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Circadian Rhythms in Locomotor Activity of the Hagfish, *Eptatretus burgeri* VI. The Effects of Cutting the Spinal Cord

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ABSTRACT—The present study is designed to clarify the mechanism by which the circadian pacemaker controls the locomotor activity of the hagfish and also to estimate the role of brain and spinal cord in the swimming behavior of the animal. We examined the effect of cutting the spinal cord at the 6 different positions on the circadian rhythm and the locomotor behavior of the animal. The most frontal cut was located between the brain and spinal cord, and the other 5 cuts were given to every 1/6 the length of the spinal cord. The relation between the locomotor activity and the cut position of spinal cord was summarized as follows. (1) When the ratio of frontal part before the cut was 0/6–1/6, the animal locomoted under initiative of caudal part, in random direction at the bottom and showed neither nocturnal rhythm in LD nor circadian rhythm in DD. (2) When the ratio of the frontal part before the cut was 4/6–5/6, the animal swam up to the surface under initiative of frontal part, and showed both nocturnal rhythm in LD and circadian rhythm in DD. (3) When the frontal ratio of spinal cord was 2/6 or 3/6, the animal showed both kinds of swimming behavior of (1) and (2). These results suggest that the descending system from the brain enable the hagfish to swim up to the surface and to express the rhythmicity of locomotor activity under control of the circadian pacemaker when at least frontal 2/6 of the spinal cord is connected to the brain by neuronal networks not by humoral factors.

INTRODUCTION

The hagfish shows a clear nocturnal rhythm of locomotor activity in 12 hr light: 12 hr dark (LD), and a stable circadian rhythm in constant darkness (DD) (Ooka-Souda *et al.*, 1985; Ooka-Souda and Kabasawa, 1990). We have indicated the pacemaker of the hagfish may be located in the preoptic nuclei (PON) of the hypothalamus (Ooka-Souda *et al.*, 1993). We have also shown that in hagfish the most important Zeitgeber for the entrainment is the light information via the pretectal areas (Ooka-Souda *et al.*, 1995). The characteristic response to light changes after removal of the fore- and mid-brain, and the animal shows direct reaction to external light stimuli via the photoreceptor of the skin (Ooka-Souda *et al.*, 1988).

In this paper, we wanted to know at first whether the circadian pacemaker controls the rhythm of the swimming activity by way of neuronal networks or humoral factors, and also to know the qualitative and quantitative relationship between the brain and spinal cord including the motoneurons used for

locomotion of the hagfish in spontaneous and controlled movements. For this purpose, we examined the effect of cuts in the spinal cord on the circadian rhythm and the locomotor behavior of the hagfish.

MATERIALS AND METHODS

The hagfish were collected in the sea at depth of 10–50 m near Misaki Marine Biological Station, the University of Tokyo, on the coast of Sagami Bay. Prior to the experiments, the animals (body length: 32–42 cm) were kept in a large aquarium for more than 1 week under 12L:12D (light on 07:00–19:00). In the experiments, an individual unit system was used so that one hagfish at a time occupied a small aquarium (60×45×45 cm). The water temperature of the aquarium was kept at 15°C and no food was given throughout the experiment. The locomotor activity of the animal was measured by means of an infrared photocell system which was located parallel to the water surface (Fig. 1). The outer diameter of an infrared lamp (IR lamp: PH 2U, Hokuyo Electric Co.) is 20 mm. The photocell system was connected to a computerized recording system (Paramecom 6, Kokusai Electronics Co.).

All surgical operations were done under binocular microscope while the animals were lightly anesthetized with MS222 (3-aminobenzoic acid ethyl ester). The skin and the fibrous connective tissue covering the spinal cord were cut longitudinally along the median axis 0.5 cm long. The spinal cord was cut with a pair of scissors keeping

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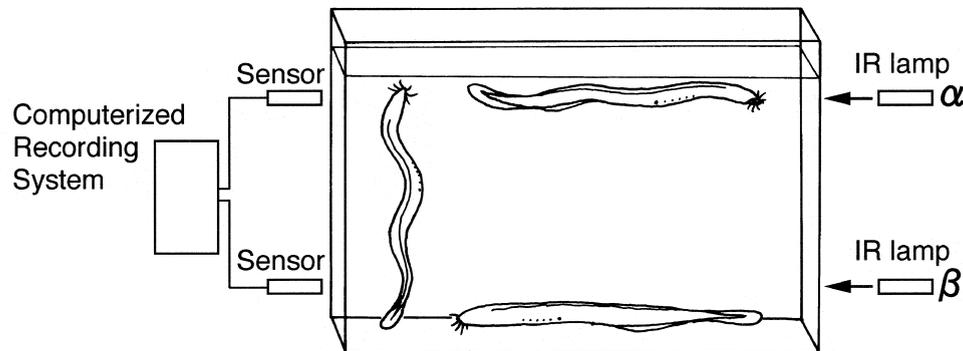


Fig. 1. The swimming activity of a hagfish in an aquarium and the position of the sensor of infrared photocell system. α and β indicate the positions of the sensor at near the surface of water and at near the bottom of aquarium, respectively.

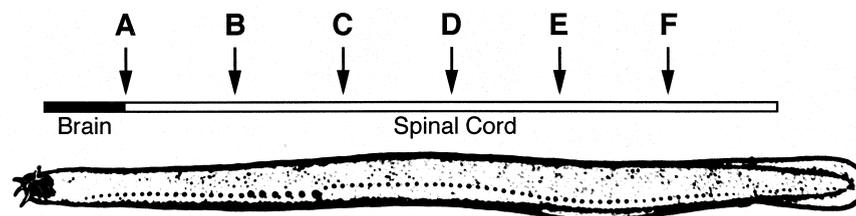


Fig. 2. The positions of cutting of the spinal cord in hagfish.

the ventral and dorsal roots untouched. The connective tissue was replaced as it was originally, and the skin was sutured after operation. In sham operated animals, the skin and the fibrous connective tissue covering the spinal cord were cut, but the spinal cord was not disturbed. In 5 sham-operated animals, the swimming behavior was almost the same as that in intact ones. The recording of the locomotor activity started 1–3 days after operation. The swimming behavior was recorded for 7–15 days in LD and 7–25 days under DD.

We examined the effects of cutting the spinal cord at multiples of 1/6 the length of the spinal cord on the circadian rhythm and the locomotion behavior of the animal (Fig. 2). The first cut (A) was located between the brain and spinal cord, and the other 5 cuts (B, C, D, E, F) were given so that the frontal part before the cut occupied 1/6 or 2/6, 3/6, 4/6, 5/6 of the spinal cord.

The behavior of intact hagfish could be divided into active and inactive stages (Ooka-Souda *et al.*, 1985). In the inactive stage, they remained almost motionless on the bottom. In striking contrast to this, the hagfish swam very actively in the active stage. They first held up the head, then undulated the body and swam up to the surface of the water along the edge of the aquarium. The animals would swim at the surface of the water along the wall of the aquarium in one direction, going around several times and then sinking to the bottom gently, keeping the body straight. After resting for a short time on the bottom of the aquarium, they would start to swim up again and would repeat this behavior over and over during the active stage, and they never swam in the middle reaches of the aquarium.

Animals with the spinal cord cut could be expected to show different swimming behavior from that in intact animals. Therefore, in preliminary experiments, we monitored the swimming behavior of 16 animals with an infrared beam at 4 different locations for 6 days each: at the surface of the water, at 1/2 and 1/3 the depth of the water, and at the bottom of the aquarium. We confirmed the content of the computerized recordings and the style of behavior by simultaneous visual observations under light and dim light. From these preliminary experiments, we found that the operated animals showed only two kinds of locomotor behavior; one was swimming at the surface like intact animals and the other was locomotion on the bottom; they did

not swim in the middle reaches of the aquarium. Then we examined the locomotor activity of operated animals using two infrared light beams at the same time, one at the surface and the other at the bottom of the aquarium (Fig. 1). All of the operated animals were put under LD and then some of them were put in DD. We first examined the effects of the spinal cut on the swimming pattern and activity rhythm visually, and then analyzed the results in the light of the recordings made with the infrared photocell system.

RESULTS

The results are summarized in Table 1 and 2. The typical locomotor activity pattern of these operated animals is shown in Fig. 3A–F.

After a cut at A (Fig. 2) between brain and spinal cord,

Table 1. Effects of cutting the spinal cord on swimming activity and locomotion with nocturnal rhythm in 12L:12D (numerals are the number of animals).

	Cut locations					
	A (n=9)	B (n=10)	C (n=11)	D (n=10)	E (n=11)	F (n=8)
a+	0	0	11	13	11	8
a-	0	0	0	0	0	0
b+	0	0	0	0	0	0
b-	9	10	11	13	0	0

a+: animals showed swimming activity with nocturnal rhythm at the surface of water

a-: animals showed swimming activity without nocturnal rhythm at the surface of water

b+: animals showed locomotion with nocturnal rhythm at the bottom of aquarium

b-: animals showed locomotion without nocturnal rhythm at the bottom of aquarium

Table 2. Effects of cutting the spinal cord on swimming activity and locomotion with free-running rhythm in DD (numerals are the number of animals).

	Cut locations					
	A (n=4)	B (n=5)	C (n=6)	D (n=6)	E (n=6)	F (n=4)
a+	0	0	6	6	6	4
a-	0	0	0	0	0	0
b+	0	0	0	0	0	0
b-	4	5	6	6	0	0

a+: animals showed swimming activity with free-running rhythm at the surface of water

a-: animals showed swimming activity without free-running rhythm at the surface of water

b+: animals showed locomotion with free-running rhythm at the bottom of aquarium

b-: animals showed locomotion without free-running rhythm at the bottom of aquarium

the each animal showed distinctly different behavior from intact animals; it would move in a random direction, mainly during the light phase, at the bottom only, undulating the body independently of the head. No activity at the surface of the water was recorded (Fig. 3A- α , Table 1A-a, 2A-a). At the bottom in LD, the animal was active mainly in the light phase, but some activity was also observed in the dark phase (Fig. 3A- β , Table 1A-b). In DD, the activity at the bottom was random (Fig. 3A- β , Table 2A-b).

After a cut at B, no activity was recorded at the surface (Fig. 3B- α , Table 1B-a, 2B-a). The activity at the bottom was similar to that after the cut at A (Fig. 3B- β , Table 1B-b, 2B-b).

After a cut at C, under LD, the animals in the light phase moved on the bottom by undulating the part behind the cut rhythmically as after a cut at A or B, keeping the part in front

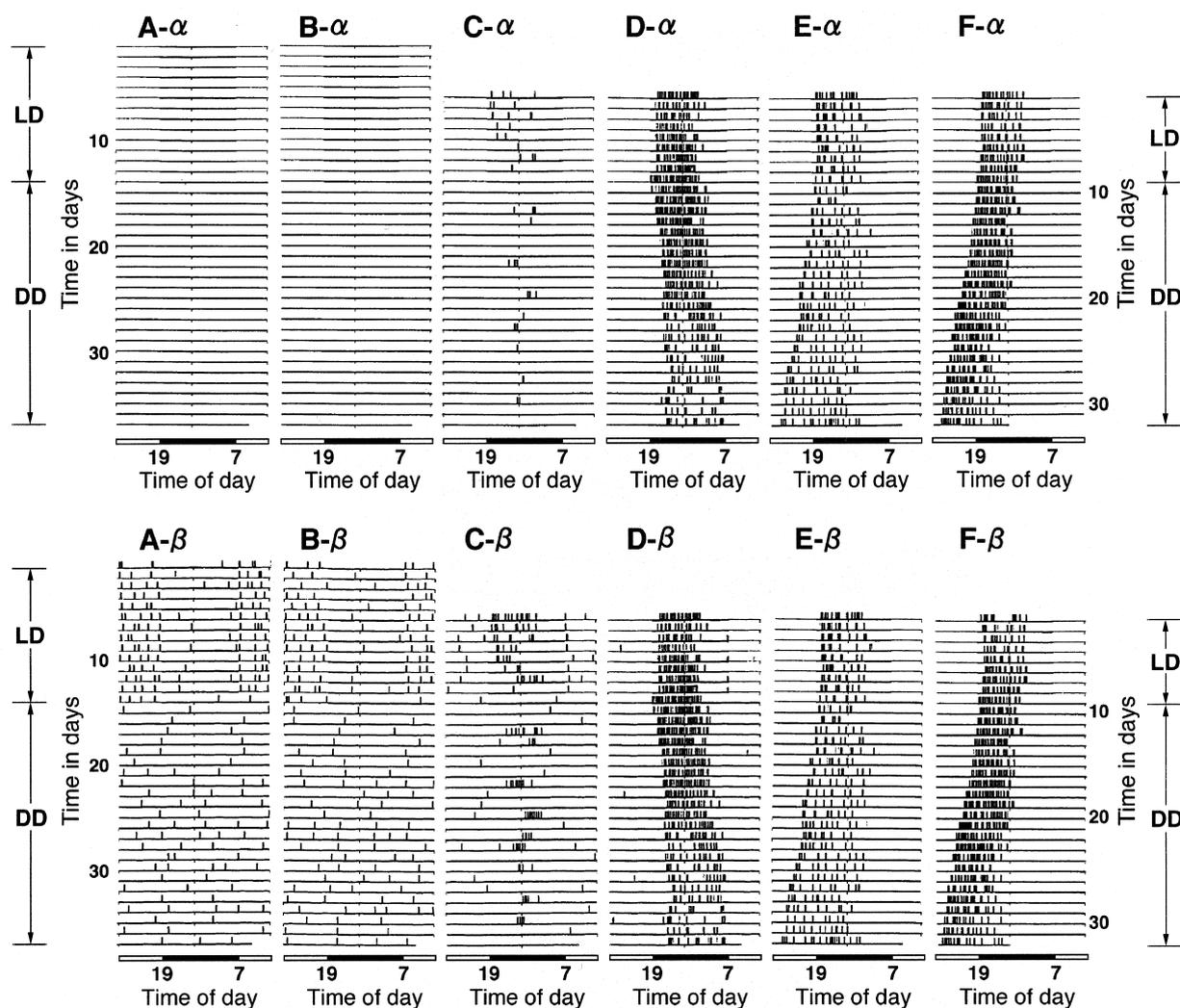


Fig. 3. The activity recording under LD and in DD of the hagfish with surgical cuts in spinal cord as shown in Fig. 2. The records in successive days after operation are plotted from top to bottom (longitudinal axis), and each record is arranged on 24-hr time base (horizontal axis). The first row in each column indicates the first day of recording after cutting the spinal cord. The light conditions were changed from LD to DD at the 14th day of recording in the cases of A- α , A- β , B- α , B- β and at the 9th day in the cases of C- α , C- β , D- α , D- β , E- α , E- β , F- α , F- β . A-F indicate the locations of cuts in the spinal cord shown in Fig. 2. α and β indicate the positions of the sensor at near the surface of water and at near the bottom of aquarium, respectively, shown in Fig. 1. The activity recorded by the sensor at position β includes not only the locomotor activity on the bottom but also the signals when animals interrupt the beam vertically while swimming up and sinking down during swimming at the surface. We named the latter signals false ones.

of the cut straight. In the dark phase, the part in front of the cut became active, and the animal held up its head and tried to swim up to the surface along the edge of the aquarium and sometimes succeeded. At the surface (Fig. 3C- α), we could see the nocturnal activity record in LD (Table 1C-a) and the free-running circadian rhythm in DD (Table 2C-a). On the bottom recording (Fig. 3C- β), we could recognize that two types of signal are mixed. One was a true signal recorded when the animal interrupted the infrared beam by horizontally moving on the bottom during the light phase as after a cut at A or B. The other was a false signal recorded when the animal interrupted the beam vertically by swimming up and sinking down in the dark phase. In DD we could also see mixture of the true signals of random activity on the bottom as after a cut at A or B and the false signals depended on vertical swimming with free-running rhythm which was similar to that at the surface.

After a cut at D, under LD, the animal was observed to move on the bottom sometimes in the light phase by undulating the part behind the cut, keeping the frontal part still. In the dark phase the frontal part became very active and swam up to the surface along the edge of the aquarium. The animal would sink down after swimming for a moment at the surface, but it would swim up again soon after sinking. The nocturnal rhythm was recorded at the surface clearly (Fig. 3D- α , Table 1D-a), and some activity signals were also recorded at the bottom in the light phase (Fig. 3D- β , Table 1D-b). In DD clear circadian rhythm was recorded at the surface of the water (Fig. 3D- α , Table 2D-a). The signals recorded in Fig. 3D- β were also mixture of the true signals of movement on the bottom and the false signals depended on vertical swimming, as mentioned above.

After a cut at E, the part behind the cut was observed to move during the light phase under LD, but this movement could not propel the whole body. The animal clearly showed both nocturnal rhythm in LD (Table 1E-a) and free-running rhythm in DD (Table 2E-a) at the surface of water (Fig. 3E- α). In Fig. 3E- β , only the false signals were recorded because animals could not move around on the bottom any more.

After a cut at F, the swimming behavior was very similar to that of intact animals. Both the nocturnal rhythm in LD (Table 1F-a) and the free-running rhythm in DD (Table 2F-a) were almost the same as that of intact animals (Fig. 3F- α).

DISCUSSION

The relation between the locomotor activity and the location of cutting the spinal cord can be summarized as follows. When the ratio of frontal part before the cut was relatively short (0/6 at A and 1/6 at B), the animals could move, propelled by the caudal part, in a random direction only at the bottom and showed neither nocturnal rhythm in LD, nor circadian rhythm in DD (Figs. 3A, 3B). When the relative length of the frontal part was great (4/6 at E and 5/6 at F), the animals could swim to the surface propelled by the frontal part, and showed both nocturnal rhythm in LD and circadian rhythm in DD (Figs. 3E, 3F). When the cut was located near the middle

of the spinal cord (2/6 at C and 3/6 at D), the animals showed both kinds of swimming behavior seen in the animals cut at A, B and at E, F. These results indicate that, in all cases, the frontal part before the cut of the animals keeps both rhythm and direction in swimming activity, and the caudal part behind the cut loses both of them in locomotor activity.

From these results, we postulate the role of central nervous system on the swimming activity and the circadian rhythmicity as follows. (1) The hagfish gets propulsive force by lateral undulation which can be generated without a descending system from the brain to the spinal cord. The descending system from the brain is necessary for the command of the direction of locomotor behavior (steering), and the animal can swim up to the surface when at least frontal 2/6 of the spinal cord connected to the brain by neuronal networks. (2) The circadian pacemaker controls locomotor activity by way of the descending system not humoral factors from the brain to the spinal cord, because the pacemaker could not control the locomotor activity of the part behind the cut point.

The undulating activity in the part behind the cut is more active in the light phase than in the dark phase. From the present and previous studies (Ooka-Souda *et al.*, 1988; Ooka-Souda and Kabasawa, 1990; Ooka-Souda *et al.*, 1993; Ooka-Souda *et al.*, 1995; Ooka-Souda *et al.*, 1997), we hypothesize that locomotor activity is controlled by the circadian system and external light stimuli as shown in Fig. 4.

In intact animals, the circadian pacemaker in the PON of the hypothalamus (Ooka-Souda *et al.*, 1993), adjusted to the external L-D cycle by retinal information via the pretectum (Ooka-Souda *et al.*, 1995), evokes locomotor activity in the dark phase and represses activity in the other phase (Ooka-Souda *et al.*, 1988; Ooka-Souda and Kabasawa, 1990). When the spinal cord is cut, the part behind the cut, released from the control of the pacemaker, shows undulating activity in the light phase in reaction to external light stimuli via the photoreceptors in the skin (Ross, 1963; Ooka-Souda *et al.*, 1988). It has been reported that after severing the spinal cord, local stimulation of the skin of the part behind the cut evokes reflex movements posterior to the cut (Ross, 1963).

The descending spinal projections of the mesencephalic tegmentum (MT) and the rhombencephalon (RH) have been reported in hagfish by tracer experiments with horseradish peroxidase (Ronan, 1989). We have shown that a hagfish with

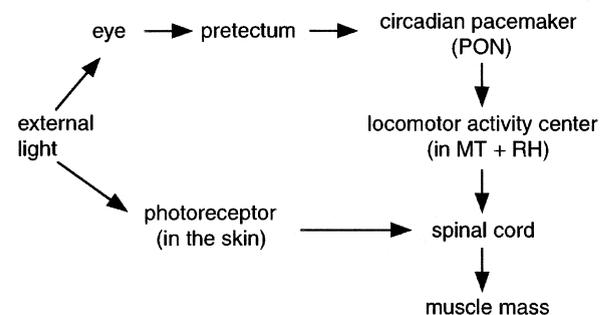


Fig. 4. The hypothetical scheme of the relation between the external light stimuli and the circadian rhythmicity of locomotor activity.

all the brain ablated except the MT and RH shows almost normal swimming behavior, but loses circadian rhythm (Ooka-Souda *et al.*, 1988; Ooka-Souda and Kabasawa, 1990). A similar descending system has been also shown in lampreys (Ronan, 1989). Grillner *et al.* (1995) reported that in *in vitro* experiments with the spinal cord of lamprey, descending system from the brain is not necessary to undulating activity and suggested that the system probably controls the direction, initiation, cessation, frequency, and intensity of movement.

Many reports have suggested a role of humoral factors, especially of melatonin, in the circadian rhythm of locomotor activity in lower vertebrates (Underwood, 1990; Morita *et al.*, 1992). But recently, in lampreys, the circadian rhythm of locomotor activity has been shown to occur independent of the melatonin level in blood and the pineal gland (Samejima *et al.*, 1994). However, it has been reported that the hagfish has no pineal gland (Wicht and Nieuwenhuys, 1997). Moreover, the recent autoradiographic analysis of melatonin receptors showed that no specific binding was detected in the brain of hagfish, whereas intense specific binding was found in the optic tectum and preoptic nucleus (or suprachiasmatic nucleus) of lamprey, skate and trout (Vernadakis *et al.*, 1998). The present study suggests that information from the pacemaker might be conducted by a descending neural system from the brain, not by a humoral system, as far as locomotor activity is concerned in hagfish.

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