African Research Review

An International Multi-Disciplinary Journal, Ethiopia

Vol. 4 (1) January, 2010

ISSN 1994-9057 (Print) ISSN 2070-0083 (Online)

Spontaneous Electrical Activity in the Nervous System and its Modification by Biogenic Amines in the Silkworm *Bombyx Mori* (L) (Pp 174-189)

Josthna, P. - Department of Biotechnology, S.P. Mahila University, Tirupati, India

Sahitya Chetan, P. - Department of Zoology, S.V. University, Tirupati, India

Murali Mohan, P. - Department of Biology, Bahir Dar University, Bahir Dar, Ethiopia

E-mail: drpmuralimohan@yahoo.com

Abstract

The present study was carried out to examine the effects of biogenic amines on the spontaneous electrical activity of the nervous system in the silkworm Bombyx mori. The activity recorded from different segments of the ventral nerve cord differed in the frequency and number of spike categories firing. The activity was highest between the subesophageal ganglion and 1st thoracic ganglion. Lower activity was recorded in the thoracic part of the cord and the activity increased in the abdominal cord. More or less the same level of activity was recorded from all the abdominal segments of the cord. The above trend was the same from the 1st day to the 7th day of the 5th instar of the silkworm. However, the overall level of activity increased from the 1st day to the 7th day in tune with the increasing complexity of the peripheral nervous system in the silkworm as the 5th instar progressed. Treatment of the cord with solutions of putative neurotransmitter substances, viz. epinephrine (EP), norepinephrine (NEP), dopamine (DA) and 5-hydroxytryptamine (5-HT) at different concentrations from $1x10^{-8}$ M to $1x10^{-4}$ M showed elevation at lower concentrations and decrease at $1x10^{-4}$ M. The concentration at which maximum elevation could be *elicited varied between the above transmitters. Biochemical analyses revealed the presence of these biogenic amines in the silkworm nervous system. Thus the possibility remains that one or more of these substances could act as neurotransmitters in the silkworm nervous system.*

Keywords: Silkworm**;** 5th Instar**;** Spontaneous electrical activity**;** Biogenic amines**;** Neurotransmitters.

Introduction

It has been reported (Vanderkloot, 1955) that the central nervous system (CNS) shows significant changes in its electrical activity during metamorphosis of certain lepidopteron insects. Spontaneous activity of the CNS is known to be influenced by neurohormones (Ozbas and Hodgson, 1958; Weiant, 1958). Investigation on the electrical activity of neurosecretory cells of *Bombyx mori* brain was reported by Miyazaki and Shun-Ichi (1980). Effects of a wide variety of neurotransmitters and precursors have been tested in a series of studies (Schantz and Goyffon, 1970; Goyffon *et al*., 1980; Goyffon and Niaussat, 1975). On the basis of electrophysiological data, the transmitter role of monoamines was suggested in the CNS of some insects (Florey, 1967). Madhusudhana (1995) studied the effects of different transmitter candidates such as epinephrine (EP), nor-epinephrine (NEP), dopamine (DA) and 5-hydroxytryptamine (5-HT), and of drugs such as imipramine, picrotoxin and reserpine on the spontaneous activity in the scorpion. The present investigation was undertaken to examine the spontaneous electrical activity of the double ventral nerve cord and to correlate it with the ongoing morphological and biochemical changes in the CNS during the 5th instar of *Bombyx mori*. Further, the action of selected biogenic amines on the spontaneous activity during the 5th instar was also examined.

Methodology

Larvae of the 5^{th} instar of NB_4D_2 hybrid of *Bombyx mori*, reared as per standard procedures (Krishnaswami, 1978), were used in the experiments. Depending on the need, the experiments were conducted from the 1^{st} to the 7^{th} day of the 5^{th} instar up to the point when the larvae were just about to start spinning the cocoons.

Preparation of Amine Solutions: 10^{-1} M solutions of the biogenic amines, viz. epinephrine (EP), nor-epinephrine (NEP), dopamine (DA) and 5-hydroxytryptamine (5-HT) were prepared in cold *Bombyx* Ringer and stored in a refrigerator. They were brought to laboratory temperature before use and diluted according to the need with *Bombyx* Ringer.

Measurement of Spontaneous Electrical Activity: The ventral nerve cord (VNC) of *Bombyx mori* was exposed from the dorsal side and the recordings of spontaneous electrical activity were made between SG - 1TG, 1TG - 2TG, 2TG - 3TG, 3TG - 1AG, 1AG - 2AG, 2AG - 3AG and 3AG - 4AG, 4AG - 5AG, 5AG - 6AG and 6AG - 7/8AG (expansions for these abbreviations are given in figure legend). The recordings were made at the same time every day, although in a metamorphosing animal the interference of diurnal variations is unclear. 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 readings were made using a Nihon Kohden RLG-6201 oscilloscope camera.

Effects of Amine Solutions on Spontaneous Electrical Activity: The 7^{t}h day larvae of the 5th instar were chosen for the study of the effects of the chosen biogenic amines. The effects were examined at 2AG-3AG, since on the 7th day of the 5th instar this segment of VNC is the longest and so maneuver with electrodes was easy. Further, it did not make any difference as to from which abdominal segment the activity was recorded, since all abdominal segments had more or less the same level of activity (Figs. 1 and 2., Tables 1 and 2). Different concentrations of amine solutions ranging from $1x10^{-8}$ M to $1x10^{-2}$ M were prepared in *Bombyx* Ringer. Each amine was tested for its effect at these concentrations starting from $1x10^{-8}$M and going up. The control activity was recorded first, and then the VNC was soaked for 3 minutes in $1x10^{-8}$ M solution of the amine under question, and the activity was recorded again. Following this, the VNC was thoroughly washed with *Bombyx* Ringer for 5 minutes to check if there was any reversal of the effect to the control level. In this way the spontaneous electrical activity was recorded after treatment with each concentration of the different amines, and after washing with Ringer.

Analysis of Spontaneous Electrical Activity: Analysis of spontaneous electrical activity in the control and experimental conditions was made by counting the number of spikes present in a 50 cm long film slip on which the spontaneous activity was recorded. Spikes from recorded film strips from 6 separate experiments were thus counted and the mean was taken. The data was then converted into spikes/sec. The number of categories of spikes firing in the controls and under each experimental condition was approximated basing on their amplitudes.

Results

Spontaneous electrical activity from 1st day of the 5th instar: The firing of spikes in the VNC varied from segment to segment (Fig. 1; Table 1). Higher activity was recorded between the subesophageal ganglion (SG) and the 1st thoracic ganglion (1TG), with a spike frequency of 212/sec and 7 spike categories (Fig. 1a; Table1). The activity decreased in the next segment, i.e. between 1TG - 2TG, with a frequency of 70 spikes/sec and 3 spike categories. Thereafter the activity increased slightly between 2TG - 3TG and 3TG - 1AG with a frequency of 88 and 125 spikes/sec, and 3 spike categories each respectively. The activity in the abdominal cord was higher than in the thoracic part. However, the activity between different segments of the abdominal cord was more or less of the same level, with minor differences. As such, only the recordings made form 1AG - 2AG, 2AG - 3AG and 6AG - 7/8AG are shown in the Fig. 1 to avoid redundancy. Between 1AG - 2AG, 2AG - 3AG and 6AG - 7/8AG the activity recorded had a frequency of 164, 154 and 155 spikes/sec and 6, 6 and 4 spike categories respectively.

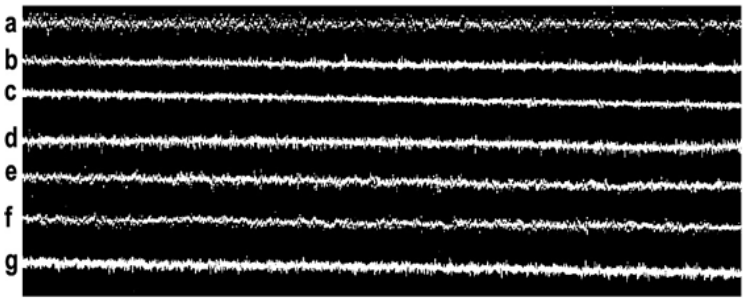

Fig. 1: *Oscilloscopic recordings showing the spontaneous electrical activity from different segments of the ventral nerve cord of Bombyx mori on the 1st day of the 5th instar.*

a) Between the subesophageal ganglion & 1st thoracic ganglion (SG-1TG);

b) Between the 1st thoracic ganglion & 2nd thoracic ganglion (1TG-2TG);

c) Between the 2nd thoracic ganglion & 3rd thoracic ganglion (2TG-3TG);

d) between the 3rd thoracic ganglion & 1st abdominal ganglion (3TG-1AG);

e) between the 1st abdominal ganglion & 2nd abdominal ganglion (1AG-2AG);

f) Between the 2nd abdominal ganglion & 3rd abdominal ganglion (2AG-3AG);

g) Between the 6th abdominal ganglion & 7/8 abdominal ganglion (6AG-7/8AG).

Calibration: Vertical: 1 cm = 50 µv; Horizontal: 1 cm = 100 msec.

Note: Recordings from 3AG-4AG, 4AG-5Ag and 5AG-6Ag are not presented to avoid redundancy of the same level of activity of these abdominal segments as of 2AG-3AG and 6AG-7/8AG.

Spontaneous electrical activity from the 7th day of the 5th instar: Spontaneous activity was recorded from different segments of the VNC of the 7th day larva of the 5th instar (Fig. 2; Table-2). The activity was in fact examined on all days of the instar, from which it was apparent that there is an increase in activity throughout the VNC from the 1st day to the 7th day. However, the trend of variation in activity between different segments of VNC was the same on all the days as on the 1st day. The data pertaining to the activity in different segments is presented in Table 2.

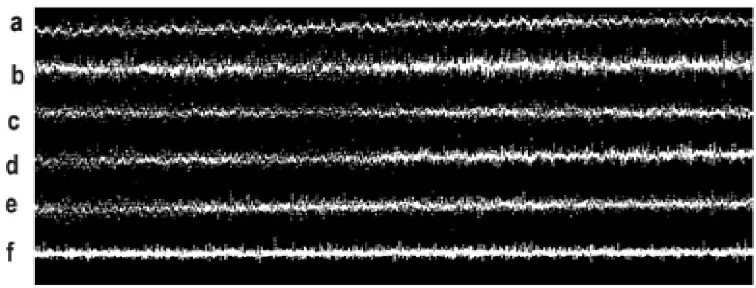

Fig. 2

Oscilloscopic recordings showing the spontaneous electrical activity from different segments of the ventral nerve cord of Bombyx mori on the 7th day of the 5th instar. The remaining legend is the same as for Fig. 1.

Effect of epinephrine (EP): Treatment of the VNC with EP in general had an elevatory effect in terms of spike frequency and spike categories (Table 3). The elevation gradually increased from 1 x 10^{-8} M onwards, with maximum elevation occurring with an EP concentration of 1x10^{-5} M. Thereafter, further increase in the concentration of EP resulted in a gradual decrease at 1x10^{-4} M and above. Washing the VNC with *Bombyx* Ringer following treatment with each concentration of EP could effect recovery towards the control (data not shown).

Effect of nor-epinephrine (NEP): The data on the effect of NEP on spontaneous activity is presented in Table 4. Treatment with NEP caused effects which were different from those with NEP. Treatment with 1x10^{-8} M NEP caused maximum increase in activity. Thereafter, treatment with 1x10^{-7} M, 1x10^{-6}M, 1x10^{-5} M and 1x10^{-4} M progressively recorded a decrease from this elevation in terms of spike frequency and spike categories. Washing the VNC with *Bombyx* Ringer following treatment with each concentration of NEP could cause recovery towards the control (data not shown).

Effects of dopamine (DA): Results on the treatment with different concentrations of DA are presented in Table 5. Concentrations from 1x10^{-8} to 1x10^{-6} caused elevation of the activity, with maximum elevation in activity on treatment with 1x10^{-6} M DA. Thereafter treatment with higher concentrations caused a progressive decrease from this elevation in activity. Washing the VNC with *Bombyx* Ringer following treatment with each concentration of DA could affect recovery towards the control (data not shown).

Effects of 5-hydroxytryptamine (5-HT): Results on the effects of different concentrations of 5-HT are presented in Table 6. The trend of 5-HT effects was similar to that of treatment with NEP. Highest elevation in was activity recorded at a 5-HT concentration of 1x10^{-8} M. Following this, treatment with 1x10^{-7} M and higher concentrations caused a progressive decrease from this elevation in activity. Washing the VNC with *Bombyx* Ringer following treatment with each concentration of DA could bring about recovery towards the control (data not shown).

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 (Bullock and Horridge, 1965). With reference to circadian rhythms it has been demonstrated that the spontaneous electrical activity is higher during the periods of the day when the animal is active and lower during the periods of lower active state of the animal (Vijayalakshmi *et al*., 1977; Rajarami Reddy *et al*., 1978). The above observations lead to the assumption that the spontaneous activity could show alteration in its levels with the increase in overt activity of the silkworm as it proceeds from the 1st day to the 7th day of the 5th instar, leading towards the spinning activity. In consonance with this assumption, an increase in spontaneous activity in different segments of the VNC from the 1st day to the 7th day of this instar was noticed in the present investigation.

Siva Prasad (1987) reported that during metamorphosis, the CNS of the silkworm undergoes a series of morphological changes such as an increase in the size of the brain and ventral ganglia, lengthening and shortening and final disappearance of certain ganglionic connectives culminating in the final coalescence of certain ventral ganglia to form anterior and posterior ganglionic complexes. The branching and innervation pattern of the segmental nerves gradually increases during metamorphosis, with the establishment of more and more synaptic contracts with the segmental muscles (Siva Prasad and Murali Mohan, 1998). The increase in spontaneous activity from the 1st day to the 7th day of the 5th instar as noticed in the present investigation could be related to these factors in addition to the increase in the complexity of the nervous system and other intrinsic and extrinsic factors which may play a role. There was no gradient in activity in the abdominal segments, and the activity level was more or less similar in all the abdominal segments. Maximum activity was recorded from AG2-AG3. This pattern was essentially the same from the 1st day to the 7th day of the 5th instar, with only quantitative changes depending upon the day of the instar, viz., early, middle or late (Figs. 1 & 2; Tables 1 & 2).

The above observations indicate a pattern wherein the cerebral part (brain + sub esophageal ganglion) of the nervous system shows higher activity followed by the abdominal cord, with less activity in the thoracic segments. Thus, greater peripheral input and central motor output seem to be concentrated in the cephalic region, with the silkworms actively involved throughout in feeding, and from the 7th day onwards during the 5th instar in spinning the cocoon. Higher activity in the abdominal region could be attributed to the active abdominal movements and the peripheral sensory input thereof. Higher activity in the abdominal region compared to the thoracic region seems to be a general feature in insects and arachnids (Bullock and Horridge, 1965).

Tyshtchenko and Mandelstam (1965) reported in oak silkworm that the highest level of spontaneous electrical activity with reference to both frequency and amplitude is present in the abdominal ganglia. Experiments on spontaneous electrical activity were performed on the abdominal cord in cockroach (Vijayalakshmi *et al*., 1977; Jacob Doss, 1993) owing to the higher activity which could be pharmacologically modified effectively. Further, the activity in the abdominal cord was found to be higher between the 2^{nd} and 3^{rd} abdominal ganglia. Thus, the pattern of activity along the ventral nerve cord seems to have been pre-fixed in the silkworm *Bombyx mori*, with only quantitative changes occurring as the metamorphosis progressed. This is in line with the profusion of peripheral branches of the nervous system, showing the highest activity by about the time the 5^{th} instar larva starts spinning the cocoon.

Effects of biogenic amines: Spontaneous activity in the abdominal cord was highest between the 2^{nd} and 3^{rd} abdominal ganglia. This segment was also the longest in the VNC of the 5^{th} instar. For these reasons the effects of neurotransmitters were examined in this segment for the facility of maneuverability with electrodes and clear visualization of changes in activity with the neurotransmitters.

Norepinephrine as a sympathetic neurotransmitter in the vertebrate nervous system is known to be excitatory (Cooper *et al*., 1982). Acetylcholine, epinephrine and norepinephrine were shown to have a stimulatory effect on the neurally mediated hindgut contractions in the crab (Florey, 1954). This amine was identified and quantitatively measured in the nervous system and hemolymph of the crayfish, and an excitatory role for it was implicated (Elofsson *et al*., 1982). However, Goyffon (1978) reported inhibition of electrical activity in the scorpion by norepinephrine. In line with this, Madhusudhana (1995) reported a decrease in the spontaneous electrical activity in the scorpion *Heterometrus fulvipes* at a concentration of $1x10^{-3}$ M of norepinephrine. This observation coincides with the decrease observed in the present study at concentrations of norepinephrine higher than $1x10^{-8}$ M. Thus, there seems to be a concentration-dependent alteration of spontaneous activity by NEP wherein $1x10^{-8}$ M elevates the activity significantly, and concentrations higher than this cause progressively lesser activation.

Epinephrine (EP) was shown to cause significant effects either in the nervous system or its innervated organs in various other invertebrates including insects. It augments the rhythmic concentrations of wing muscles mediated by the metathoracic ganglion in *Locusta* (Voskresenskaya, 1950). It increases the heartbeat in the scorpion *Palamnacus bengalensis* (Kanungo, 1957). It has a stimulatory effect on the neurally mediated hindgut contractions in the crab

(Florey, 1954). However, the concentration of EP in the central nervous system is known to be relatively low, approximately 5 to 17% of the NEP content, implicating a relatively less important role as a neurotransmitter compared to NEP (Cooper *et al.,* 1982).

Using a high concentration of $1 x 10^{-3}$ M dopamine (DA), Madhusudhana (1995) demonstrated a strong inhibitory action on the spontaneous activity in the ventral nerve cord of the scorpion *Heterometrus fulvipes*. In contrast to this, Janet and Ritzmann (1992) noticed an increase in the amplitude of giant neuron-evoked excitatory postsynaptic potentials with lower concentrations of DA in the cockroach *Periplaneta americana*. This report is in coherence with the excitation recorded with DA in the present study.

Inhibition in the spontaneous activity of prosomian nervous system of the scorpion *Androctonus maritanicus* with 5-hydroxytryptamine (5-HT, serotonin) was reported by Goyffon (1978). However, in certain other regions 5-HT caused pronounced activation and discharge rate. Therefore it was concluded that 5-HT causes mainly, if not exclusively, inhibitory effects (Cooper *et al.,* 1982). Thus, all the biogenic amines examined in the present study caused elevation of spontaneous activity of the VNC in a concentration dependent fashion. The concentration at which maximum elevation was caused varied from compound to compound. The elevation could be recognized in two ways, viz., an increase in the overall frequency of the spikes, and an increase in the firing of spikes with larger amplitudes. The latter could be visualized through an increase in the number of spike categories. In all cases the spike categories were also higher when the spike frequency was high.

It is possible that some of the compounds examined in the present study for their effects on spontaneous activity could be acting as neurotransmitters in *Bombyx mori* besides subserving other functions. An earlier biochemical investigation carried out by us (Josthna and Murali Mohan, 2002) suggests interesting implications for the present investigation. Based on that study, epinephrine may be exempted from the possibility of acting as a transmitter substance, since it was neither detected in the nervous system nor in hemolymph throughout the 5^{th} instar, although it did cause an elevation in spontaneous activity in the present study. Dopamine as the precursor for norepinephrine was present in the nervous system and hemolymph of *Bombyx mori* throughout (Josthna and Murali Mohan, 2002), and could very well be a transmitter candidate. Norepinephrine was present in the hemolymph throughout, but made its appearance in the nervous system only on the 5th day, three days before cocoon spinning, and was present during the rest of the instar (Josthna and Murali Mohan, 2002). This gives room for speculation on its role as a neurotransmitter in spinning activity. Serotonin in contrast was present in the nervous system throughout but appeared in the hemolymph from the 4th day only (Josthna and Murali Mohan, 2002). The reason for its release into the hemolymph during the second half of the instar, four days before the spinning, is not clear, although one may speculate feedback actions on the nervous system by chemicals (neurohormones, neurohumors etc.) released by itself. Instances for such actions are available in literature on other invertebrates (Rao and Gopalakrishna Reddy, 1967; Vijayalakshmi *et al*., 1977; Rajarami Reddy *et al*., 1978). Further studies are required to consolidate the above assumptions.

Conclusions

From the present study, it can be concluded that the spontaneous electrical activity of the nervous system of the silkworm *Bombyx mori* shows variations in its level in different segments of the ventral nerve cord without any specific gradient. The activity shows progressive increase as the metamorphosis progress from the 1st to the 7th instar, in tune with the increase in the complexity of the nervous system. Different biogenic amines examined for their effects on the spontaneous activity cause elevation in activity in a dose-dependent fashion. The possibility remain that one or more of the biogenic amines tested in the present study could act as transmitter substances in the silkworm nervous system, and further studies are needed to consolidate this assumption.

References

Bullock, T.H. and Horridge, G.A. (1965). Structure and function in the nervous system of invertebrates. W.H. Freemann and Company, San Francisco and London.

Cooper, R.J., Bloom, F. E. and Roth, H.R. (1982). The biochemical basis of neuropharmacology (Fourth edition). Oxford University Press, New York, Oxford.

Elofsson, R., Laxmyr, L., Rosengren, E. and Hanson, C. (1982). Identification and quantitative measurements of biogenic amines and GABA in the central nervous system and hemolymph of the crayfish, *Pacifasticus leniusculus* (Crustacea). *Comp. Biochem. Physiol*.71C: 185-201.

Florey, E. (1954). Uber die Wirkung von Acetylcholine, Adrenalin, Noradrenalin, Faktor I und anderen Substanzen auf den isolierten Enddarm des Flusskrebses *Cambarus Clarkii Girard. Z. Vergl. Physiol*. 36: 1-8.

Florey, E. (1967). Neurotransmitters and modulators in the animal kingdom. *Fed. Proc.* 26: 1164-1178.

Goyffon, M. and Niaussat, P. (1975). Interrelationships between cholinergic and monoaminergic mechanisms in the determinism of spontaneous electrical activity in the central nervous system of scorpion. *Annales d'endocrinologie (Paris)*, 36: 101-2.

Goyffon, M. (1978). Amines biogenes et activitè électrique spontanee du systemè nerveuse prosomien du scorpion. *Comp. Biochem. Physiol.* 59C: 65-74.

Goyffon, M., Drouct, J., and Francaz, J.M. (1980). Neurotransmitter amino acids and spontaneous electrical activity of the prosomian nervous system of the scorpion (*Androctonus australis).Comp. Biochem. Physiol.* 66C: 59-64.

Jacob Doss, P. (1933). *Long hair sensilla in the scorpion, Heterometrus fulvipes: Central projections, physiology and behaviour.* Ph.D. thesis, S.V. University, Tirupati, Andhra Pradesh, India.

Janet, L.C. and Ritzmann, R.E. (1992). Biogenic amines modulate synaptic transmission between identified giant interneurons and thoracic interneurons in the escape system of the cockroach. *J. Neurobiol.* 23: 644-655.

Josthna, P. and Murali Mohan, P. (2002). Monoamine levels in the central nervous system and haemolymph during the 5^{th} instar of the silkworm, *Bombyx mori* L. J. Adv. Zool. 23: 17-22.

Kanungo, M.S. (1957). Cardiac physiology of the scorpion *Palamnaeus bengalensis* C. Koch. Biol. Bull. Woods Hole 113:135-140.

Krishnaswami, S. (1978). New Technology of silkworm rearing. Bulletin No. 2, Central Sericultural Research and Training Institute, Mysore, India.

Madhusudhana, L. (1995). *Activity rhythms in the scorpion, Heterometrus fulvipes with special reference to extra cholinergic systems.* Ph.D. thesis, S.V. University, Tirupati, India.

Miyazaki and Shun-Ichi (1980). Ionic mechanism of action potentials in neurosecretory cells and non-neurosecretory cells of the silkworm *B. mori. J. Comp. Physiol. A Sens Neural Behav. Physiol.* 140: 43-52.

Ozbas, S. and Hodgson, E.S. (1958). Action of insect neurosecretion upon central nervous system *in vitro* and upon behaviour. *Proc. Natl. Acad. Sci. Wash.* 44: 825-830.

Rajarami Reddy, G., Pavan Kumar, T., Murali Mohan, P. and Sasira Babu, K. (1978). Diurnal variations in physiological activities in the garden snail *Cryptozona ligulata. J. Comp. Physiol.* 125: 59-66.

Rao, K.P. and Gopalakrishna Reddy. (1967). Blood-borne factors in circadian rhythms of activity. *Nature* Lond. 213: 1047-1048.

Schantz, R. and Goyffon, M. (1970). Neurotransmitter amino acids and spontaneous electrical activity of the prosomian nervous system of the scorpion. *Comptes rendus des séances de la societe de biologie* (Paris), 164:1225-1227.

Siva Prasad, S. (1987). *Neurobiological studies on the silkworm Bombyx mori* L. Ph.D. Thesis, S.V. University, Tirupati, India.

Siva Prasad, S. and Murali Mohan, P. (1998). Morphological changes in the nervous system during metamorphosis of the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) *Indian J. Seric.* 37: 21-28.

Tyshtchenko, V.P. and Mandelstam, J.E. (1965). A study of spontaneous electrical activity and localization of cholinesterase in the nerve ganglia of *Antheria pernyi* Guer at different stages of metamorphosis and in pupal diapause. *J. Insect Physiol.* 11: 1233-1239.

Vanderkloot, W.G. (1955). The control of neurosecretion and diapause by physiological changes in the brain of the cecropia silkworm. Biol. Bull. 109: 276-294.

Vijayalakshmi, S., Murali Mohan, P. and Sasira Babu, K. (1977). Circadian rhythmicity in the nervous system of the cockroach, *Periplaneta Americana. J. Insect Physiol.* 23: 195-202.

Voskresenskaya, A.K. (1950). On "sympathetic" innervation of skeletal muscles in insects. *Sechenov J. Physiol.* (Fiziol. Zh. SSSR) 36: 176-183 (in Russian).

Weiant, E.A. (1958). Control of spontaneous efferent activity in certain nerve fibers from metathoracic ganglion of the cockroach. *Proc.* 10th *Int. Congr. Ent.* 2: 81-82.

Table 1: Analysis of spontaneous electrical activity (spikes/sec) in different segments of the ventral nerve cord (VNC) of *Bombyx mori* on the 1st day of the 5th instar

The activity is the mean ± standard deviation (SD) of six separate experiments.

Segment of VNC	Number of spikes	Number of spike categories
SG - 1TG	212 ± 12	7
1TG - 2TG	70 ± 7	3
2TG - 3TG	88 ±16	3
3TG - 1AG	125 ±17	4
1AG - 2AG	164 ± 10	6
2AG - 3AG	154 ± 8	6
6AG - 7/8AG	155 ± 12	4

Table 2: Analysis of spontaneous electrical activity (spikes/sec) in different segments of the ventral nerve cord (VNC) of *Bombyx mori* on the 7th day of the 5th instar

The activity is the mean ± standard deviation (SD) of six separate experiments. Since the activity level was the same in all abdominal segments, the data for 1AG-2AG alone is presented in the table.

Segment of VNC	Number of spikes	Number of spike categories
SG - 1TG	275± 18	9
1TG - 2TG	103± 14	4
2TG - 3TG	157± 13	7
3TG - 1AG	162± 8	5
1AG - 2AG	241± 13	6

Table 3: Analysis of the effects of epinephrine (EP) at different concentrations ($1x10^{-8}$ M to $1x10^{-4}$ M) on the spontaneous electrical activity (spikes/sec) of the ventral nerve cord (VNC) in 7th day larva of the 5th instar of *Bombyx mori*. The activity is the mean ± standard deviation (SD) of six separate experiments. The signs + or - indicate % changes from the control.

Nature of treatment	Number of spikes	Number of spike categories
Ringer (Control)	213±14	5
$1x10^{-8}$M	243±15 + 14.18*	6
$1x10^{-7}$M	285± 26 + 33.89***	7
$1x10^{-6}$M	298±19 - 40.09***	8
$1x10^{-5}$M	340±18 + 60.00***	8
$1x10^{-4}$M	216±20 + 1.31 NS	5

*** = P < 0.001; * = P < 0.05; NS: Not significant.

Table 4: Analysis of the effects of nor-epinephrine (NEP) at different concentrations ($1x10^{-8}$ M to $1x10^{-4}$ M) on the spontaneous electrical activity (spikes/sec) of the ventral nerve cord (VNC) in 7th day larva of the 5th instar of *Bombyx mori*. The activity is the mean ± standard deviation (SD) of six separate experiments. The signs + or - indicate % changes from the control.

Nature of treatment	Number of spikes	Number of spike categories
Ringer (Control)	210 ± 13	5
$1x10^{-8}$M	443 ± 18 + 110.86***	11
$1x10^{-7}$M	292 ± 16 + 39.24***	6
$1x10^{-6}$M	275 ± 17 + 30.86***	6
$1x10^{-5}$M	223 ± 13 + 6.38NS	4
$1x10^{-4}$M	198 ± 14 + 5.62NS	6

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

Table 5: Analysis of the effects of dopamine (DA) at different concentrations ($1x10^{-8}$ M to $1x10^{-4}$ M) on the spontaneous electrical activity (spikes/sec) of the ventral nerve cord (VNC) in 7th day larva of the 5th instar of *Bombyx mori*

The activity is the mean ± standard deviation (SD) of six separate experiments. The signs + or - indicate % changes from the control.

Nature of treatment	Number of spikes	Number of spike categories
Ringer(Control)	217±21	5
$1x10^{-8}$M	248±12	6
	+ 14.47*	
$1x10^{-7}$M	265±14	8
	+ 22.21***	
$1x10^{-6}$M	314±8	9
	+ 44.88***	
$1x10^{-5}$M	259±15	5
	+ 19.45***	
$1x10^{-4}$M	204±14	5
	- 5.81NS	

*** = P < 0.001; * = P < 0.05; NS: Not significant.

Table 6: Analysis of the effects of 5-hydroxytryptamine (5-HT) at different concentrations ($1x10^{-8}$ M to $1x10^{-4}$ M) on the spontaneous electrical activity (spikes/sec) of the ventral nerve cord (VNC) in 7th day larva of the 5th instar of *Bombyx mori*

The activity is the mean ± standard deviation (SD) of six separate experiments. The signs + or - indicate % changes from the control.

Nature of treatment	Number of spikes	Number of spike categories
Ringer (Control)	214±16	5
$1x10^{-8}$M	324±13 + 51.45***	6
$1x10^{-7}$M	289± 20 + 34.83***	6
$1x10^{-6}$M	277± 12 + 29.13***	4
$1x10^{-5}$M	266± 10 + 24.37***	4
$1x10^{-4}$M	233± 10 + 8.96*	4

* = $P < 0.05$; *** = $P < 0.001$.