

Morphometric Changes of the Central Nervous System of Oligomelic *Tegenaria atrica* Spiders

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Oligomely is an anomaly manifested in the morphology of spiders (except for deformations of the prosoma and exoskeleton), by the absence of one or more appendages, and in their anatomy by the absence of neuromeres. This study was aimed at determining whether there is a correlation between the absence of a neuromere or its half in the subesophageal ganglia and the volume of the prosoma. Morphometric studies involved oligomelic specimens of *Tegenaria atrica* with the absence of one walking leg and two walking legs. Volumetric analysis concerned with nymph stage II of spiders obtained after exposing the embryos to alternating temperatures of 14 and 32°C. The results were compared with those obtained from the histological analysis of the prosoma and central nervous system of control individuals. It was found that there was no relationship between the absence of half or an entire neuromere and the volume of the prosoma of oligomelic specimens. The volume of the central nervous system decreased but the volume change was not proportional to the changes in the prosoma volume. During studies, it was found that the lack of neuromeres resulted in an increase in the volume of remaining neuromeres.

Key words: Alternating temperatures, anomaly, oligomely, Arachnida, morphometric studies, CNS.

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The central nervous system of spiders, contained entirely in the prosoma, is characterized by a high concentration of ganglia. The metameric structure of this system can only be seen in embryos and in adult Mesothelae spiders (FOELIX 1996). All originally paired ganglia are fused in two ganglia: a smaller, supraesophageal called the brain, and a much larger, subesophageal with a distinct star-like shape. The boundary between these two is marked by the esophagus. The oval brain is situated in the anterior part of the prosoma, over the fused subesophageal ganglia. At the front of the brain there is a deep long furrow dividing it in two (BABU 1965, 1969, 1975; BABU & BARTH 1984; PUNZO 2007). The brain is the superior structure, containing the most important optical and associative centers (SATIJA *et al.* 1970a, 1970b, 1980; BABU & BARTH 1984, WELTZIEN & BARTH 1991; STRAUSFELD & BARTH 1993; STRAUSFELD *et al.* 1993; BARTH 2002; HILL 2006; LOESEL *et al.* 2011).

The subesophageal ganglia form by the fusion of pairs of ventral ganglia. Its anterior, larger, star-like part is composed of neuromeres of walking

legs, arranged in a specific order. The posterior part is much smaller, narrower and contains opisthosomal ganglia. From the fused opisthosomal ganglia run nerve fibers that converge towards the back forming a thick cauda equina, which passes through the petiolus to opisthosoma (BABU 1965; WEGERHOFF & BREIDBACH 1995; PARK & MOON 2013; PARK *et al.* 2013).

In terms of histological structure, the central nervous system is divided into a marginal outer layer forming a cortex and a central, dense and thick mass of nerve fibers forming a neuropil (BABU 1969). During postembryonic development, the volume of the neuropil increases significantly in contrast to the cortex, where the number of neurons remains the same. As a result an increase in the volume of the central nervous system is caused mainly by an increase in the volume of the neuropil. Neurons were classified and described in detail (BABU 1975; BABU & BARTH 1984).

Currently, the spatial position of the nervous system of spiders as well as its anatomical and histological structure seem to be well understood.

However, an interesting topic of changes in the nervous system observed during embryonic development still needs more recognition. The results of numerous teratological experiments indicate that it is possible to obtain a range of bodily malformations in spiders, which are reflected in their internal structure (JACUŃSKI *et al.* 2005; NAPIÓRKOWSKA *et al.* 2010; NAPIÓRKOWSKA *et al.* 2013). Temperature is one of the teratological factors which may cause defects in the prosoma and opisthosoma of *Tegenaria atrica* embryos. JACUŃSKI (1969, 1971) analyzed the impact of higher than optimum temperature (supra-optimal) (32°C), as well as the impact of alternating temperatures (JACUŃSKI 1984). The exposure of the embryos to temperature changed at regular intervals (lower and higher than optimum) led to a higher number of individuals with anomalies and more diverse and complex changes in the prosoma than when a single thermal shock was applied.

The morphology of spiders with different deformities has been analyzed in detail (JACUŃSKI *et al.* 2002a; JACUŃSKI *et al.* 2004; TEMPLIN *et al.* 2009; NAPIÓRKOWSKA & TEMPLIN 2013). In addition, the anatomy of the spiders (particularly their nervous and digestive systems) was evaluated for structural anomalies (JACUŃSKI 1983; JACUŃSKI *et al.* 2002b; NAPIÓRKOWSKA *et al.* 2010; NAPIÓRKOWSKA *et al.* 2013). The incidence of oligomelic specimens in the group exposed to the teratological factor is quite high (JACUŃSKI 1984; JACUŃSKI *et al.* 2005). Oligomely manifests itself as a unilateral or bilateral reduction in the number of legs and affects both pedipalps and walking legs. As for walking legs, usually one leg is missing although when the anomaly is more serious, two, three or more walking legs may be missing. In such cases significant changes in the shape and size of the prosoma are observed. Since oligomely is associated with metamerism and thus with neuromery, the lack of appendages should lead to a reduced total volume of the brain and/or subesophageal ganglia proportionally to changes in the volume of the prosoma. To verify the hypothesis, morphometric studies of individuals without a single walking leg (oligomely 4/3) and two walking legs (oligomely 3/3) have been undertaken. The aim of the study was to examine the relationship between the absence of a neuromere (or its half) in the subesophageal ganglia and volume of the prosoma.

Material and Methods

The study involved specimens of *Tegenaria atrica* C. L. Koch (1843) (Agelenidae). In our experiment, sexually mature males and females collected in August and September (2008-2014) near

the towns of Chełmża (18°36'E; 53°12'N) and Toruń (18°37'E; 53°02'N) (Poland) were maintained at constant temperature of 21°C and relative humidity of 70%. Each spider, kept in a separate glass container with a capacity of 500 ml, was provided with a constant supply of oxygen and a suitable amount of water, and fed twice a week larvae of *Tenebrio molitor* L., 1758. A sexually mature male was introduced twice into the container with a female for fertilization.

Shortly after the eggs had been laid, we removed the embryos from the cocoons, counted them and divided them into two groups: the control group, maintained in conditions optimal for the embryonic development of this spider species, and the experimental group exposed to temperatures of 14 and 32°C (both significantly deviating from the optimum) applied alternately every 12 hours. The procedure continued for ten days, until the first metameres of the prosoma appeared on the germ band. Subsequently, all experimental specimens, similarly to the control ones, were incubated under optimal conditions. Hatching took place approximately 20 days after the eggs were laid.

From the material exposed to a teratogenic factor we selected larval specimens with oligomely of walking legs. For morphometric studies we chose 20 larvae with the absence of one walking leg on the left side of the prosoma (unilateral oligomely 4/3), 20 larvae with the absence of two walking legs (bilateral oligomely 3/3), and 20 larvae from the control group. The selected specimens were cultured in separate Petri dishes, provided with water and food (freshly caught spider eggs and *Tribolium* sp. larvae) until they reached the nymph stage II.

Subsequently, the spiders were fixed in Bouin's fluid prepared according to ZAWISTOWSKI (1970). Following the procedures we prepared histological sections (thickness of 7 µm) using the paraffin method, and stained them with Mayer's hematoxylin and eosin. Each section was photographed with a digital camera and the images were further analyzed using ImageJ (freeware by W.S. Rasband, U.S National Institutes of Health, Bethesda, Maryland, USA, <http://rsb.info.nih.gov/i>).

We measured surface areas (mm²) of each section of the prosoma and nervous system, separately for the brain and subesophageal ganglia and their components, i.e. cortex and neuropil. Then we calculated the volumes (mm³), multiplying the surface areas by the thickness of the sections. By summing up the results obtained for the control and oligomelic specimens we determined the average volume of the prosoma, the entire nervous system, the brain and the subesophageal ganglia. The average volumes of the cortex and neuropil were also calculated. On the basis of the results we

determined the average share of the volume of the nervous system in the volume of the prosoma, the average share of the volume of the brain and the subesophageal part in the nervous mass, and finally the average share of the volume of the cortex and neuropil in the volume of the subesophageal part for the control specimens as well as oligomelic specimens 4/3 and 3/3. To assess the statistical significance of the results we used ANOVA and post-hoc Tukey tests.

Results

Our teratological experiments carried out during six breeding seasons involved a total of approx. 10 thousand embryos, half of which constituted a control group. In the control group no developmental abnormalities were observed (Fig. 1 A) and embryonic mortality was about 4%. From the teratological material we selected 674 individuals with various deformities of appendages (Table 1). The largest group consisted of individuals with oligomely, i.e. unilateral or bilateral absence of appendages (453 specimens), primarily of walking legs (287 specimens) (Table 2), with the majority lacking one leg (141 specimens) – (unilateral oligomely 3/4 and 4/3) (Fig. 1 B) or two legs (bilateral oligomely type 3/3) (63 specimens) (Fig. 1 C). The mortality of embryos treated with alternating temperature was high and amounted to about 20%. No abnormalities in the position and number of the

Table 1

Observed cases of developmental anomalies in the prosoma of *Tegenaria atrica*

Kind of anomaly	Number of individuals
Oligomely	453
Heterosymely	64
Schistomely	61
Reduction in length	45
Polymely	23
Symely	15
Complex anomalies	13
Total	674

Table 2

Frequency of various kinds of oligomely in the prosoma of *Tegenaria atrica*

Kind of oligomely	Number of individuals
Walking legs	287
Feeding legs	123
Walking and feeding legs	43
Total	453

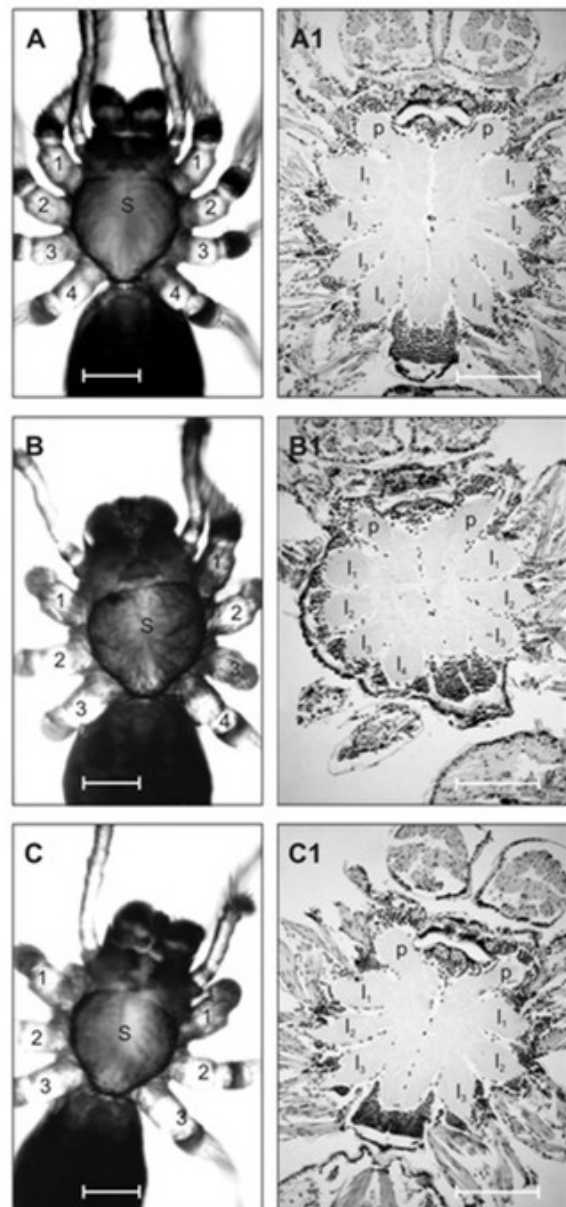


Fig. 1. A-C – The prosoma of *Tegenaria atrica* individuals from control (A), oligomelic 4/3 (B) and 3/3 (C) groups (ventral view): 1-4 walking legs, s – sternum, scale bar: 0.26 mm. A1-C1 – the horizontal sections through the prosoma with fused subesophageal ganglia: p – ganglia of pedipalps, l₁-l₄ – ganglia of walking legs from control (A1), oligomelic 4/3 (B1) and 3/3 (C1) individuals. Scale bar = 0.22 mm.

ganglia forming the subesophageal part of the nervous system were observed in the histological sections of specimens from the control group (Fig. 1A1). Major changes in neuromery were found in specimens affected by oligomely. The absence of one walking leg (oligomely 4/3) was associated with the absence of half of a neuromere in the fused subesophageal ganglia. The symmetry of this part of the nervous system was slightly impaired: it was arched to the opposite side, which contained all neuromeres corresponding to the number of exist-

ing legs (Fig. 1B1). Specimens affected by bilateral oligomely (3/3) lacked an entire neuromere of walking legs in the subesophageal part of the nervous system. Its absence did not disturb the symmetry of this part, but it was considerably shorter than that of the control specimens (Fig. 1C1). It should be noted that the absence of half or an entire neuromere did not disrupt the continuity of the nervous system: all ganglia were closely interconnected.

In order to assess the statistical significance of differences among the results, ANOVA and a post-hoc test were used. The volume of prosoma significantly differentiated the investigated groups ($F_{2,27} = 10.41$, $P = 0.0004$) (Table 3). The highest average volume of the prosoma was observed in the control group, lower was recorded in oligomelic individuals 4/3, and the lowest one in the case of oligomely 3/3. The tests revealed statistically significant differences between the control group and oligomelic individuals 3/3. However, the tests did not show statistically significant differences between individuals with the absence of half of a metamere and individuals lacking an entire neuromere (post-hoc). The average volume of the entire nervous system also differentiated the investigated groups ($F_{2,27} = 9.51$, $P = 0.0007$). The volume of the nervous system was greater in con-

trol spiders than in both oligomelic groups, which did not differ among themselves (post-hoc). The average share of the nervous system in the volume of the prosoma also differed among the compared groups ($F_{2,27} = 12.76$, $P = 0.0001$), however the distribution of differences was shaped up otherwise. All groups were significantly different from each other, with the largest average share of nervous system in the volume of the prosoma observed in oligomelic individuals 3/3 and the lowest in the case of oligomely 4/3.

These results show that when two walking legs were missing, a significant decrease of the prosoma volume occurred. Despite lacking a neuromere, the decrease of the CNS volume was much smaller than the decrease of the prosoma volume. So, the average volume of the brain and subesophageal ganglia in the tested groups was compared. Control specimens had the largest brains, while oligomelic specimens 4/3 had the smallest, but these differences were not statistically significant ($F_{2,27} = 20.88$, $P = 0.2434$). Whereas statistically significant results were obtained in the average volume of the subesophageal ganglia ($F_{2,27} = 13.59$, $P = 0.0001$). The volume of that part of the nervous system was the highest in the control group and considerably decreased in oligome-

Table 3

The average volumes of the prosoma and the central nervous system and its components (the brain, subesophageal ganglia: cortex and neuropil) (mm^3), and the average share of the volume of the nervous system in the volume of the prosoma, the volume of the brain in the volume of the nervous system, the volume of the subesophageal ganglia in the volume of the nervous system, the volume of cortex and neuropil in the volume of the fused subesophageal ganglia (%) (\pm - SD)

	Control specimens	Oligomelic specimens 4/3	Oligomelic specimens 3/3
Average volume of the prosoma (mm^3)	0.205 \pm 0.036	0.170 \pm 0.021	0.145 \pm 0.011
Average volume of the nervous system (mm^3)	0.081 \pm 0.015	0.062 \pm 0.004	0.062 \pm 0.004
Average share of the volume of the nervous system in the volume of the prosoma (%)	39.51 \pm 2.84	36.47 \pm 3.76	42.75 \pm 2.84
Average volume of the brain (mm^3)	0.028 \pm 0.003	0.024 \pm 0.003	0.025 \pm 0.002
Average share of the volume of the brain in the volume of the nervous system (%)	34.57 \pm 2.75	38.71 \pm 2.77	40.32 \pm 2.30
Average volume of the subesophageal ganglia (mm^3)	0.053 \pm 0.011	0.038 \pm 0.002	0.037 \pm 0.003
Average share of the volume of the subesophageal ganglia in the volume of the nervous system (%)	65.43 \pm 2.75	61.29 \pm 2.77	59.68 \pm 2.30
Average volume of the cortex in the subesophageal ganglia (mm^3)	0.030 \pm 0.008	0.021 \pm 0.003	0.019 \pm 0.003
Average share of the volume of the cortex in the volume of the subesophageal ganglia (%)	56.60 \pm 5.24	55.26 \pm 6.42	51.35 \pm 5.14
Average volume of the neuropil in the subesophageal ganglia (mm^3)	0.023 \pm 0.005	0.017 \pm 0.002	0.018 \pm 0.002
Average share of the volume of the neuropil in the volume of the subesophageal ganglia (%)	43.40 \pm 5.24	44.74 \pm 6.42	48.65 \pm 5.14

lic specimens 4/3 and 3/3, but the difference between the two oligomelic groups was minor and was not statistically significant (post-hoc).

The cortex volume in the subesophageal ganglia also differentiated the studied groups ($F_{2,27} = 15.81$, $P < 0.0001$). In the control group, the average cortex volume in the subesophageal ganglia was significantly higher than in both experimental groups (post-hoc). The volume of the neuropil in the subesophageal ganglia was also calculated. It was different between particular experimental groups ($F_{2,27} = 4.20$, $P = 0.0258$). The volume of the neuropil was the highest in the control group and differed significantly from oligomelic individuals 3/3 (post-hoc). Despite the small and insignificant difference in neuropil volume between oligomelic individuals (post-hoc), the volume of the neuropil in the group without the entire neuromere was higher than in the group without half of a neuromere.

The average share of the cortex and neuropil at the volume of subesophageal ganglia was also calculated. Both of these parameters differentiated the tested groups ($F_{2,27} = 3.95$, $P = 0.0313$). The neuropil share at the volume of the subesophageal ganglia was the highest in oligomely 3/3, while the lowest in the control group.

Discussion

Morphological abnormalities are common in crustaceans (DE OLIVEIRA DIAS 1999; FOLLESA *et al.* 2008; FEULLASSIER *et al.* 2012) and in many other animal taxa (ĆURČIĆ *et al.* 1991; REINERT 1999; MITIĆ & MAKAROV 2007; FERREIRA 2008, 2011; MITIĆ *et al.* 2011; KOZEL & NOVAK 2013). They include deformities of the head, abdominal epimera, pleopods, telson and uropods in crustaceans (FERNANDEZ *et al.* 2011) and mispaired tergites, shrunk segments, variously deformed sclerites, bifurcated trunk and defects of spiracles in centripedes (LEŚNIEWSKA *et al.* 2009). In insects the majority of anomalies affect antennae, mandibles, legs and exoskeleton (ASIAIN & MÁRQUEZ 2009). Many biological and non-biological factors can be considered as possible causes of anomalies in morphology including mutations of the germ or somatic cells, as well as mechanical, physical, and chemical factors which can disturb the embryonic development of the arthropods (MILIČIĆ *et al.* 2013).

Temperature is a teratogenic factor which negatively affects the embryonic development of spiders and significantly alters their morphology. Many years of research into the effect of temperature on embryos of *Tegenaria* indicate that stable temperature is essential for optimal growth, and

any deviation from the norm, such as exposing embryos to alternating temperatures, result in developmental monstrosities (JACUŃSKI 1984).

Oligomely, one of the most common developmental anomalies, is extremely easy to notice. Changes in oligomelic individuals can already be identified during embryonic development, when metameres form on the germ band: the entire metameres or their halves are missing (JACUŃSKI *et al.* 2005). The anomaly is associated with metamerism and neuromery of the body, and all morphological modifications result in serious changes in the internal structure, especially the structure of the nervous system. Because oligomely of walking legs was the most frequently observed, the experiment was based on specimens affected by oligomely of walking legs types 4/3 and 3/3 in which we expected a proportional loss of nervous mass in the subesophageal ganglia compared to the volume of the prosoma.

The morphology of such individuals has been thoroughly described by TEMPLIN and NAPIÓRKOWSKA (2013). Based on biometric studies, the authors determined the changes in the external skeleton (carapace length and sternum area) in subsequent stages of postembryonic development. These studies focused on anatomical changes related to the subesophageal ganglia.

In the control specimens the subesophageal mass was wide, symmetrical and contained all the ganglia of walking legs. The structure of this part of the nervous system was typical of teratologically unchanged spiders. In contrast, the absence of legs caused significant changes in the structure of the subesophageal ganglia, corresponding to the number of missing legs. In individuals with unilateral oligomely 4/3 half a neuromere was missing, which was related to the asymmetry of the subesophageal ganglia. In individuals with regular, bilateral oligomely 3/3, more severe problems were diagnosed including the symmetrical absence of two ganglia - one on the right and one on the left side of the prosoma. Moreover, the subesophageal ganglia were shorter.

In our experiment the control specimens had the largest volumes of the prosoma and the central nervous system. The absence of one or two legs resulted in a proportional reduction in the volume of the prosoma and the nervous system. The absence of one leg reduced the volume of the prosoma by approx. 17% compared to the control specimens, while the absence of two legs reduced the volume of the prosoma by approx. 30%. The results concerning the average volume of the nervous system were different. It was visibly reduced, and the difference between the control and oligomelic specimens 4/3 was approx. 24%. The same percentage

difference was observed between the control and oligomelic specimens 3/3.

The results indicated that the volume of the nervous system decreased dramatically when half a metamere and thus half a neuromere were missing but there was no difference in volume of the nervous system between oligomelic individuals 4/3 and 3/3 when a half neuromere and the whole neuromere were absent.

To verify the presented hypothesis, we measured the volume of the subesophageal ganglia, which was the aim of the experiment, and also the volume of the brain. The results indicated that oligomelic individuals 3/3 had a smaller brain than the control group, but bigger than oligomelic individuals 4/3.

It can be assumed that a slight increase of brain volume in oligomelic individuals 3/3 compared to individuals 4/3 was the result of the specific development of brain neuropil, where new synaptic connections emerged. Such a development of neuropil probably aims to integrate all the life processes, which have been impaired by the loss of as many as two walking legs. Therefore, the average share of the brain volume in the volume of the nervous system in oligomely 3/3 was higher than in oligomely 3/4, and was even higher than in the control.

However, the differences in the volume of the subesophageal ganglia constitute the most important parameter indicating changes in the nervous system in individuals with oligomely of the walking legs. As was assumed before the experiment, considerable differences should be detected in this part of the nervous system. Statistically significant differences were recorded between the control specimens characterized by the largest average volume of the subesophageal ganglia and the oligomelic individuals 4/3 and 3/3 characterized by a considerably smaller volume of these ganglia. No differences between the two groups of oligomelic individuals were observed (post-hoc). Individuals without a half of a neuromere had only slightly larger subesophageal ganglia than individuals without the entire neuromere. The volume of the cortex decreased (control specimens → oligomelic specimens 4/3 → oligomelic specimens 3/3), whereas the volume of the neuropil increased. The highest volume of the neuropil was observed in the specimens from the control group, and the lowest in the oligomelic specimens 4/3. In the individuals lacking two legs, and hence the entire neuromere, the neuropil had a higher average volume than in the individuals lacking only one leg and half a neuromere. The decrease of the cortex volume can be explained by the fact that the lack of legs and neuromere caused a lack of sensory receptors innervated by neurons, and this implies a lack of extensions: neurites and dendrites. Because a slight increase in volume of the neuropil in the case of ab-

sence of two legs was observed, it had to be a particular increase in the neuropil of existing leg ganglia.

In summary, the results of this study on oligomelic specimens indicate a number of major anomalies in the structure of the central nervous system, mainly in the subesophageal ganglia, where one or two ganglia were missing. The level of anatomical changes was adequate to the number of missing legs. Oligomelic individuals had smaller subesophageal ganglia in comparison with control individuals. However, no significant differences in the volume of the subesophageal ganglia were noted between individuals with one leg missing and individuals with two legs missing. The absence of two ganglia was compensated by the increased size of the remaining ones, or, more precisely, their neuropil.

This seems obvious because the neuropil mass is the most important element of the nervous system in invertebrates: it is the center of neuronal contacts where processes of functional integration occur. A significant loss of neurons causes a more intense growth of the neuropil: axons extend, their diameter increases, new dendrites appear, and new connections between neurons are created in order to perform the tasks of the central nervous system. Brain reduction is also important. This part of the nervous system undergoes modifications reflecting the changes in the subesophageal ganglia.

The results have demonstrated a lack of a close relationship between the absence of half or entire neuromere and the type of oligomely. The volume of the central nervous system decreases, but the change is not proportional to changes of the entire volume of the prosoma. It should be noted that the absence of the ganglion/ganglia in the subesophageal part does not result in the division of the nervous system. All the remaining ganglia are still interconnected, so the morphological and physiological continuity of the system is maintained.

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