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Noradrenergic and Cholinergic Nerves in the Uterus of the Japanese Long-Fingered Bat, *Miniopterus schreibersii fuliginosus*, Change with Reproductive Cycle

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ABSTRACT—The pattern of uterine innervation by noradrenergic (NA) and acetylcholinesterase-positive (AChE) nerves in different reproductive stages of the adult Japanese long-fingered bats were investigated histochemically and immunohistochemically. In the non-pregnant bat, the uterine horn was supplied with abundant NA and AChE nerves. These two types of nerves were closely associated with the uterine arteries and myometrial smooth muscles. In the pregnant bat, NA and AChE nerves supplying the uterus did not degenerate much during hibernating period, but reduced markedly after arousal. In the postpartum bat, the density of nerves recovered progressively. The significant change in the innervation pattern of uterine NA and AChE nerves in the pregnant bats under and after hibernation, and in the postpartum bat must be considered in relation to the adrenergic and cholinergic controlling mechanisms on the uterine function that is matched for the unique reproductive cycle of this bat.

Key words: bat, uterus, innervation, noradrenalin, acetylcholinesterase

INTRODUCTION

It has been well established that the uteri of mammals are innervated by sympathetic noradrenergic (NA) (Papka *et al*., 1985; Moustafa, 1988; Renegar and Rexroad, 1990; Zoubina *et al*., 1998) and parasympathetic acetylcholinesterase positive (AChE) (Moscarini *et al*., 1982; Papka *et al*., 1985; Moustafa, 1988; Renegar and Rexroad, 1990) nerves. The distribution and density of these two types of nerves differ among mammalian species. It has been reported that both types of nerves in pregnant animals degenerate rapidly under influence of progesterone, while they regenerate after parturition (Owman and Stjernquist, 1988).

In mammals, the gestation period is generally fixed for each species, but the period in some species of heterothermic bats varies according to individual sensitivity and/or outer environmental conditions (Orr, 1970; Racey, 1982). The miniopterine bats are widely distributed over the temperate, subtropical and tropical zones of the Old world. In the Japanese long-fingered bats, *Miniopterus schreibersii fuliginosus*, copulation, ovulation and fertilization occur in autumn with a quick succession (Mōri and Uchida, 1980, 1981a, b), and implantation and subsequent embryogenesis is proceed very slowly, owing to the heterothermic hibernation (Kimura and Uchida, 1983). However, the speed of embryonic development is hastened prominently after arousal from hibernation in early spring (Kimura and Uchida, 1984), and the young are born in early summer (Uchida, 1957). Thus, it is an interesting subject to investigate the neurogenic mechanisms responsible for the regulation of the uterine function in the unique reproductive cycle of this bat. Our previous study revealed the rich innervation of NA and AChE nerves in the uterus of the immature Japanese long-fingered bat (Sugasawa *et al*., 2000). In the present study, we report the significant changes of uterine NA and AChE innervations in the adult Japanese long-fingered bats during non-breeding season, pregnant bats under and after hibernation, and mothers soon after parturition.

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MATERIALS AND METHODS

Twenty-seven adult female bats were collected at Ohsedō cave (32.5°N) in the Kumamoto prefecture at evening in September 1998 - August 1999. They were examined in this study on the day subsequent to their capture. The animals were anesthetized with ethyl ether, and perfused through the left ventricle with Ringer's solution, followed by 30 ml of ice-cold 4% buffered formaldehyde. Reproductive tracts were quickly removed from the pelvic region, postfixed with the same fixative for 1 hr at 4°C, and washed thor-

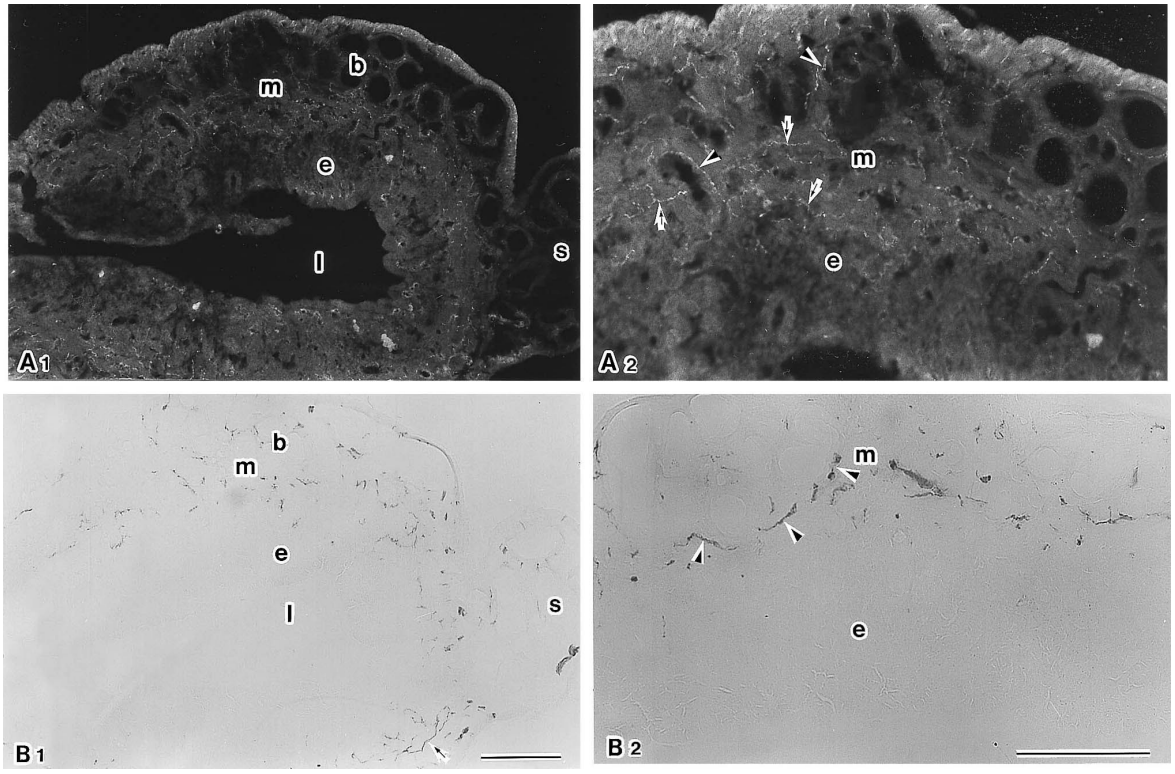

Fig. 1. NA (A) and AChE nerves (B) of the uterus in non breeding season in September. Arrow heads indicate nerves associated with blood vessels. Arrows indicate nerves running in the myometrium. b, blood vessel; endometrium; l, lumen; m, myometrium; s, mesometrium Bar=200 μm.

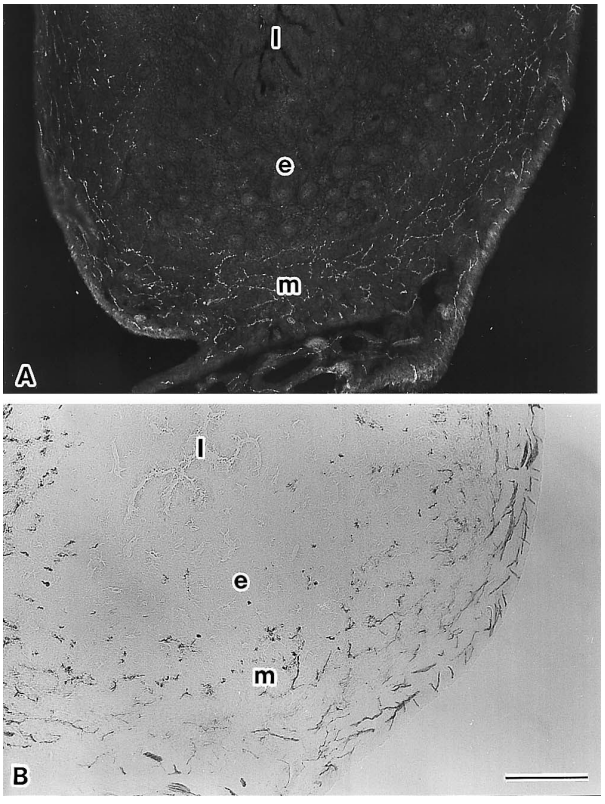

Fig. 2. NA (A) and AChE nerves (B) in the cervix in September. e, endometrium; l, lumen; m, myometrium. Bar=200 μm.

oughly with 0.1 M phosphate buffer (PB, pH 7.4). The reproductive tracts were carefully stripped free of the fat and other connective tissues covering them. Thereafter, the materials were sequentially immersed in PB containing 10% and 20 % sucrose for two days each. The uterus was dissected out from the reproductive tract, quickly frozen in isopentane chilled with dry ice, and serially sectioned at a thickness of 15 μm on a freezing microtome in a cryostat. Consecutive cross-sections were thereby prepared from the uterus and mounted on nonfluorescent glass slides coated with poly-L-lisin. One of each two serial sections was immunostained for dopamine-β-hydroxylase (D β H), a key enzyme in the synthesis of noradrenalin, and the other was histochemically stained for AChE, the enzyme hydrolyzing acetylcholine.

Immunohistochemical and histochemical procedures

For demonstration of neurons immunoreactive for D β H, sections were placed in 0.01 M phosphate-buffered saline containing 0.3 % Triton-X (PBST) over night at 4°C. They were then first incubated for 3 days at 4°C in rabbit D β H antiserum (Eugen Tech International, Ridgefield Park, NJ) at a dilution of 1:200. The specificity of D β H polyclonal antibody used in this study has been already confirmed elsewhere (Zoubina *et al*., 1998) They were washed with PBST and incubated for 2 hr at 37°C in sheep anti-rabbit IgG conjugated with fluorescein isothiocyanate (FITC; Capple, West Chester, PA) diluted 1:200. They were rinsed in PB, overlaid with glycerol-PB (1:1), covered with cover glasses and viewed under a fluorescent microscope.

For demonstration of neurons positive for AChE, sections were preincubated in substance (acetylcholine iodide, Sigma Chemical)-free Karnovsky's medium (Karnovsky and Roots 1964) for 30 min at 4°C, and then incubated in the complete medium containing 2×10^{-4} tetraisopropyl pyrophosphoramide (Sigma Chemical, USA), an inhibitor of non-specific cholinesterase, for 1 hr at 20°C (Andō

1981).

RESULTS

In the adult bats collected in early September where copulation has not yet been occurred, an abundant number of NA and AChE nerves, which came from the mesometrium, was found in the horn of the uterus (Fig. 1A1, B1) (Table 1). The innervation patterns of these two types of uterine nerves were similar to each other. They were predominantly distributed over the outer parts of myometrium, and mainly associated with a varying size of uterine arteries (Fig. 1A2, B2). A substantial amount of thin NA and AChE nerve fibers also entered into the myometrial proper from the vascular layer, and ran in close proximity to the long axis of non-vascular smooth muscles. There were no NA and AChE nerve axons with close association to the endometrium. NA and AChE nerves in the uterine cervix were much more numerous than those seen in the uterine horn (Fig. 2A, B). They were frequently observed at the myometrial arterial walls and non-vascular smooth muscles but not in the endometrium.

The distribution and density of NA and AChE nerves supplying the uterus showed no significant change during early pregnant period from October to February, and were basically similar to those demonstrated in the adults at September (Table 1). In the process of pregnancy from February to March, both types of uterine nerves decreased gradually from the anti-mesometrial side toward the mesometrial side, whereas those in the cervix did not changed significantly (Fig. 3A, B) (Table1).

The reduction of NA and AChE nerves in the uterine

Table 1. Relative density of AChE and NA nerves in the right uterine body of the adult Japanese long-fingered bat.

Date captured	Myometrium	Endometrium
Non breeding season		
3 September 1998 (3)		
AChE	+++	+
NA	+++	+
Pregnancy		
28 October 1998 (3)		
AChE	+++	+
NA	+++	+
14 December 1998 (2)		
AChE	+++	+
NA	+++	+
3 Jnuary 1999 (2)		
AChE	+++	+
NA	+++	+
22 Feburary 1999 (2)		
AChE	+++	+
NA	+++	+
27 March 1999 (3)		
AChE	++	−
NA	++	+
16 Aprill 1999 (3)		
AChE	+	−
NA	+	−
2 May 1999 (2)		
AChE	+	−
NA	+	−
12 June 1999 (2)		
AChE	+	−
NA	+	−
Postpertum		
3 July 1999 (3)		
AChE	+++	+
NA	++	+
5 August 1999 (2)		
AChE	+++	+
NA	++	+

−, nerves not found; +, few nerves; ++, moderate numbers of nerves; +++, numerous nerves. (n).

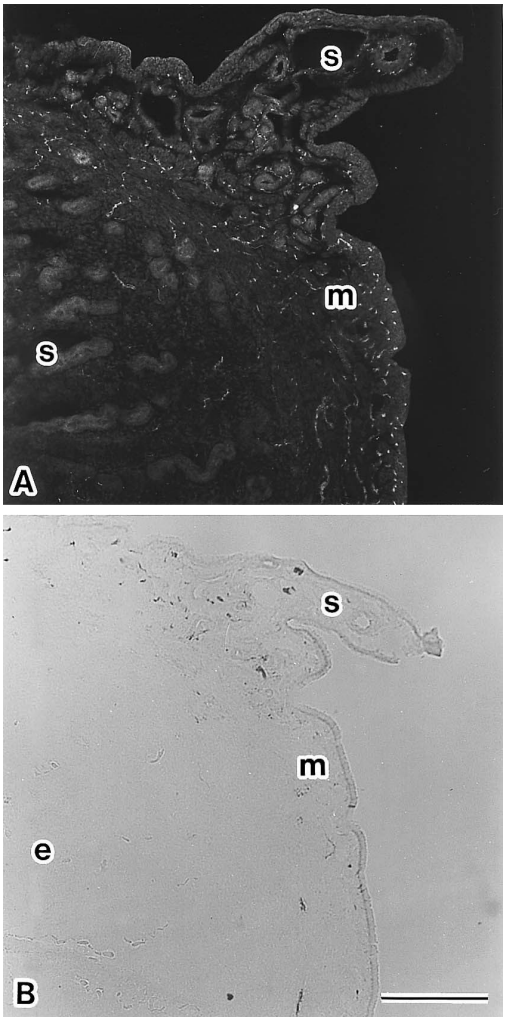

Fig. 3. NA (A) and AChE nerves (B) in the uterus just before the end of the delayed embryogenesis in March. e, endometrium; m, myometrium; s, mesometrium. Bar=200 μm.

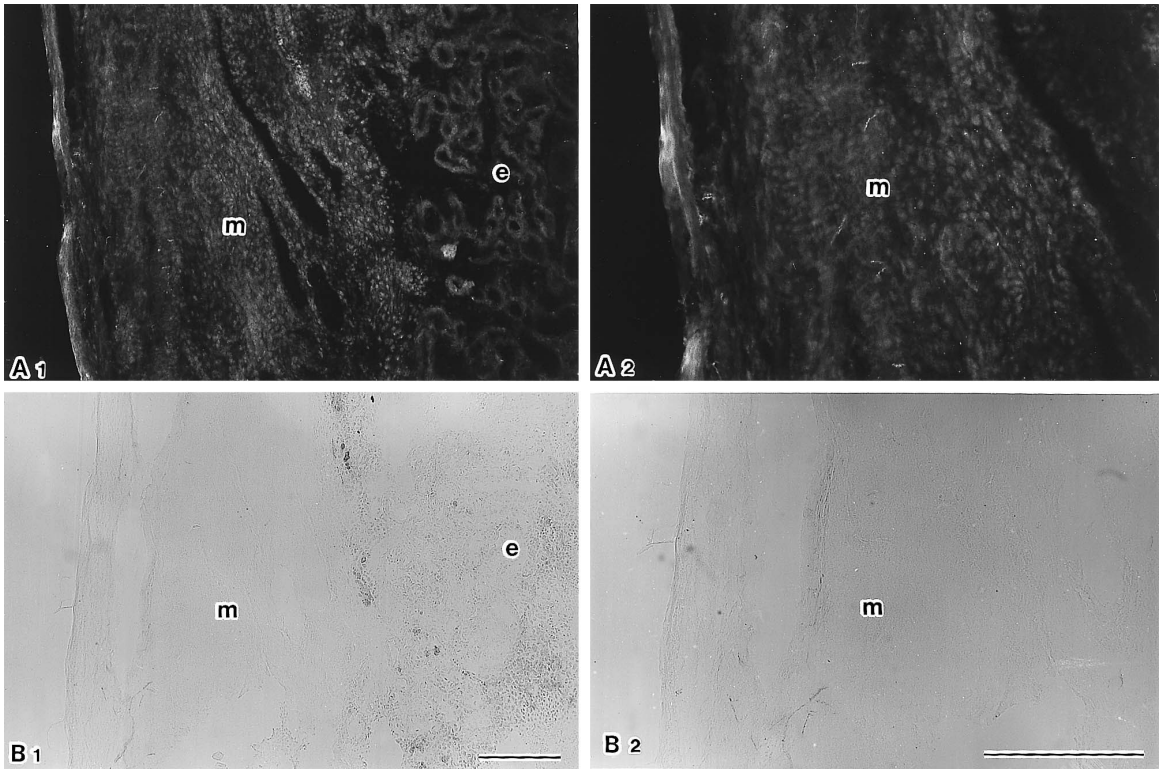

Fig. 4. NA (A1) and AChE (B1) nerves in the uterus just after the start of rapid embryogenesis in April. (2) Higher magnification of (1). e, endometrium; m, myometrium. Bar=200 μm.

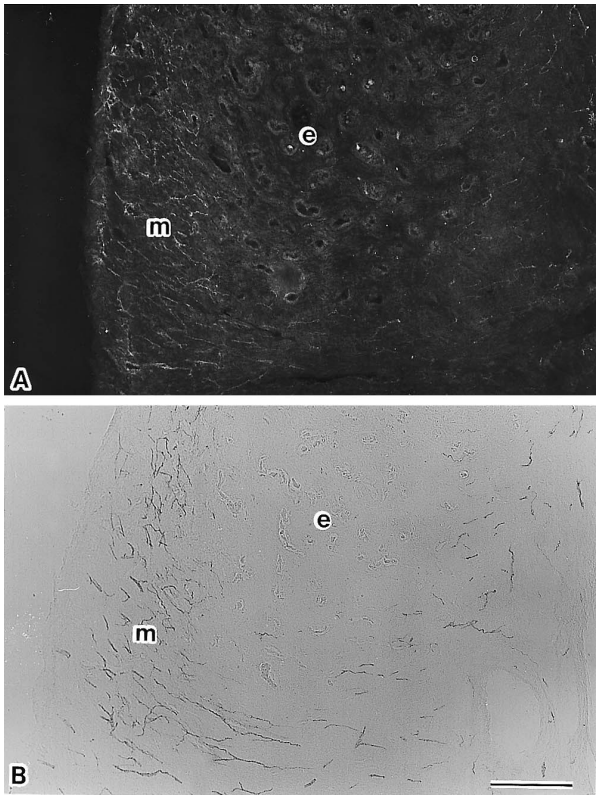

Fig. 5. Abundant NA (A) and AChE nerves (B) of the cervix in April. endometrium; l, lumen; m, myometrium. Bar=200 μm.

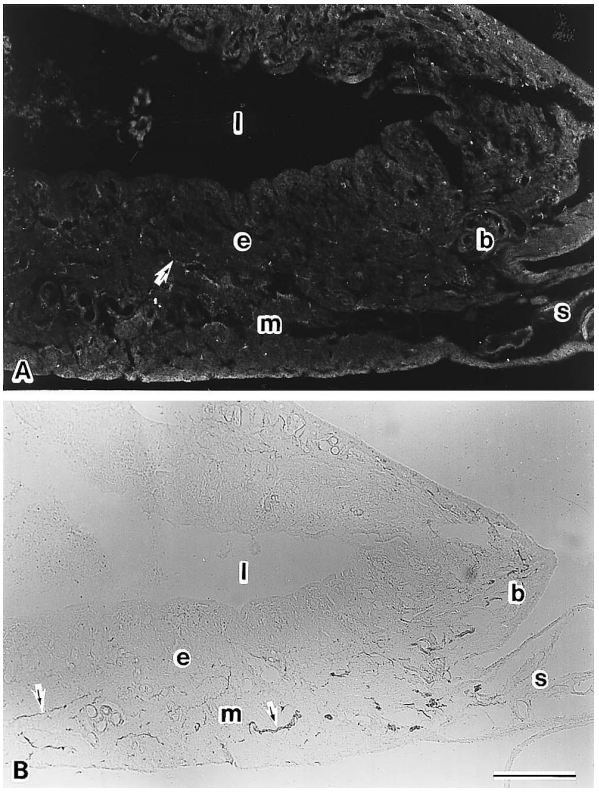

Fig. 6. NA (A) and AChE (B) nerves in the uterus just after parturition in July. Arrows indicate nerves running in the myometrium. b. blood vessel; e, endometrium; l, lumen; m, myometrium; s, mesometrium. Bar=200 μm.

horn at April, of which the size now enlarged greatly as the result of the accelerated embryogenesis, became markedly prominent (Fig. 4A, B). During further late period of pregnancy between May and June, NA and AChE nerves supplying the horn maintained a low level of density similar to that seen in April (Table 1). From April to June, the abundant supply of NA and AChE nerves was consistently found in the myometrium of the cervix (Fig. 5A, B).

In the shrunk uterine horn just after parturition examined at July and August, density and distribution of NA and AChE nerves began to increase from mesometrial side toward antimesometrial side (Fig. 6A, B) (Table 1). These two types of nerves were preferentially distributed to the myometrial arterial system and non-vascular smooth muscles, and rarely encountered at the endometrium.

DISCUSSION

In the present study, we showed that NA and AChE innervation in the uterus of the adult Japanese long-fingered bat changes greatly with its own characteristic reproductive cycle. In the non-breeding season, an abundance of NA and AChE nerves were closely associated with the uterine arteries and myometrial smooth muscles. The density of two types of uterine nerves did not decrease much during early pregnancy. In little brown bats, *Myotis lucifugus*, (Buchanan *et al*., 1988) rats (Melo and Machado 1993), cats (Alm *et al*., 1986) and sheep (Renegar and Rexroad, 1990), it has been reported that these two types of uterine nerves mediate the tone of myometrial vascular as well as non-vascular smooth muscles. Accordingly, the rich NA and AChE innervation focused on the myometrium of the Japanese long-fingered bat during early pregnancy seems to involve in some specific functions of this site such as transport and spacing of conceptus in addition to the regulation of vasomotor action.

In the Japanese long-fingered bat, a significant increase in progesterone concentration and the volume of the corpus luteum, concomitant with an elevation of metabolic activity, has been revealed in the pregnant animals just after arousal from hibernation (Kimura *et al*., 1987). Thus, there is a clear correlation between the degeneration of uterine NA and AChE nerves and rise of luteal activity in late period of pregnancy after hibernation. This suggests that progesterone acts as the major factor for pregnancy-induced nerve reduction in the bat as has been reported for the rat (Moustafa, 1988; Hasse *et al*., 1997), guinea pig (Moustafa, 1988) and sheep (Reneger and Rexroad, 1990). Reduction of NA and AChE nerves from the bat uterus in late pregnancy, as has been suggested in the rat (Moustafa, 1988; Hasse *et al*., 1997), guinea pig (Moustafa, 1988) and sheep (Reneger and Rexroad, 1990), may be closely related to a regressive reorganization of neuronal influence that is relevant to the formation of placental circulation, and to quiescence of uterine contractility during pregnancy. It may also prevent neuronal elements from mechanical damage during the violent contractile activity of myometrium at the onset of labor. On the other hand, uterine NA and AChE nerves in the little brown bat do not degenerate during pregnancy, despite of a high plasma concentration of progesterone (Buchanan *et al*., 1988). It is remained to be elucidated whether difference in the sensitivity of uterine neurons to this steroid hormone exists in chiropteran species.

The supply of NA and AChE nerves to uterine vascular and non-vascular smooth muscles in the Japanese long-fingered bat recovers again from the mesometrial side toward the antimesometrial side after parturition where the concentration of progesterone decreases markedly (Kimura *et al*., 1987). Since it has been shown that NA nerves exert a trophic effect on uterine tissues (Brauer *et al*., 1992), the regeneration of uterine NA and possibly AChE nerves in the post partum bat might be related to the regain of uterine structure and function for its next reproductive cycle. Recently, it has been suggested that hormone-induced neurotrophic factors, which are produced by uterine tissues, can regulate the state of uterine innervation of the rat (see Brauer *et al*., 2000). In addition to this, it has been shown that the concentration of nerve growth factor, the major trophic agent essential for the survival of NA neurons, and its mRNA within the uterus decrease during pregnancy, but return to the normal level after parturition (Varol *et al*., 2000). Seasonal differences in the amounts of such target-specific trophic molecules, which depend on hormonal influence, may play a critical role in the degeneration and regeneration of uterine NA and AChE nerves within the uterus that are required for the establishment of the unique estrus cycle of the Japanese long-fingered bat.

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