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Modification of Spontaneous Electrical Activity by Neural and Blood-Borne Factors in the Silkworm *Bombyx mori* L.

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Abstract: The objective of the present study was to examine the variations in the levels of spontaneous electrical activity during the 5th instar development of the silkworm *Bombyx mori* and the possible mode of its regulation. The spontaneous electrical activity of the ventral nerve cord (VNC) showed an increase from the 2nd day to the 7th day of the 5th instar. Treatment of the VNC with extracts of the central nervous system (CNS) from the 2nd day larvae or hemolymph from the 7th day larvae elevated the spontaneous electrical activity of the VNC in both the 2nd and 7th day larvae, while extracts from the 7th day larvae or hemolymph from the 2nd day larvae did not cause any perceptible effect. Acetylcholine (ACh, 10⁻⁷M) elevated the activity in both the 2nd and 7th day larvae. It is suggested that synthesis and release of neuromodulators from the CNS into the hemolymph could modify the activity of the nervous system at different stages of the life cycle and consequently the behavior in the silkworm.

Keywords: *Bombyx mori*, Neural extracts, Hemolymph, Spontaneous electrical activity, Spinning behavior

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Introduction

Electrical activity of the nervous system in various silkworms during metamorphosis showed considerable variation (Koshtoyants *et al.*, 1954; Van der Kloot, 1955; Tyshtchenko and Mandelstam, 1965; Yamaoka and Hirao, 1973, 1977; Siva Prasad *et al.*, 1992). Despite the availability of investigations on the electrical activity of the nervous system in the insects (Nitz *et al.*, 2002; Suenobu *et al.*, 2004; Ellen *et al.*,

2012), research on the changes in electrical activity during different stages of development in the silkworm and the possible reasons responsible for them is very scanty, except the reports by Siva Prasad *et al.* (1992) and Siva Prasad and Murali Mohan (1998). The present study was performed to examine the differences in spontaneous electrical activity of the nervous system between the early and spinning stages of the 5th instar of

Bombyx mori, and the possible influence of neural and blood-borne factors on this activity.

Materials and Methods

The larvae of the 5th instar of NB₄D₂ hybrid of *Bombyx mori*, reared as described by Krishnaswami (1978), were used in the experiments. The experiments were carried out on the 2nd day larvae of the 5th instar and on the larvae that were just about to start spinning the cocoons or those which had just started spinning.

Preparation of the neural extracts:

The nervous tissue, consisting of the brain-subesophageal ganglion complex, thoracic and abdominal ganglia along with the interganglionic connectives from the 2nd day larvae of the 5th instar and also from the larvae that were just about to start or had just started spinning was pooled up by dissection. Tissue from several larvae was pooled into cold *Bombyx* Ringer (Yamaoka *et al.*, 1971). 1% homogenates in cold Ringer were prepared separately for these two isolates. The homogenates were centrifuged at 600 g for 5 min and the supernatants were stored in the refrigerator. The extracts were brought back to room temperature before use. The extracts were used within 24 h after preparing them.

Collection of hemolymph:

Hemolymph from the 2nd day larvae of the 5th instar and the pre-spinning larvae or from those just started spinning was collected into pre-chilled glass tubes separately by cutting the first pair of abdominal legs, and was used immediately.

Preparation of acetylcholine (ACh) solutions:

A 10⁻¹M solution of acetylcholine chloride (AChCl) was prepared in cold *Bombyx* Ringer and stored in the Refrigerator. It was brought back to the laboratory temperature before use and diluted with *Bombyx* Ringer according to the need.

Recording of spontaneous electrical activity:

The ventral nerve cord (VNC) was exposed from the dorsal side and the recordings were made from the connective between the 2nd and 3rd

abdominal ganglia. Paired platinum hook electrodes were used to monitor the spontaneous electrical activity. The potentials were fed through a Nihon Kohden AVB-21 preamplifier and displayed on a Nihon Kohden VC-11 memory oscilloscope. Photographic recordings were made using a Nihon Kohden RLG-6201 oscilloscope camera.

Effects of neural extracts and hemolymph on the spontaneous electrical activity:

The 2nd day larvae of the 5th instar and the larvae which just started to spin were chosen for the study of the effects of the neural extracts, hemolymph and ACh. The VNC of these larvae were treated separately with neural extracts and hemolymph of the 2nd day larvae as well as the spinning larvae. To study the effect of the extracts and hemolymph on spontaneous electrical activity, the control activity was recorded first, and then the VNC was soaked for 3 min in the extract/hemolymph, and the activity was recorded again. Following this, the VNC was then thoroughly washed with *Bombyx* Ringer for 5 min, to check if there was any reversal to the control level of activity.

Effect of ACh on spontaneous electrical activity:

For examining the effect of ACh, after recording the control activity the nerve cord was soaked in 10⁻⁷M ACh solution for 3 min and the spontaneous activity was recorded. After this the cord was washed with Ringer for 5 min and the activity was recorded again to check if there was a reversal of the activity to the control level.

Analysis of the spontaneous electrical activity:

Analysis of the spontaneous electrical activity in the control and experimental conditions was made by counting the number spikes present in a 20 cm long film strip on which the spontaneous activity was recorded. Spikes from recorded film strips from 6 separate experiments were thus counted separately and the means were taken. The data were then converted into spikes/sec. The number of categories of spikes firing in the controls and

under each experimental condition was approximated basing on the spike amplitudes.

Results

Spontaneous electrical activity in control larvae:

The spontaneous activity was present continuously as background activity in the normal condition. The spontaneous impulses were both monophasic and biphasic, with steep ascending and descending phases and smooth contour. In controls the potentials were firing at a frequency which varied from about 180 impulses per second in the 2nd instar larvae to about 210 impulses per second in the 5th instar larvae. The potentials differed in amplitudes of these impulses which varied from a minimum of 10 μ v to a maximum of 125 μ v in the controls. Basing on their amplitudes the spikes could be discerned into different categories wherein the potentials having the same amplitude were reckoned as belonging to one category. The activity recorded from the 2nd day larvae had a frequency of about 180 spikes/sec (Table 1). However, the activity consisted of potentials which were mostly of lower amplitude. In contrast to this, the control activity recorded from the spinning larvae had a higher frequency of about 210 spikes/sec (Table 1). The activity consisted of some potentials which were of higher amplitude than those recorded from the 2nd day larvae. Basing on the amplitudes of the potentials the spike categories in the activity from the 2nd day larva were discerned as 7, while 9 spike categories could be identified in the spinning larvae (Table 1).

Effects of neural extracts and hemolymph on spontaneous electrical activity:

After recording the control activity, the ventral nerve cord was soaked separately with extracts of the CNS and hemolymph from the 2nd day larva as well as the 7th day (spinning) larva. The treatment was done for the nerve cord both in the 2nd day larva as well as in the spinning larva. It was noticed from these treatments that neural extract from the 2nd day larva and hemolymph from the 7th day larva have an elevating effect on the

spontaneous electrical activity both in the 2nd day larva and the spinning larva (Table 1). With neural extract the activity was elevated by 22.56% and 21.43%, respectively in the 2nd day larva and the spinning larva. With hemolymph also the activity was elevated by 26.11% and 32.38%, respectively in the 2nd day larva and the spinning larva. These increases were statistically significant. The increase in activity was in terms of both the frequency of the potentials and their amplitudes.

The effects of neural extract from the spinning larva and hemolymph from the 2nd day larva, however, contrasted the effects of 2nd day neural extracts and 7th day hemolymph. The extracts from the spinning larva and hemolymph from the 2nd day larva did not cause any change in the control activity (Table 1). Thus, the 2nd day larva and the spinning larva recorded non-significant changes of +1.11% and -3.81%, respectively on treatment of the VNC with the neural extract from the spinning larva. The hemolymph from the 2nd day larva also caused non-significant changes of -2.22% and -2.38% in the 2nd day larva and in the spinning larva, respectively. After each of the above treatments, washing the VNC with Ringer for about 5 min could retrieve the activity back to the control level.

Effect of acetylcholine (ACh) on spontaneous electrical activity:

Treatment of the VNC with ACh (10^{-7} M) significantly elevated the spontaneous activity in the 2nd day larva as well as in the spinning larva (Table 1). The increase was in terms of both the frequency of firing of impulses as well as amplitudes of the potentials. The frequency recorded an increase of 40% in the 2nd day larva and 42.85% in the spinning larva. Most of the potentials firing after treatment with ACh were of higher amplitude compared to those in the controls or after treatment with either the neural extract or hemolymph. The spike categories also were found to be higher on treatment with ACh.

As in the case after treatment with the extracts or hemolymph, washing the VNC with Ringer for 5

Table 1: Effects of neural extracts and hemolymph from the 2nd day larvae and spinning larvae, and 10⁻⁷M acetylcholine on the spontaneous electrical activity (number of spikes/second) of the ventral nerve cord in 2nd day larva and spinning larva of the 5th instar of the silkworm *Bombyx mori*. The activity is the mean $\pm$ standard deviation (SD) of six separate experiments

S. No.	Treatment		2 nd Day larva		Spinning larva	
			No. of spikes	No. of spike categories	No. of spikes	No. of spike categories
1.	Ringer (Control)	Mean SD	180 ± 4	7	210 ± 7	9
2.	Neural extract from 2 nd day larvae	Mean SD % Change	224 ± 8 +22.56***	8	255 ± 9 +21.43**	7
3.	Neural extract from spinning larvae	Mean SD % Change	182 ± 4 +1.11 ^{NS}	6	202 ± 5 -3.81 ^{NS}	7
4.	Hemolymph from 2 nd day larvae	Mean SD % Change	176 ± 3 -2.22 ^{NS}	7	205 ± 5 -2.38 ^{NS}	8
5.	Hemolymph from spinning larvae	Mean SD % Change	227 ± 7 +26.11***	9	278 ± 12 +32.38***	10
6.	ACh	Mean SD % Change	252 ± 9 +40.00***	12	300 ± 12 +42.85***	12

*** = P < 0.001; ** = P < 0.01; NS: Not significant

min after treatment with ACh could reverse the effects of ACh and bring the spontaneous electrical activity back to the control level.

Discussion

Spontaneous electrical activity is an endogenous neural activity which occurs in neurons without any apparent stimulation. However, this activity is generally reckoned as a reflection of the active state of the animal (Prosser and Brown, 1962; Bullock and Horridge, 1965). This feature has been repeatedly demonstrated in several animals. In the present investigation, it may be stated that the overt activity and the internal neural activity of the silkworm are probably less on the 2nd day of the 5th instar compared to the 1st day of spinning. The difference in activity between the 2nd day and spinning day seems to be more in terms of the amplitude and frequency of firing of the impulses. The absence of large amplitude potentials in the silkworm as noticed in the present study is in coherence with the sluggish nature of the

silkworm. The increase in spontaneous electrical activity from the 2nd day to the spinning day of the 5th instar could also be due to the increasing morphological and functional complexity of the nervous system (Siva Prasad *et al.*, 1992; Siva Prasad and Murali Mohan, 1998).

Neural extract from the 2nd day had an elevating effect on the spontaneous electrical activity both in the 2nd day larva as well as in the spinning larva, while the extract from the spinning larva did not show any effect. In contrast, hemolymph from the spinning larva elevated the spontaneous electrical activity in both the 2nd day and spinning larvae, while hemolymph from the 2nd day larva did not show any effect. This shows that the spontaneous electrical activity could be modulated by blood-borne factors which may be of neural origin. The spontaneous electrical activity of the CNS is known to be influenced by neurohormones and neurohumors (Ozbas and Hodgson, 1958; Weiant, 1958; Milburn *et al.*,

1960; Milburn and Roeder, 1962; Vijayalakshmi *et al.*, 1977). Thus, in the present study it is possible that neural factors, humoral and/or hormonal, could be synthesized in the CNS and released at appropriate times to modulate the spontaneous electrical activity and consequently the overt behavioral activity of the silkworm with reference to feeding and spinning. The fact that the 2nd day neural extract elevated the spontaneous electrical activity while the spinning day extract did not indicates the relative abundance of neuromodulators in the nervous system on the 2nd day. Similarly, the effects of hemolymph from the 2nd day and spinning larvae indicate the relative abundance of blood-borne neuromodulators in the hemolymph of the spinning larvae.

Synthetic and releasing phases for neurohumoral and neurohormonal factors have been reported earlier in other invertebrates (Rao and Gopalakrishna Reddy, 1967; Vijayalakshmi *et al.*, 1977; Rajarami Reddy *et al.*, 1978). With regard to the neurohumoral factors, special emphasis has been laid on the neurotransmitters such as ACh. ACh has been suggested to be an important neurohumor in silkworms (Subhashini *et al.*, 1983; Siva Prasad *et al.*, 1992; Sasikala *et al.*, 1998). The present results also showed that ACh has a significant elevating effect on the spontaneous electrical activity. Oscillations in the behavioral activity of the animal may result from complex interactions between the nervous system, hormones, chemicals etc. in the blood and a variety of other factors (Strumwasser, 1967). Thus, it may be reasonably surmised that apart from a possible role for other neurohumors, fluctuations of ACh levels in the nervous system as well as in the hemolymph probably play a role in the changes on spontaneous electrical activity during the development of the silkworm and consequently modulate the overt behavioral activity.

Conclusion

From the present study we conclude that synthesis and release of blood-borne neuro-

modulators from the CNS into the hemolymph could modify the activity of the nervous system at different stages of the life cycle and consequently the behavior in the silkworm.

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