1987) and other species (Marler, 1991) have been cumulated. Operant conditioning can diversely generate the performance pattern, complexity and difficulty of the task within and across experiment(s) by selecting reinforcement schedules and by operating variables such as cue, interval and frequency of response, and magnitude and quality of outcomes (O'Brien, 1968). Despite the fact that it is one of the most robust forms of associative learning observed in the animal laboratory, developmental process of operant learning function has not been systematically assessed; therefore, neural structures and regions of the brain for controlling operant learning function, and timing of maturation of operant learning function are still poorly understood.

Thus, we examined the learning abilities of operant task of young male animals at different ages and identified the point of onset of adult-like operant performance of young rats, using different fixed ratio (FR) response requirements (FR1, FR2 and FR20) to manipulate the difficulty of the task.

MATERIALS AND METHODS

Animals

Five groups of young rats and one group of adult rats were used for the experiments. Ten pregnant Long Evans (LE) rats on gestation day (GD) 8, the first day of the presence of a copulatory plug was defined as GD0, were obtained from the Institute for Animal Reproduction (Ibaraki, Japan). Each animal was individually housed in a polyethylene cage under controlled environment condition (24 ± 1°C, 45 ± 5% humidity, a 12-hr light/12-hr dark cycle, and filtered air) in a barrier facility. All pregnant rats delivered naturally on GD22 or GD23. The day of birth was designated as PD1. The number of live pups, sex ratio at birth, and gross appearance of the pups were examined. The litter size was standardized to six male and two female pups, if possible, on PD1. Five male pups each were randomly selected from one litter and used for experiment. Each male out of five pups was assigned either one of the five experimental groups. These animals were housed with their littermates and their dams until weaning (PD23), and accommodated individually afterward. Eight LE males, 40 days of age, were purchased from the Institute for Animal Reproduction (Ibaraki, Japan) and used for experiments as adult animals after 20 days of acclimatization.

The body weights of all animals were recorded everyday throughout the experimental period and were compared to animals in the same age group with no food-restriction (counterparts). The body weights of young animals and adult animals were maintained within 90 and 80% of standard body weights, respectively. The development of body weights of young animals were based on the body weight recording in our pilot study. The standard weight of adults was employed from the breeder's background data. The daily food (Cat. Code: CA-1, Japan CLEA, Tokyo, Japan) consumption was restricted to 20 g per day for the adult animals. Daily food for young animals was not restricted. All animals had *ad libitum* access to water. All experiments were conducted in accordance with the *Guidelines for the Care and Use of Animals* of the National Institute for Environmental Studies (Tsukuba, Japan).

Motor function test

Motor function was tested with vertical pole and wire hang tests on PD25 and PD35 for young animals, and on PD60 for adult animals. Both vertical and wire hang tests were induced before daily session of the operant test.

Vertical pole test

In the vertical pole test, a rat was placed facing up on a cloth-tape- covered pole (1.9 cm diameter, 43 cm long), which was horizontally placed on the floor covered with wooden bedding. The opposite end of the pole on where the rat stayed was then gradually lifted and moved up to vertical position until the position of the rat was brought 30 cm above the floor. Duration of staying on the pole of the rat was recorded for a maximum by 60 sec. The duration was converted to a pole test score as follows: fell before the pole reached 45 and 90 degree of angles = 0 and 1, respectively, fell in 0-10 sec = 2, 11-20 sec = 3, 21-30 sec = 4, 31-40 sec = 5, 41-50 sec = 6, 51-60 sec = 7; stayed on 60 sec and climbed halfway down the pole = 8, climbed to the upper half of the pole = 9.

Wire hang test

The wire hang test was performed to measure balance and grip strength of rat. Each rat was placed on a wire cage top which was taped around the edge, and raised 30 cm above the cage. The lid was shaken three times and then slowly inverted a foot above an empty cage strewed with wooden bedding. The amount of time which the rat took to fall from the top to the cage was recorded. The time of the rat which gripped the top longer than 60 sec was recorded as 60 sec.

Operant test

Experimental apparatus

Operant learning function of an animal in PD60 group was examined using an operant conditioning chamber (500 mm wide x 280 mm depth x 325 mm high, Model: MSK-002R, Muromachi Kikai, Tokyo, Japan, Fig. 1A). Two levers were mounted 80 mm above the floor and 60 mm from the center of the front wall of the chamber. A recessed feeder receptacle was mounted between the levers and 40 mm above the floor. For young animal all tests were carried out with a standard operant chamber for mice (370 mm wide x 280 mm depth x 280 mm high, Model MSK-002M, Muromachi Kikai, Tokyo, Japan, Fig. 1B). A recessed feeder receptacle was located in the middle of two levers. The levers were equipped 60 mm above the floor and 55 mm apart from each other.

The operant chamber was individually accommodated in a double-walled, insulated, sound-attenuating chamber (ICM Implement, Tsukuba, Japan). Air in the chamber was ventilated with a fan equipped behind the chamber. As rewards, Dustless Precision Pellets (Rodent Purified Diet, BioServe, NJ, USA) were used in operant tests. Sizes (diameter x thickness) and weights of pellets were as follow; 1.5 mm x 1.0 mm and 14 mg for young animals, and 4.0 mm x 4.0 mm and 45 mg for adult animals. When the pellet was delivered, a feeder light in the ceiling of the feeder was turned on for 0.5 sec. A house light

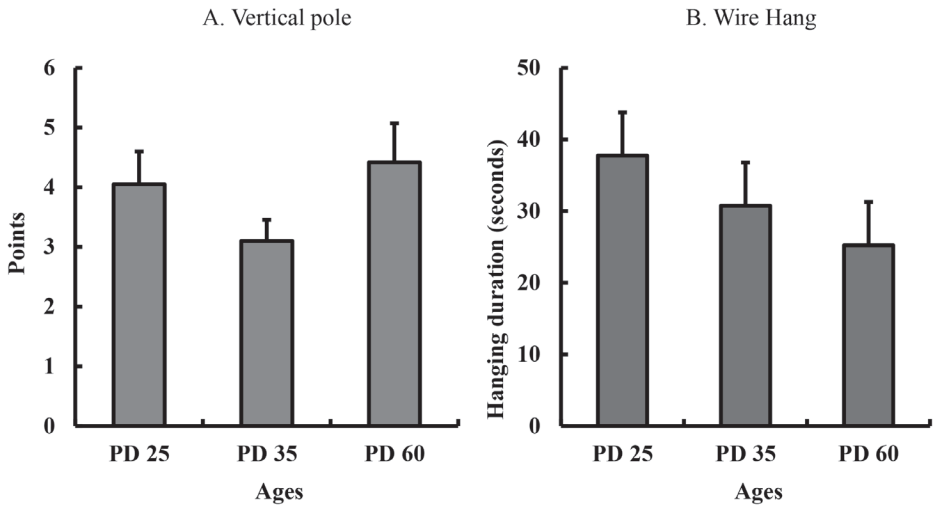

Fig. 1. The results of simple motor test in (A) vertical pole, in which the time rat stayed on the vertical pole, recorded a maximum of 60 sec. the time was converted to a test score; Fell before the pole reached 45 to 90 degree of angles = 0 or 1, respectively; fell in 0-10 sec = 2, 11-20 sec = 3, 21-30 sec = 4, 31-40 sec = 5, 41-50 sec = 6, 51-60 sec = 7; stayed on 60 sec and climbed half way down the pole = 8, climbed to the lower half of the pole = 9. Values are the mean ± S.E.M.

mounted in the center on the front wall was turned on when an experiment was started. Task operation and data acquisition were accomplished with a programming package ComPACT (Muromachi Kikai, Tokyo, Japan) running on a Windows XP. Time resolution for data acquisition was 10 ms.

Procedure of the operant test

Young animal in each group started the operant test at the different days of age, on PD16, PD19, PD22, PD25, or PD28. Adult animals were 60 days of age at the first day of the operant test. In the operant test, daily sessions including trainings were conducted 7 times a week, started at 9:00. Fixed ratio (FR) operant tasks, in which a food pellet is delivered as a reward when a rat presses a lever with a certain number, were used in the test. FR tasks used in the present study were three kinds, which were FR1, FR2 and FR20.

Three days before the operant test, acclimation training was conducted. In the acclimation training, young animals in PD22, PD25, and PD28 groups were required to eat 10 pellets within 60 min from the feeder of the operant chamber after mild food deprivation (for three hours). For the animals younger than those in PD22 group, breast milk deprivation was fixed at one hour and 5 pellets were fed at outside of the operant chamber. We carefully checked whether the animals ate the pellets or not, and counted the number of the rest of pellets if they were not consumed. Afterwards, these animals were entered in the operant chamber and fed 5 more pellets from the feeder. Adult animals were required to eat 30 pellets within 60 min from the feeder of the operant chamber. If the animal did not eat more than 80% of the maximum pellets (40 out of 50 pellets) within 60 min, the same training was repeated on the next day. All adult animals ate all pellets within 60 min by the second day of the acclimation training. After the acclimation training, bait training began. In the bait training, kneaded experiment pellet was pasted on the levers to promote a rat accessing the lever. The bait training was finished with either one of the following events occurred; when the duration of the training session reached to 60 min, or when an animal acquired 10 pellets with lever pressing. In case that the animal did not perform the lever pressing 10 times in 60 min, the same training was repeated on the next day. All animals acquired the lever pressing in two days.

On the first day of the operant test, FR1 task, which required an animal to push lever once for a food pellet, was started. The daily session was terminated by either one of the following events which occurred first; when animal ate the maximum number of pellets (50 pellets), or when the duration of the session achieved a scheduled experimental time (30 min). When animals did not eat more than 40 pellets in a daily session, the same schedule was repeated on the next day. A learning acquisition criterion, a timing in which FR1 task shifted to FR2 on the next day, was defined as follows; when an animal produced more than 40 rewards for two consecutive days. FR2 task, in which each second lever pressing is reinforced by a food pellet, used the same learning acquisition criterion as FR1 and FR20. Although the learning achievement criterion of FR20 task was the same as FR1 and FR2 tasks, FR20 task was not conducted more than three times even if the rat did not satisfy it.

Data analysis

Body weight difference between experiment animals and their counterpart (data derived from our pilot study) in each age group was analyzed by two-way analysis of variance (ANOVA), between factor was food restriction treatment and no treatment, and within factor was days of experiment. Results of the vertical pole and the wire hang tests of animals on PD23, PD35, and 60 were analyzed by one-way ANOVA. Means of the number of rewards and the response rates (the number of responses per minute) in daily sessions in each FR task were individually analyzed by one-way ANOVA. The number of sessions until animal met the learning acquisition criterion in FR1, FR2 and FR20 tasks were analyzed by one-way ANOVA as well. As post hoc analysis, Turkey's test was adopted. All statistical analyses performed using the SPSS program version 14.0 (SPSS Japan Inc., Tokyo, Japan). The probability level required for significance was set at $p < 0.05$.

RESULTS

There was no difference in body weights of young animals between the food-restricted and the counterpart (no food-restriction) group at each age (data not shown). In adult animals, mean body weight of animals in the experimental group was kept within 80% of animals with the standard body weight (no food-restriction) throughout the experimental period.

Motor function test

In the vertical pole (Fig. 2A) and the wire hanging (Fig. 2B) tests, there were no differences among groups.

Operant test

In FR1 task, there were group differences in means of the response rates and of the number of session until animals met the learning acquisition criterion. Post hoc

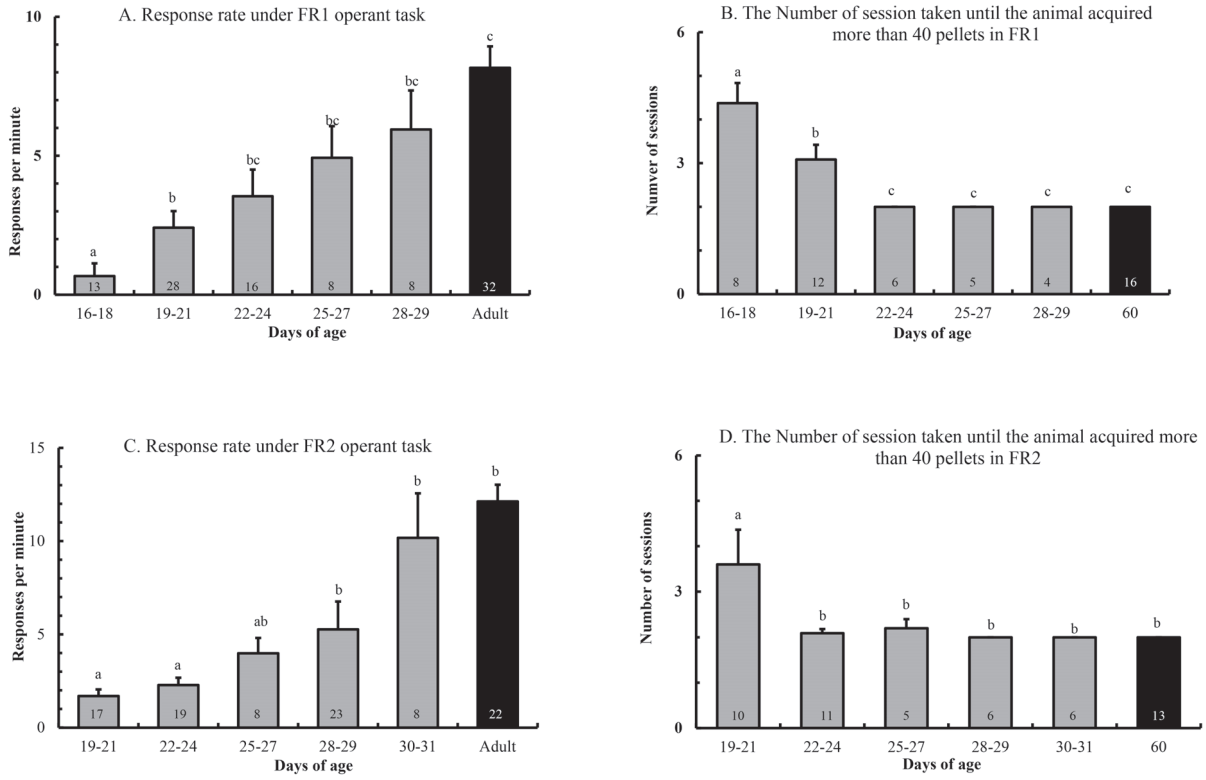

Fig. 2. Behavioral changes of male animals on FR1 and FR2 schedules. Response rate in FR1 (A), the number of session taken until the animal acquired more than 40 pellets in FR1 (B), response rate (C) in FR2 and the number of session taken until the animal acquired more than 40 pellets in FR2 (D) at each days of age. Values are the mean ± S.E.M. The numerical in each column indicates the number of animals used in each schedule. Values with different letters are significantly ($p < 0.05$) different from each other.

test indicated that the mean of the response rate of animals tested on PD16-PD18 was significantly lower than those of other young animal groups, and that animals tested on PD19-PD21 showed a significantly lower response rate than adult animals, tested on PD60. Animals tested on PD16-PD18 needed a significantly higher number of sessions to satisfy the learning acquisition criterion for FR1 task than those of young animal in other groups. The number of sessions to satisfy the learning acquisition criterion of animals tested on PDs19-21 was also significantly higher than that of animals tested on PD60 in FR1 task. Response rates [$F(5, 45) = 9.654$, $p < 0.05$] (Fig. 3C) and the number of sessions until animal met the learning acquisition criterion [$F(5, 74) = 10.211$, $p < 0.05$] (Fig. 3D) in FR2 task also showed group differences. Mean response rate of animals tested on PD19-PD21 was significantly lower than that of animals in PD28-PD29 and PD30-PD31 groups, and that of animals tested on PD22-PD24 was significantly lower than that of animals tested on PD60. In addition, animals tested on PD19-PD21 spent a significantly higher number of sessions to satisfy the learning acquisition criterion in FR2 task than animals in other groups.

In FR20 task, one-way ANOVA revealed differences among groups in a percentage of animals which satisfied the learning acquisition criterion (Table 1). The percentage of animals in PD22-PD29 group which met the learning acquisition criterion for the FR20 task was significantly smaller than those of PD32-PD33 and PD60 animals. These results indicated that 90% of animals tested on PD32-PD33 satisfied the criterion as well as adult animals (87.5%). On the other hand, the percentage of animals in PD30-PD31 group which acquired FR20 task was 50% and did not differ from those of animals in other young groups. One-way ANOVA revealed group differences [$F(3, 11) = 9.139$, $p < 0.05$] in the response rate in FR20 task. Post hoc test indicated that the response rate of animals tested on PD22-PD27 was significantly lower than

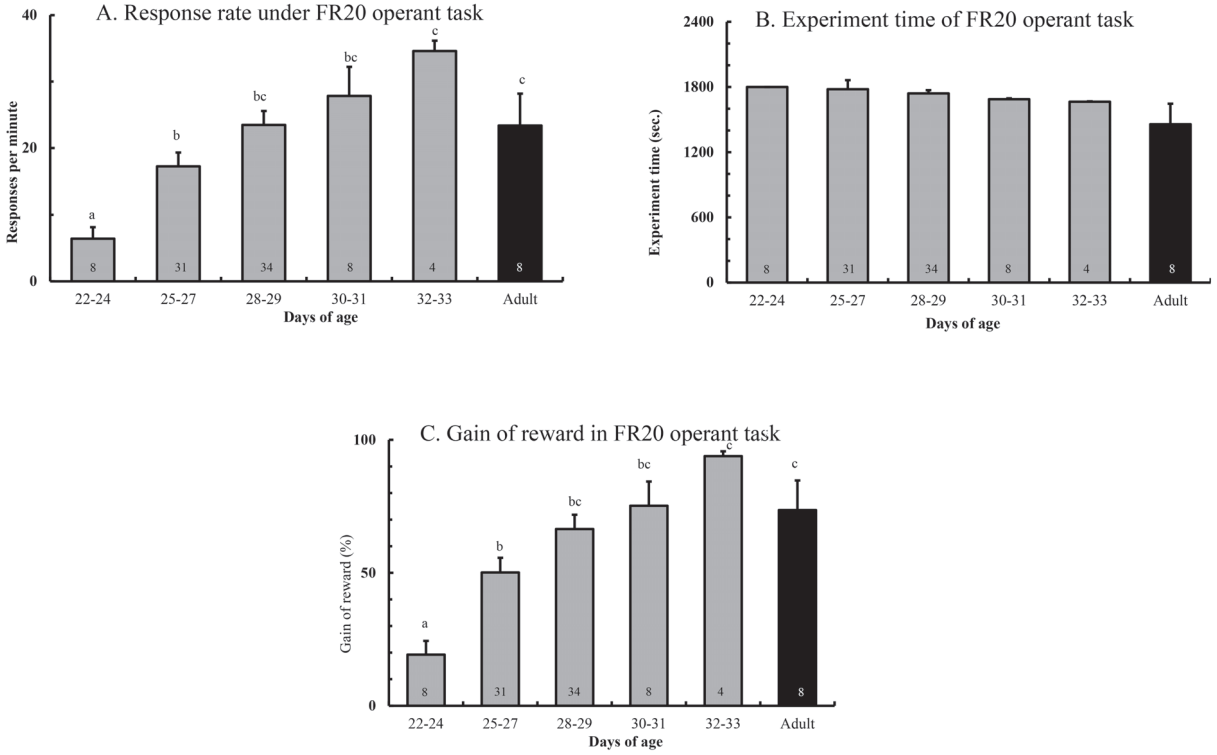

Fig. 3. Age differences in response rates (A), experiment time (B), and gain of reward (C) operant learning test. Gain of reward was expressed as the percentage of the number of reward pellets acquired, with the maximum rewards of 50 pellets being 100%. Values are the mean ± S.E.M. The numerical in each column indicates the number of animals used. Values with different letters are significantly ($p < 0.05$) different from each other.

Table 1. Age difference in the percentage of animal acquired the operant learning behavior.

Days of age at FR20	Total number of animals	Number of animal acquired	Number of animal not acquired	Percent of animal acuired of total animals
22-24	8	0	8	0[a]
25-27	31	8	23	25.8[a]
28-29	34	15	19	44.1[a]
30-31	8	4	4	50[ab]
32-33	20	18	2	90[b]
60	16	14	1	87.5[b]

Values of percent with different characters were significantly ($p < 0.05$) different from each other. Acquisition of learning in each animal was difined , if animal obtained more than 40 out of 50 reward pellets.

those in PD32-PD33 and PD60 groups (Fig. 3A). Animals tested on PD22-PD24 showed a lower response rate than that of PDs25-27 group. There was no significant difference in the experiment time of FR20 task among groups (Fig. 3B). There were significant [F (3, 12) = 4.000, $p < 0.05$] differences in the mean gain of reward (%) in FR20 task among groups (Fig. 3C). Post hoc test indicated that the gain of reward of animals in PDs22-27 group was significantly lower than those of animals in PD32-PD33 and PD60 groups. In addition, animals tested on PD22-PD24 showed a significantly lower gain of reward than that of animals tested on PD25-PD27.

DISCUSSION

A major finding of the present study was that response

pattern of LE young rats tested on PD32 showed the same pattern as adult animal under FR20 task. This result suggests that the learning function responsible for the FR20 task matured on PD32 in LE rat. For responding to associative learning such as the FR20 task, basal ganglia (Sutton and Barto, 1998) and hippocampus (Dumas, 2004) may play an important role. According to Brown *et al.* (2005), the brain structure which is responsible for associative learning such as FR20 operant task in rat becomes functionally, fully mature on the fourth week of age in the rat. This occurs because higher order memory functions depend on late-developing brain systems such as prefrontal cortex (Freeman and Stanton, 1992) and the hippocampal formation (Altman *et al*., 1973; Nadel and Zola-Morgan, 1984; Rudy, 1993; Stanton, 2000).

Maturation for two-way avoidance reflex is observed also on PD32 (Kudryashova, 2006). Also, in the place navigation test using the Morris water maze, only animals older than PD40 showed typical adult behavior, swimming directly toward the platform without any cue (Schenk, 1985). The conditioned two-way avoidance reflex and the spatial navigation are both complex cognitive abilities that mature in the hippocampus. This suggests that maturation of learning function for FR20 operant task requires the same level of development of the CNS formation as that of higher-order cognitive functions in these two tasks.

The phenomenological similarity of the developmental patterns of learning functions between two higher-order learnings and FR-operant learning can be especially compatible memory processes such as short-term and long-term memory formations and/or recall (Dumas, 2005). FR20 task may require animals "higher order" memory functions the same as spatial working memory (Freeman and Stanton, 1991) and configural learning (Rudy, 1993). The higher order memory functions emerge later in ontogeny than reference memory or learning of elemental associations (Rudy, 1993; Stanton, 2000). In general, animals in experiments evaluated higher-order cognitive functions such as Morris Water Maze (Morris, 1984) or delayed alteration task (Bachevalier and Beauregard, 1993) show adult-like performance at the weaning corresponding to the maturation of hippocampus. Likewise, under Pavlovian conditioning, a standard conditioning using visual cue CS emerges adult-like freezing between PD17 and PD24 (Stanton, 2000; Paczkowski *et al*., 1999). However, when visual cue CS is paired with brief foot shock US with a trace interval of 10 sec or longer, subsequent freezing to the light is impaired at PD25 and adult-like visual trace condition emerges at PD30 (Moye and Ruby, 1987). In this regard, the developmental delay in the present study probably relates to maturation of short-term memory process within the specific sensory system such as ventral striatum and the amygdala (Squire and Zola, 1996), or late development of short-term associative learning ability, which are supported by different mechanisms (Ivkovich and Stanton, 2001); the task acquisition for FR20 and conditioning with trace requires maturation of the memory function for retention.

In addition, not only cognitive functions but also other physiological or functional changes probably affect the change of behavior (Feinberg, 1983). According to the definition by Spear and Brake (1983), rats at PD30-PD32 are categorized into adolescence as the ontogenetic period including approximately 10 days prior to the onset of puberty (which occurs at about PD40 in the rat) and few days thereafter. Particular marked alterations are found in the forebrain regions (cerebral cortex, hippocampus and striatum), neurotransmitter systems such as mesolimbic dopaminergic (Campbell *et al*., 2000) and basal forebrain cholinergic systems (Scattoni *et al*., 2003; Ricceri, 2003), proliferation and maturation of axon terminals, and synapses (Stamford, 1989; Teicher *et al*., 1995) are seen in this period. Dopamine receptors play a role of reinforcement effect (Jocham, 2011) in the striatum and sexual hormonal systems in early adolescence (Sisk and Foster, 2004) are activated in this period. The result in the present study that learning ability for FR20 task matured on PD32 may have contributed to the dramatic change mentioned above.

Animals younger than PD18 showed impairment of the acquisition of the learning in the FR1 schedule. Usually, ability to association using a visual cue CS with an electric shock US becomes apparent within a few days of eyelid parting, which occurs at PD14-PD15 (Moye and Rudy, 1985; Paczkowski *et al*., 1999). Therefore, simple association ability seems to be emerged at early developmental stage. However, not like the conditioning which required little motor activity, FR1 learning task may require some other functions, for instance, habituation for a novel environment and motor abilities. Both habituation and allover activity levels are dependent on the development of the muscarinic cholinergic system, which is markedly activated after PD16, and is not established before PD18 (Bâ and Seri, 1995). This suggests that ability for FR1 learning may be more complicated and mature than that for conditioning.

Another explanation is immature memory system. Rudy and Morledge (1994) have shown that after one fear conditioning trial, rats at PDs 18-32 showed the same

amount of conditioned freezing when tested immediately after conditioning but PD18 rats showed much less conditioned freezing than the older rats when the retention interval was 24 hr. These findings imply memory consolidation and hippocampal formation develop after PD18.

Response rate of the animal in PD22-PD24 group did not reach the criterion for the acquisition of the FR2 learning task. Impairment of the acquisition in FR2 learning task does not seems to be due to the immaturity of motor functions, because rats aged PD23 did not show any dysfunction of motor activity in the present study. The inability of learning acquisition in the FR2 learning task may be related to the properties of the task. Unlike FR1, FR2 learning task is a partial reinforcement in which not all responses are reinforced by food. "Disruptive responses" meaning non-rewarded responses, emerge in partial reinforcement (Sangha *et al*., 2002; McComb *et al*., 2002). Younger animals have more difficulty associating a response with a conditioned stimulus (Brennan and Baron, 1976; Potash and Ferguson, 1977). In FR2 learning task, the disruptive response is conditioned stimulus for the rewarded response which is directly associated to food reward (Schulteis *et al*., 2005). Smith and Bogomolny (1983) reported that only PD23 rats were able to acquire the maze task with milk only, but not preweanling rats aged PD10-PD15. Smith and Bogomolny (1983) concluded that the development of foraging strategies might be necessary for the expression of maze behaviors reinforced with milk alone. Both studies of Smith and Bogomolny (1983) and the present study may show inability to learn the association between response-appetitive outcome, or inability to retain its memory.

In summary, results suggest that structure of the CNS responsible for associative learning for FR20 operant task in rat become functionally, fully mature on PD32. This suggests that FR20 operant task requires cognitive functions to mature later than general higher-order cognitive functions because of short-term memory process or late development of short-term associative learning ability, which are supported by different mechanisms. Immature learning function for FR1 of animals younger than PD18 may be due to immaturity of the muscarinic cholinergic system, or immature memory system. The fact that animals of PD22-PD24 group did not reach the criterion for the acquisition of the FR2 learning task might show difficulty associating a response with a conditioned stimulus as required by the task; inability to learn the association between response-appetitive outcome; or inability to remember the motivation of food reward. In conclusion, the present result suggested that each stage of a series of FR learning is connected with the structure of the memory system in the hippocampus. However, temporal correlation in improvement across tasks may be related to different neural mechanisms supporting each task or ability.

Conflict of interest---- The authors declare that there is no conflict of interest.

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