

RESEARCH ARTICLE

The function of the ophiuroid nerve ring: how a decentralized nervous system controls coordinated locomotion

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ABSTRACT

Echinoderms lack a centralized nervous control system, yet each extant echinoderm class has evolved unique and effective strategies for locomotion. Brittle stars (Ophiuroidea) stride swiftly over the seafloor by coordinating motions of their five muscular arms. Their arms consist of many repeating segments, requiring them to use a complex control system to coordinate motions among segments and between arms. We conducted *in vivo* experiments with brittle stars to analyze the functional role of the nerve ring, which connects the nerves in each arm. These experiments were designed to determine how the ophiuroid nervous system performs complex decision making and locomotory actions under decentralized control. Our results show that brittle star arms must be connected by the nerve ring for coordinated locomotion, but information can travel bidirectionally around the nerve ring so that it circumvents the severance. Evidence presented indicates that ophiuroids rely on adjacent nerve ring connections for sustained periodic movements. The number of arms connected via the nerve ring is correlated positively with the likelihood that the animal will show coordinated locomotion, indicating that integrated nerve ring tissue is critical for control. The results of the experiments should provide a basis for the advancement of complex artificial decentralized systems.

KEY WORDS: Ophiuroidea, Decentralized control, Locomotion, Nervous system

INTRODUCTION

Most bilaterians have a central integrated nervous structure such as a brain to process sensory information, make decisions and coordinate movements (Arendt et al., 2008; Martín-Durán et al., 2018). Centralized control is highly conserved within the Bilateria. The evolution of decentralized nervous control (Cobb, 1987; Watanabe et al., 2012) in echinoderms from a centralized ancestral state (Denes et al., 2007; Arendt et al., 2008; but see Martín-Durán et al., 2018) represents one of the most radical body plan alterations in the history of complex animals. Locomotion is a complex hierarchical process involving the transmission and integration of sensory information and

the coordination of many moving parts. All crown-group echinoderm classes have evolved unique and varied strategies for coordinating locomotion, which is necessary for behaviors such as feeding, defense against predators, and reproduction. The advent of decentralized control in echinoderms may have been associated with the evolution of pentameral symmetry (Sly et al., 2002), as metamorphosis from the bilateral larval stage to a pentameral adult impacts the organization of the nervous system (Díaz-Balzac and García-Arrarás, 2018). The pentameral echinoderm crown group is thought to have evolved from bilateral ancestors, and the stem group includes several fossil taxa with bilateral and other non-pentameral forms of symmetry (Smith and Zamora, 2013); deciphering the structure of the nervous system can help to illuminate the transition from bilateral to pentameral symmetry. Both the steps underlying and the evolutionary drivers behind the development of decentralized control are poorly understood. We analyzed the control system underlying locomotion in brittle stars (phylum Echinodermata, class Ophiuroidea) as a first step towards understanding how it functions.

Brittle stars have five thin arms that radiate from a circular body (the 'disk') (Fig. 1). Both the disk and arms have an internal skeleton of high-magnesium calcite (Chave, 1954; Weber et al., 1969) made up of units called ossicles. Most brittle star arms are made of repeating five-ossicle segments, over 100 per arm in some species (LeClair, 1996). Four large muscles adhere to both the proximal and distal surfaces of each arm segment (Wilkie, 1978; Byrne, 1994; Clark et al., 2017, Fig. 1). For locomotion, ophiuroids rely primarily on a bilaterally symmetrical gait using coordinated oscillations of their muscular arms to push themselves across the substrate (Astley, 2012). This is in contrast to sea stars, their closest extant relatives (Reich et al., 2015), which rely primarily on their tube feet (Smith, 1947; Kerkut, 1953) and move much slower than ophiuroids.

Ophiuroids utilize locomotory gaits in which each arm takes on one of three roles: as center limb, forelimb or hindlimb (Fig. 2) (Astley, 2012). Each role is associated with a repetitive set of actions. Role assignments are based on the arm's position relative to the direction of motion (Arshavskii et al., 1976a,b; Astley, 2012). Two gaits predominate: 'rowing' and 'reverse rowing' (Astley, 2012). In rowing, the center limb points in the direction of motion and is flanked by forelimbs, which oscillate synchronously to push against the substrate and move the body forward (Astley, 2012). The hindlimbs are directed posteriorly relative to the direction of motion and typically rest or oscillate alternately. In reverse rowing, the center limb points away from the direction of motion, and the forelimbs are in front. The forelimb and hindlimb pairs oscillate synchronously, and the center limb trails behind (Astley, 2012). In order to change direction, the ophiuroid does not turn; rather, the roles of the arms are reassigned (Arshavskii et al., 1976a; Astley, 2012). We refer to the center limb during rowing and forelimbs during reverse rowing as 'leader arms', as they are anterior to the direction of motion. 'Astleyan locomotion' is defined here as a

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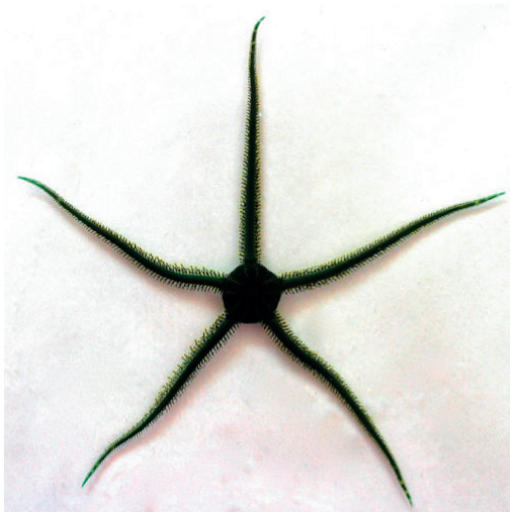


Fig. 1. The ophiuroid *Ophiarachna incrassata*.

locomotion strategy in which the position and roles performed by the arms are as in Astley (2012) and include one center limb during rowing or two forelimbs during reverse rowing (Fig. 2). Specimens do not require five arms to engage in Astleyan locomotion. Forms of locomotion observed here that were not defined explicitly by Astley (2012) are referred to as ‘non-Astleyan’.

Ophiuroids with partially or entirely removed arms continue to execute systematic and coordinated gaits (Arshavskii et al., 1976a; Matsuzaka et al., 2017; Kano et al., 2017a). Even ophiuroids with only one arm can locomote (Arshavskii et al., 1976a; Matsuzaka et al., 2017; Kano et al., 2017a). When all arms are removed, ophiuroids move using tube feet on the oral surface of the disk (Arshavskii et al., 1976a). This resilience is a testament to the versatility of the ophiuroid nervous control system.

The ophiuroid system for coordinating and synchronizing arm movements, recovering from damage, and performing omnidirectional locomotion has been analyzed for insights to improve robotic devices (Lal et al., 2008; Watanabe et al., 2012; Kano et al., 2012, 2017a). Robotic models also provide a tool for analyzing these unique aspects of ophiuroid locomotion (Watanabe et al., 2012; Kano et al., 2012, 2017a). For example, Lal et al. (2008) constructed an ophiuroid-based robot with modular arms to investigate the coordination mechanism of the arm segments. Mao et al. (2014) built a robot based on the asterozoan body plan to emulate their ability to traverse varying terrain and navigate obstacles.

Robots with decentralized systems have many advantages, such as the ability to adapt quickly to physical damage (Kano et al., 2017a). *In vivo* behavioral observations of locomotion in living ophiuroids with

fewer than five arms were performed and integrated into a mathematical model that served as the basis for the control setup of an ophiuroid-based robot that reproduced the behavior observed (Kano et al., 2017a). Mobile ophiuroid-based robots of this kind can execute gait changes for omnidirectional locomotion by reassigning the roles of the arms in response to external stimuli as in a living ophiuroid (Watanabe et al., 2012; Kano et al., 2012). This research using mathematical modeling and robotics provided important evidence of the nature of the decentralized control mechanisms underlying arm coordination during locomotion and direction change (Watanabe et al., 2012; Kano et al., 2012). However, many questions remain. It is still unclear, for example, which parts of the nervous system are responsible for critical aspects of ophiuroid locomotion, including processing sensory information, determining which locomotory actions are necessary and delegating specific tasks to the arm segments.

The ophiuroid nervous system is composed of two major parts: the five radial nerves and the circumoral nerve ring (Mashanov et al., 2016). The radial nerves run along the oral surface of each arm between two ossicles: the vertebra and the ventral plate (Wilkie, 1978). The circumoral nerve ring connects to each of the five radial nerves. Branches of the circumoral nerve ring innervate other parts of the disk (Cobb and Stubbs, 1981, 1982). The length of the axons in the circumoral nerve ring is equal to or less than a fifth of its total length, i.e. the longest axons do not extend further than the distance connecting adjacent radial nerves (Cobb and Stubbs, 1982). Anatomical studies have suggested that the circumoral nerve ring is not a centralized control center, but functions primarily to connect the radial nerves (Cobb and Stubbs, 1982; Cobb, 1987).

In order to execute coordinated locomotion in response to a stimulus, ophiuroids must process sensory information, make decisions regarding the optimal direction and speed of movement, and implement those decisions effectively. The way in which these hierarchical processes operate under decentralized control is poorly understood. The first step in determining the setup of the ophiuroid decentralized nervous system is to identify the function of each of its parts.

A few *in vivo* studies have used behavioral experiments to investigate how the nervous system controls locomotion. Arshavskii et al. (1976a) compared motions of amputated arms with and without the adjacent area of nerve ring tissue attached. The segments of the arm were able to execute coordinated movements, suggesting that the nerve ring is not responsible for intra-arm coordination. However, only arms with associated pieces of the nerve ring could engage in locomotion, suggesting that the nerve ring is necessary. This interpretation was supported by experiments that showed that the ophiuroid was unable to initiate locomotion when the nerve ring was anesthetized (Matsuzaka et al., 2017). Based on their experimental results, Arshavskii et al. (1976a) hypothesized that a ‘nerve center’ at the base of each arm was responsible for integrating information, making decisions and relaying those decisions to adjacent arms. They suggested that during rowing the ‘nerve center’ of the center arm controlled the other arms (or at least those adjacent to it). This would explain why arms could not execute coordinated locomotion without the associated part of the nerve ring. However, anatomical studies found no evidence for a ‘nerve center’ (Cobb and Stubbs, 1982; Cobb, 1987). This hypothesis does not explain how nervous function is distributed during reverse rowing, when the position of the center limb is posterior relative to the direction of movement. Nor does it explain how the arm could respond to a negative stimulus, when the center limb is opposite the point of stimulation.

Arshavskii et al. (1976a) conducted an experiment in which the nerve ring was severed between adjacent arms. The two arms

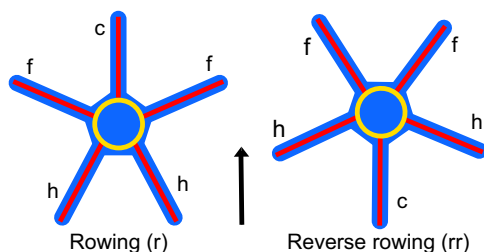


Fig. 2. Ophiuroid locomotion strategies as described by Astley (2012). The yellow circle represents the nerve ring; red lines represent the radial nerves. The black arrow indicates direction of motion. Abbreviations for arm role assignments: c, center limb; f, forelimb; h, hindlimb.

adjacent to the cut coordinated their motions poorly when one was the center arm during rowing. They interpreted this observation to mean that intact nerve ring connections between adjacent arms are necessary for proper information transmission and coordination between them. Arshavskii et al. (1976a) regarded this as supporting evidence for their hypothesis that a 'nerve center' associated with the center arm controls adjacent arms via the nerve ring. However, Arshavskii et al. (1976a) tested only the effect of a single cut, and provided little detail regarding their methods, sample size and the outcome of their experiments. A large number of questions remain regarding the control mechanism underlying ophiuroid locomotion and particularly the role of the nerve ring. This prompted us to conduct a series of experiments to elucidate the role of the nerve ring in locomotory feedback by addressing three key questions: (1) are nerve ring connections necessary for information transmission and arm coordination? (2) Can transmission of information travel bidirectionally to avoid a nerve ring cut and still allow coordinated locomotion with all arms? (3) Are adjacent nerve ring connections necessary for coordination between adjacent arms? Deconstructing the distribution of nervous function allowed us to pinpoint the function of the nerve ring in controlling brittle star locomotion.

MATERIALS AND METHODS

Experimental setup

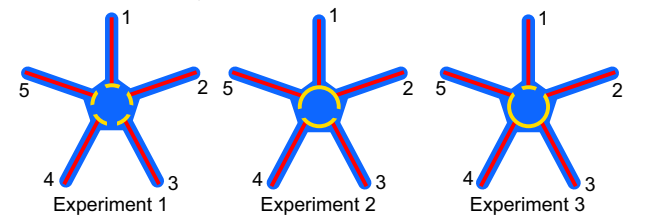
Specimens of the ophiuroid *Ophiarachna incrassata* (Lamarck, 1816) (Fig. 1) were obtained commercially (Aqua Shop Saien, Sapporo, Japan). Only specimens with arms of approximately equal length (i.e. not obviously regenerating) were used. The arms (untrimmed) ranged from 4.42 to 17.42 cm in length (mean 10.22 cm, s.d. 3.18 cm) and the disk diameter ranged from 1.29 to 4.23 cm (mean 3.21 cm, s.d. 1.13 cm) (see Table S1 for arm and disk lengths for each specimen). The ophiuroids were kept in a holding tank with artificial seawater (33 g NaCl l⁻¹) at 25–26°C. Behavioral observations were made in a 69.5×69.5 cm plexiglass tank filled with artificial seawater to approximately 7 cm deep. Stimulation was conducted using 2 mol KCl l⁻¹ mixed with black ink. Trials were filmed using a JVC GX-RX130 digital video recorder.

We recorded the behavior of each specimen before (the control trial) and after (the experimental trial) the nerve ring was cut. Seven nerve ring cut configurations were performed, and each experiment numbered separately (Fig. 3). Six controls and six experimental trials (the same specimens were used in the control and experimental trial) were conducted for each nerve ring cut configuration (i.e. six different specimens were used in each experiment). An additional specimen was used in experiment 4 (see Experimental trials). In three of the specimens in each experiment, the arms were trimmed to approximately 1/3 to 1/4 of the original length, between 1.72 and 4.74 cm (mean 2.80 cm, s.d. 0.73 cm) prior to the control trials; all the arms of an individual specimen were trimmed to approximately the same length (Table S1). All aspects of the research complied with federal and Yale University protocols for working with invertebrate animals.

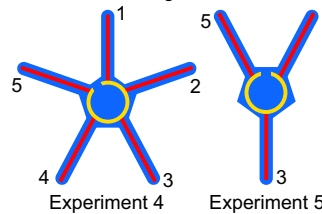
Control trials

Control trials were conducted with all specimens to observe their normal locomotory behavior before the nerve rings were cut. Specimens were placed at the center of the tank. They have a tendency to avoid open spaces, so they typically crawl towards the corners (as observed by Matsuzaka et al., 2017). Specimens were replaced at the center of the tank in a random orientation after approaching the tank's edge. Observations of the control (and experiment) lasted for 10 min or until the specimen ceased to

A Multiple nerve ring cuts



B One nerve ring cut, >2 arms



C One nerve ring cut, 2 arms

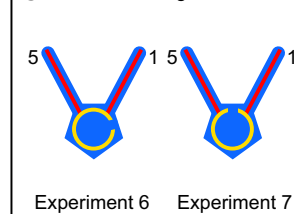


Fig. 3. Position of nerve ring cuts for experiments. (A) Experiments in which specimens have multiple nerve ring cuts. (B) Experiments in which specimens have more than two arms and one nerve ring cut. (C) Experiments in which specimens have two arms and one nerve ring cut. Arms are numbered as in the text. For more information on each experiment, please refer to the following figures: experiment 2, Fig. 5; experiment 3, Fig. 6; experiment 4, Fig. 7; experiment 5, Fig. 8; experiment 6, Fig. 9; experiment 7, Fig. 9. Colors are as in Fig. 2.

be active (see Table S2 for exceptions). Arms were periodically stimulated with the KCl/black ink solution to observe behavior upon stimulation, to initiate arm role reassignment for testing omnidirectional capabilities, or to re-energize resting specimens. Green or white circular paper markers 5 mm in diameter were affixed to the disk with superglue to allow individual arms to be identified.

Experimental trials

Immediately after the control trial, the same specimen underwent surgery to cut the nerve ring. The nerve ring was accessed by removing part of the skin on the dorsal surface of the disk and cutting through the proximal oral plate/distal oral plate (see Hendler, 1978) where the nerve ring tissue is located. We conducted seven different experiments representing seven different cut configurations (Fig. 3). Arms were stimulated with the KCl/black ink solution periodically to compare transmission of information, role reassignment, and reaction and arm coordination with the control. We assigned numbers to the arms relative to the position of the nerve ring cut (Fig. 3) to allow both the control and experiment to be analyzed.

Experiments 1–3 (multiple nerve ring cuts)

To determine whether nerve ring connections are necessary for information transmission and arm coordination (question 1), we initiated three experiments (experiments 1–3). These involved cutting the nerve ring in at least two places to investigate whether specimens with areas of the nerve ring completely separated were capable of full-body or partial-body coordination (Fig. 3A). In experiment 1, we cut the nerve ring connections between each arm (i.e. five separations). In experiment 2, we cut the nerve ring in two places, with each arm retaining at least one nerve ring connection with an adjacent arm. In experiment 3, we also cut the nerve ring in two places but on both sides of the same arm.

Experiments 4 and 5 (one nerve ring cut, >2 arms)

To determine whether information transmission could travel bidirectionally to avoid a nerve ring cut and still achieve

coordinated locomotion using all arms (question 2), we initiated two experiments (experiments 4 and 5). In experiment 4, the single cut separated two adjacent arms. In experiment 5, we removed two non-adjacent arms before running the control trial in order to understand how nerve ring connections affect the response to arm damage. Following the control trial, we cut the nerve ring between the adjacent arms (Fig. 3B).

Experiments 6 and 7 (one nerve ring cut, 2 arms)

To determine which nerve ring connections are necessary for coordination between two adjacent arms (question 3), we conducted two experiments in which all but two adjacent arms were removed before the control trial and the nerve ring was subsequently cut in one place (experiments 6 and 7). In experiment 6, we cut the nerve ring connection outside the remaining pair of arms (Fig. 3C). In experiment 7, we cut the nerve ring connection between the arms (Fig. 3C).

Six specimens from experiment 4 (with one nerve ring cut) were subsequently used in experiment 3 (with two nerve ring cuts). Fatigue did not affect the parameters we measured, as the number of specimens in which locomotion was observed after two nerve ring cuts was higher in experiment 3 than in experiment 2 despite their prior use in experiment 4. We did not directly compare any of the other results from experiment 3 with the other experiments due to their differential treatment. As one specimen with untrimmed arms did not survive experiment 4, we added a specimen, making 7 in total, so that all the specimens in experiment 3 would go through the same treatment.

Analysis

Locomotion was defined as more than one coordinated oscillation of two or more arms. In the analysis of video data, we logged the length of time each specimen spent in coordinated locomotion, noting all instances of rowing or reverse rowing and the roles executed by each arm. We recorded the identity of the arm to which every stimulation was applied, and the assignment of roles relative to the location of stimulation. As not every instance of stimulation resulted in coordinated locomotion, we developed a scale to record

the degree of reaction to the stimulation when no locomotion occurred: no reaction (NR); reaction (R; one arm reacted to the stimulus); uncoordinated reaction (UR; more than one arm reacted to the stimulus in an uncoordinated fashion); coordinated reaction (CR; at least a partial coordinated locomotory oscillation of two or more arms). Where coordinated locomotion did not occur in a given trial, we rated stimulations using this classification (Table S2).

Micro-CT scans

X-ray micro computed tomography (micro-CT) scans were used to check that the nerve ring was cut during the experiment. We randomly selected eight specimens for nervous tissue staining and scanning to ensure that the technique used to cut the nerve ring was successful. Staining methods were modified from Metscher (2009a, b). Brittle stars were anesthetized on ice, fixed with alcoholic Bouin's fixative and refrigerated overnight. After fixation, specimens were dehydrated with an ethanol series (70, 80, 90 and 100% for 30 min each). They were then stained using 1% iodine in dehydrated ethanol for 2 days. This staining procedure enhances the contrast of tissues for X-ray micro-CT scanning. After rinsing the specimens with dehydrated ethanol, the ethanol was removed from the specimens in a chamber with an evaporator (PX-52, Yamato Ltd, Japan). All chemicals were obtained from Kanto Chemical Co. (Tokyo, Japan). The specimens were micro-CT scanned either at Harvard University using a Nikon XTEK XT H225 scanner (Tokyo, Japan) with the X-ray source operated at 85 kV and 80 μ A, or at Hokkaido University using an inspeXio SMX-100CT scanner (Shimadzu Corporation, Kyoto, Japan) with the X-ray source operated at 75 kV and 40 μ A. The images were reconstructed with a voxel size of 30–50 μ m. CT image reconstruction and rendering were carried out using Nikon CTPro 3D and VG Studio MAX v. 3.0 (Volume Graphics, Heidelberg, Germany) (Fig. 4).

RESULTS

Details of each experiment are given in Table S2. The micro-CT scans confirmed that the nerve rings were completely severed in the desired location for each experiment, as revealed in the 2D image

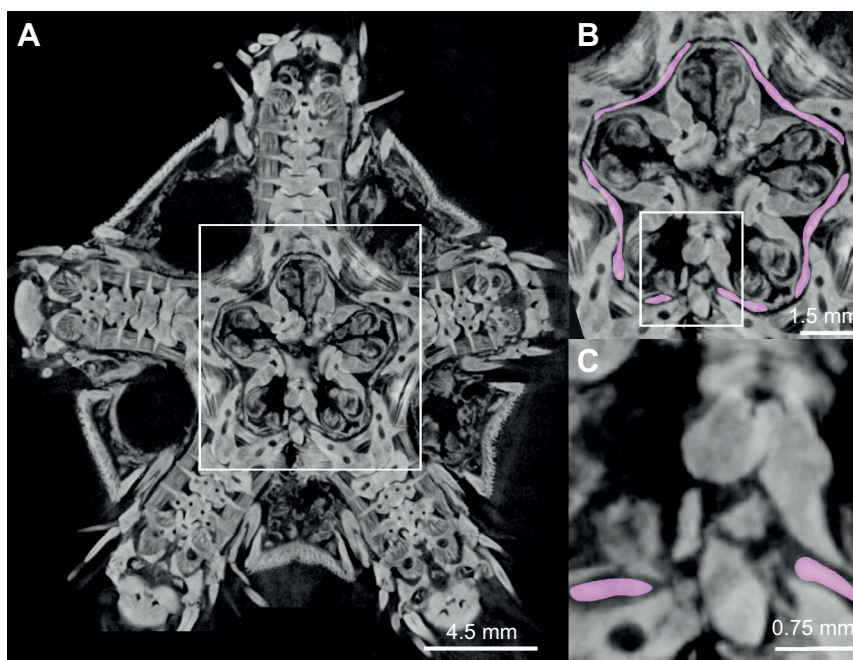


Fig. 4. 2D coronal section from a micro-CT scan of a specimen with one nerve ring cut to illustrate the location of the nerve ring cut. (A) View of the disk. (B) Close up of the nerve ring area indicated by the white box in A, with the nerve ring highlighted in purple. (C) Close up of the nerve ring cut area indicated by the white box in B.

stacks (Fig. 4). Results for each experiment are summarized in Table 1.

Experiments 1–3 (multiple nerve ring cuts)

Experiment 1

A representation of the nerve ring cuts in experiment 1 is shown in Fig. 3A. Specimens showed dramatic differences in locomotion capabilities between the control and experiment. All specimens in the control exhibited coordinated locomotion, whereas, during the experiment, neither coordinated locomotion nor reactions were observed. When a stimulation was applied to a single arm, the reaction was restricted to that arm in the vast majority of instances but movement of more than one arm was observed in every specimen at least once in the experiment (Table S2). Movement of non-stimulated arms may have been a reaction to sensing the movement of the stimulated arm or triggered by the diffused KCl, or it may be unrelated to the stimulus. Uncoordinated reactions did not often translate into an ability to escape. Only rarely did the specimen

move beyond the area of KCl, using sweeping arm motions or uncoordinated arm strokes, but such motions were not as effective as coordinated locomotion in the control. The results of experiment 1 show that nerve ring connections are required for effective transmission of information and coordination between arms.

Experiment 2

A representation of the nerve ring cuts in experiment 2 is shown in Fig. 5. No specimen exhibited coordinated locomotion using all five arms together, in contrast to every specimen during the control trials. There was no coordination between the sets of two and three limbs separated by cuts when only one set was stimulated. However, several specimens executed coordinated locomotion involving one set of connected arms. Three specimens exhibited coordinated locomotion using the three arms that remained connected by nerve ring tissue. Simultaneous coordinated locomotion of the other two connected arms, which were separated from the three, was observed in one specimen (Fig. 5C). This suggests that arms do not need to be

Table 1. Summary of experimental results

Experiment no.	No. nerve ring cuts	Max. no. arms consecutively connected by nerve ring tissue	Specimen no. (see Table S2)	Locomotion observed in control	Max. degree of coordination observed during experiment
1	5	1	13	Yes	Uncoordinated reaction
1	5	1	30	Yes	Uncoordinated reaction
1	5	1	32	Yes	Reaction/Uncoordinated reaction
1	5	1	33	Yes	Uncoordinated reaction
1	5	1	37	Yes	Uncoordinated reaction
1	5	1	38	Yes	Uncoordinated reaction
2	2	3	14	Yes	Coordinated reaction
2	2	3	17	Yes	Uncoordinated reaction/coordinated reaction
2	2	3	18	Yes	Locomotion
2	2	3	19	Yes	Locomotion
2	2	3	24	Yes	Locomotion
2	2	3	25	Yes	Coordinated reaction
3	2	4	2	Yes	Locomotion
3	2	4	3	Yes	Locomotion
3	2	4	5	Yes	Locomotion
3	2	4	6	Yes	Uncoordinated reaction
3	2	4	7	Yes	Locomotion
3	2	4	29	Yes	Locomotion
4	1	5	2	Yes	Locomotion
4	1	5	3	Yes	Locomotion
4	1	5	4	Yes	Uncoordinated reaction
4	1	5	5	Yes	Locomotion
4	1	5	6	Yes	Locomotion
4	1	5	7	Yes	Locomotion
4	1	5	29	Yes	Locomotion
5	1	3	8	Yes	Locomotion
5	1	3	9	Yes	Locomotion
5	1	3	10	Yes	Coordinated reaction
5	1	3	23	Yes	Locomotion
5	1	3	27	Yes	Coordinated reaction
5	1	3	31	Yes	Locomotion
6	1	2	15	Yes	Locomotion
6	1	2	34	Yes	Locomotion
6	1	2	35	Yes	Coordinated reaction
6	1	2	36	Yes	Locomotion
6	1	2	39	Yes	Locomotion
6	1	2	40	Yes	Uncoordinated reaction
7	1	2	16	Yes	Coordinated reaction
7	1	2	20	Yes	Locomotion
7	1	2	21	Yes	Locomotion
7	1	2	22	Yes	Uncoordinated reaction
7	1	2	26	Yes	Locomotion
7	1	2	28	Yes	Locomotion

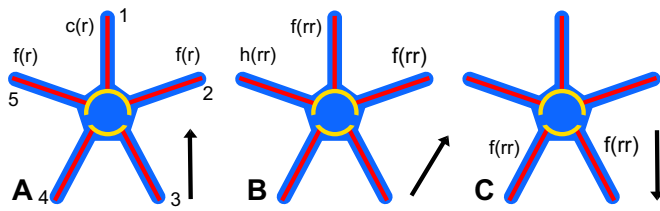


Fig. 5. Locomotion strategies observed during experiment 2 (multiple nerve ring cuts). Colors and abbreviations are as in Fig. 2.

connected by the nerve ring in both directions in order to achieve coordinated locomotion. When the three- and two-arm sets were stimulated simultaneously, there were instances when the specimens attempted locomotion in opposing directions so that the specimen remained stationary or moved slightly orthogonal to the stimulus through the motion of the arms. Such motion was less effective than locomotion observed during the control. The results of experiment 2 show that coordinated locomotion requires the arms involved to be connected by the nerve ring in at least one direction.

Experiment 3

A representation of the nerve ring cuts in experiment 3 is shown in Fig. 6. Five out of six specimens executed coordinated locomotion using the four connected arms. Locomotion using all five arms was not observed, in contrast to every specimen using all five arms to move during the control trials. Arm roles were assigned to facilitate efficient escape. Adjacent hindlimbs (Fig. 6C), for example, pushed synchronously so that the isolated limb was not a hindrance to forward locomotion. In Astleyan locomotion, one hindlimb would have served as a center limb and played a less active role in pushing the specimen forward (Fig. 2, reverse rowing). The isolated arm would have always been the center limb or hindlimb if Astleyan locomotion conventions were followed. The results of experiment 3 suggest that connected arms can respond to the isolation of a single limb. The arm separated by two nerve ring cuts presented an isolated reaction to stimuli applied directly to it in five of six specimens; the sixth specimen showed no reaction. The four other arms showed no reaction or uncoordinated reactions when the isolated arm was stimulated.

Experiments 4 and 5 (single nerve ring cut in specimens with more than two arms)

Experiment 4

A representation of the nerve ring cuts in experiment 4 is shown in Fig. 3B and results in Fig. 7. All specimens, apart from the one that did not survive (see Experimental trials above), exhibited coordinated locomotion. Five out of these specimens used both rowing and reverse rowing; one used only rowing. We identified the

leader arm(s) during locomotion to test whether the presence of a nerve ring cut shifted the arms that were positioned anteriorly relative to the direction of motion. We pooled the frequency with which a leader role was assigned to adjacent arms and compared it to the frequency with which a leader role was assigned to each combination of two non-adjacent arms (Chi-squared goodness-of-fit test, $P < 0.05$ in each case) (Fig. 7). This allowed us to reject the hypothesis that arms adjacent and non-adjacent to the cut are assigned leader roles at equal frequencies (Fig. 7). However, in one instance when the arm farthest from the cut was stimulated, the arms adjacent to the cut coordinated an escape motion as reverse rowing forelimbs. This notable exception suggests that, although there is a preference for arms non-adjacent to the cut to be the leader arms, arms without adjacent connections can assume that role and coordinate their actions if necessary.

We observed that arm 1 showed little action when arm 5, which was separated from it by a nerve ring cut, was stimulated, and *vice versa*. Arshavskii et al. (1976a) carried out a single nerve ring cut experiment on specimens with five arms and concluded that the arm separated by a cut from the one stimulated could not be involved in a coordinated response, as adjacent nerve ring connections are necessary to allow the 'nerve center' of the center limb to distribute function to the other arms. However, our observations showed that the arms next to the cut were often assigned as hindlimbs when one of them was stimulated. Thus, the limited motion observed by Arshavskii et al. (1976a) in the arm separated by the cut may be sufficient to represent coordinated locomotion, as this limited action is appropriate for arms assigned a hindlimb role. Coordinated motion occurred between arms on opposing sides of the cut at one point during this experiment (and several times during experiments 5 and 7), counter to Arshavskii et al.'s (1976a) hypothesis that locomotion is controlled by the 'nerve center' of the leader arm, which distributes function directly to the adjacent arm via nerve ring connections. The results of experiment 4 suggest that specimens with one nerve ring cut can continue to exhibit coordinated locomotion using all arms. It is possible for arms adjacent to the cut to take on leader roles, although this is significantly less frequent than for arms that are non-adjacent to the cut.

Experiment 5

A representation of the nerve ring cuts in experiment 5 is shown in Fig. 8. Four specimens exhibited coordinated locomotion, but only rowing, whereas all specimens in the control trials exhibited both rowing and reverse rowing. In the two specimens that did not exhibit coordinated locomotion, coordinated reactions were observed. The single cut between arms 1 and 5 eliminated two of the five locomotion strategies observed in the control, those where these arms occupied an anterior position relative to the direction of motion. This result indicates a preference for locomotion strategies in which the arms adjacent to the cut occupy a posterior position relative to the direction of motion, in contrast to their anterior position in the control.

Experiments 6 and 7 (single nerve ring cut in specimens with only two arms)

Experiment 6

Four out of six specimens exhibited coordinated locomotion (in contrast to all specimens in the control trials). All four exhibited reverse rowing, but only one performed rowing as well (this specimen was not the one that exhibited both gaits in the control trials). Arm role assignments for these two gaits were also observed in the control trials (control B,C in Fig. 9). The two specimens

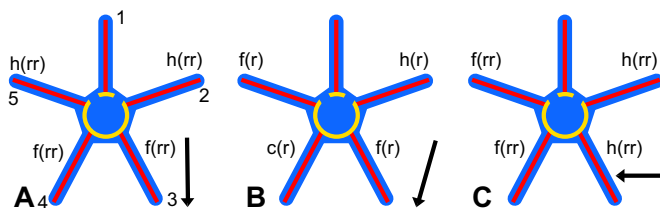


Fig. 6. Locomotion strategies observed during experiment 3 (multiple nerve ring cuts). Colors and abbreviations are as in Fig. 2.

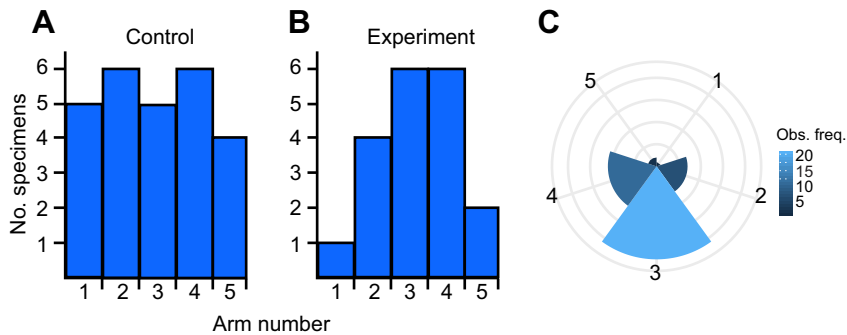


Fig. 7. Distribution of leader arms observed during locomotion in control and experiment 4 (one nerve ring cut, five arms). Arm numbers in plots refer to numbered arms in the ophiroid schematic (Fig. 3). (A) The number of specimens in which a given arm was assigned a leader role in the control. (B) The number of specimens in which a given arm was assigned a leader role in experiment 4. (C) Rose diagram represents the pooled frequency of leader arm assignments for all experimental trials (arm 1=1, arm 2=7, arm 3=21, arm 4=11, arm 5=2).

that lacked coordinated locomotion exhibited coordinated and uncoordinated reactions (Table S2). The average number of coordinated arm oscillations per instance of reverse rowing was 5.4 out of 12 instances of reverse rowing.

Experiment 7

Four out of six specimens exhibited coordinated locomotion (in contrast to all specimens in the control trials). All four used reverse rowing, and one used a non-Astleyan locomotion strategy, which was also observed in the control trial of a different specimen (Fig. 9A). The two specimens that lacked coordinated locomotion exhibited coordinated or uncoordinated responses. One of these specimens did a backflip to escape from the stimulus. The average number of coordinated arm oscillations per instance of reverse rowing was 2.1 out of 5 instances of reverse rowing.

The results of experiments 6 and 7 show that an organism can continue to coordinate adjacent arms despite a nerve ring cut between or adjacent to them. However, sustained periodic arm oscillations were less frequent when the nerve ring was cut between rather than adjacent to the arms, suggesting that connections between adjacent arms may be necessary for sustained periodic oscillations.

Sequentially connected arms

We found that the number of arms that are sequentially connected by the nerve ring is a significant predictor of the probability that they will exhibit coordinated locomotion (logistic regression, $P=0.00543$) (25/26 specimens with five arms sequentially connected exhibited coordinated locomotion, 5/6 specimens with four arms, 3/6 specimens with 3 arms and 1/6 specimens with 2 arms).

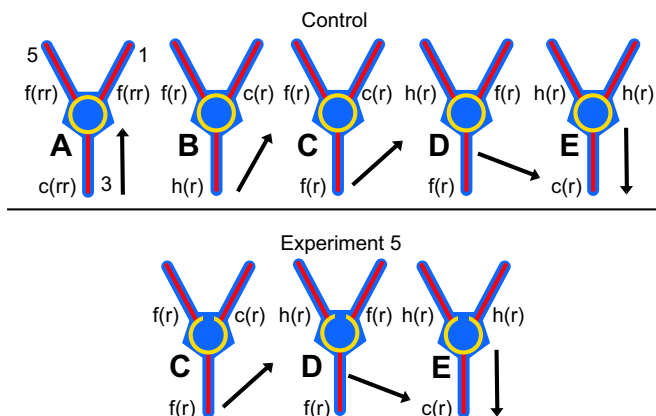


Fig. 8. Locomotion strategies observed during control and experiment 5 (one nerve ring cut, three arms). Colors and abbreviations are as in Fig. 2.

Trimmed and untrimmed arms

Each experiment was carried out with three specimens with trimmed and three specimens with untrimmed arms to provide a basis for comparison with the results of previous studies [i.e. Arshavskii et al., 1976a,b (untrimmed); Matsuzaka et al., 2017 (trimmed and untrimmed); Kano et al., 2017a (trimmed)]. We found that trimming the arms did not significantly affect the proportion of individuals displaying coordinated locomotion across experiments (Mann–Whitney U -test, $P=0.33$, data in Tables S1 and S2). All specimens, regardless of arm length, exhibited coordinated locomotion in the control trials (19/19 untrimmed and 18/18 trimmed; the same control trials applied to experiments 3 and 4). A total of 68% of untrimmed specimens (15/22) and 52% of trimmed specimens (11/21) exhibited locomotion during the experiments. The most notable disparity was in experiment 2: all untrimmed specimens exhibited locomotion in the experimental trials, whereas none of the trimmed specimens did. There was nothing unusual about the conduct of experiment 2 that would explain this difference; it may be an artefact of the small sample. If experiment 2 is not considered, the number of instances in which untrimmed and trimmed specimens did not exhibit locomotion is the same (i.e. seven instances each).

DISCUSSION

Multiple nerve ring cuts

Are nerve ring connections necessary for information transmission and arm coordination? (Experiments 1–3)

Arms that were isolated from one another by two nerve ring cuts did not exhibit coordinated locomotion, indicating that a nerve ring connection is necessary for transmission of information. There was a

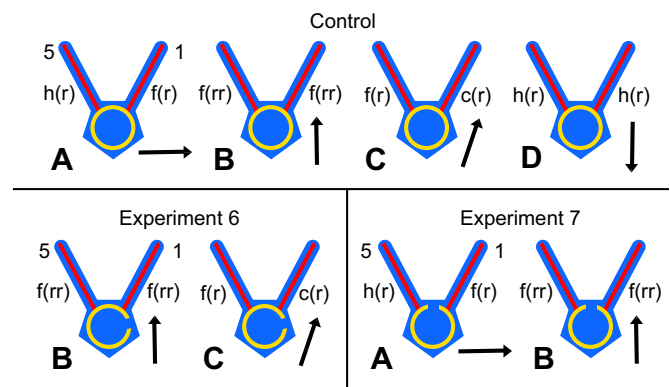


Fig. 9. Locomotion strategies observed during control and experiments 6 and 7 (one nerve ring cut, two arms). Control trials for experiments 6 and 7 are pooled. Colors and abbreviations as in Fig. 2.

significant positive relationship between the number of arms that remained connected by nerve ring tissue and the occurrence of coordinated locomotion. This shows that a greater quantity of integrated nerve ring tissue is advantageous for initiating coordinated locomotion and/or an appropriate response to a stimulus.

Single nerve ring cuts (>2 arms)

Can information travel bidirectionally to avoid a nerve ring cut and still allow coordinated locomotion using all arms? (Experiments 4 and 5)

Ten out of thirteen specimens exhibited coordinated locomotion during experiments with one nerve ring cut. In specimens of experiments 4 and 5, stimulation of an arm adjacent to the cut initiated locomotion or reactions from all arms, which indicates that ophiuroids can coordinate their arms if the nerve ring is cut in just one place. However, coordinated oscillations of arms adjacent to the cut were rare, indicating that direct connections between adjacent arms are important for this kind of motion.

Single nerve ring cuts (2 arms)

Are adjacent nerve ring connections necessary for coordination between two adjacent arms? (Experiments 6 and 7)

Coordinated locomotion occurred in four out of six specimens in both experiments 6 and 7, indicating that it is not compromised by a single cut adjacent to an arm. However, the number of coordinated oscillations per instance of locomotion was higher in experiment 6 than in experiment 7, indicating that a cut outside adjacent limbs is less detrimental for sustained periodic movements than one between them.

Role of local sensory feedback

A primary aim of this study was to test whether or not arm coordination for locomotion was driven exclusively by local feedback. If this were the case, each arm would have sensed the actions undertaken by the stimulated arm and self-assigned a role to escape the stimulation regardless of the presence or absence of nerve ring connections to other arms. However, our experiments showed that arms that were not connected by nerve ring tissue were incapable of engaging in coordinated locomotion, indicating that coordination is not exclusively driven by local feedback.

Neural control

The brittle stars were engaging in avoidance behavior in response to the KCl stimulus. Brittle stars have been hypothesized to have 'giant' neurons that are involved in the rapid transmission of motor information (Cobb and Stubbs, 1981). Transmission of information via these giant neurons may have been used for the avoidance behavior we observed. Although we found that information can be passed along either side of the nerve ring, cutting the nerve ring may have disrupted the normal transmission of information. We hypothesize that vital information may be transmitted along the nerve ring in the shortest possible distance, while non-vital information may be transmitted around the body. This may account for the reduced frequency of leader arms adjacent to the cut in experiment 4, and the reduction of the number of sequential periodic oscillations observed during locomotion when the nerve ring was cut between arms (experiment 6) versus adjacent to them (experiment 7). Future work will include using electrophysiological experiments to map the transmission pathway of the stimulation through the ophiuroid nervous network and test the giant neuron hypothesis (Cobb and Stubbs, 1981).

Resilience to damage

Our experiments showed that arms adjacent to a nerve ring cut were assigned 'leader arm' roles at a significantly lower rate than other

arms. This indicates that the organism could actively respond to damage by assigning arm roles so that the cut area was not directly anterior with respect to the direction of motion. However, a reduction in the number of arms sequentially connected by nerve ring tissue resulted in a lower frequency of coordinated locomotion, indicating a limit to the ability of the ophiuroid to respond to damage.

Mathematical models of brittle star locomotion

Previous research attempted to develop mathematical models to simulate brittle star locomotion. Watanabe et al. (2012) modeled locomotion with five arms, each with two degrees of freedom, allowing dorsoventral and lateral motion. Each arm was connected directly to the adjacent arms to represent nerve ring connections. This model was not set up to evaluate arm role re-assignment during locomotion in response to damage and could not reproduce the results of our experiments. Kano et al. (2017a) proposed a mathematical model to reproduce the behaviors of brittle stars with amputated arms. However, in the proposed model, the direction of movement is determined in a centralized manner, while brittle stars likely determine this in a decentralized manner. Hence, this model cannot describe the results of our nerve ring cut experiments. However, we believe that they can be reproduced by extending the model, which will be the subject of future work.

Most research on the echinoderm nervous system thus far has been anatomical (Díaz-Balzac and García-Arrarás, 2018). This study represents a step forward in understanding the way in which the unique echinoderm nervous system carries out integration and coordination for locomotion. As each extant echinoderm class uses a unique form of locomotion, using experimental methods to analyze these disparate strategies will provide additional data on the controls on the echinoderm nervous control system.

Other examples of decentralized nervous control in the animal kingdom include the diffused nerve nets of cnidarians (Watanabe et al., 2009) and other bilaterians such as xenacoelomorphs and hemichordates (Martín-Durán et al., 2018). Decentralized control can also be used to describe group behaviors driven primarily by local sensory feedback at the individual level, such as synchronized movement within schools of fish and flocks of birds (Breder, 1954; Couzin, 2008). Robotic models operating under decentralized control have been used to generate adaptive gait patterns found in quadrupeds (Fukuhara et al., 2018), hexapods (Owaki et al., 2017) and snakes (Kano et al., 2017b), and robots have been used to study group behaviors in schools of fish and groups of insects (Kube and Zhang, 1994; Landgraf et al., 2014).

Conclusions

Our behavioral experiments highlighted several critical features of the function of the ophiuroid nerve ring and its role in controlling locomotion. Arms must be connected by the nerve ring in order to transmit information and execute coordinated actions. However, information can be transmitted in either direction around the nerve ring; arms do not need to be connected to their immediate neighbor on both sides. Specimens with one nerve ring cut can execute coordinated locomotion using all arms, i.e. nerve connections do not need to be bidirectional. However, direct nerve ring connections between adjacent arms are important for sustained periodic locomotion. In addition, the greater the number of successive arms connected by the nerve ring, the higher the possibility of coordinated locomotion, suggesting that integrated nerve ring tissue is involved in controlling movement. The necessity for nerve ring connections between arms for coordination refutes the notion that ophiuroid locomotion is controlled by local feedback and confirms that

ophiuroids transmit and integrate information via the nerve ring. The ability of ophiuroids to continue locomotion despite significant damage to the nerve ring demonstrates the sophisticated decentralized control setup underlying ophiuroid locomotion.

Robots that can continue to function in the face of irreparable damage have important practical applications, such as exploring natural disaster sites or remote areas. Novel designs in engineering often draw inspiration from complex processes operating in nature. Our behavioral experiments have advanced our understanding of the way in which brittle stars maintain functionality despite severe damage to the major structures involved in locomotor control. Future work will integrate the results of the experiments presented in a mathematical model to explain how brittle stars maintain resilience after eliminating connections between the arms. This can be applied to improve the construction of artificial decentralized systems.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: E.G.C., D.K., T.K., D.E.G.B., A.I.; Methodology: E.G.C., D.K., T.K., H.A., D.E.G.B., A.I.; Software: H.A.; Validation: H.A.; Formal analysis: E.G.C., D.K., T.K., H.A., D.E.G.B., A.I.; Investigation: E.G.C., D.K., T.K., H.A., D.E.G.B., A.I.; Resources: T.K., H.A., D.E.G.B., A.I.; Data curation: E.G.C., T.K., H.A.; Writing - original draft: E.G.C., T.K., D.K., D.E.G.B., A.I.; Writing - review & editing: E.G.C., D.K., T.K., H.A., D.E.G.B., A.I.; Visualization: E.G.C., H.A.; Supervision: T.K., H.A., D.E.G.B., A.I.; Project administration: T.K., A.I.; Funding acquisition: E.G.C., T.K., H.A., D.E.G.B., A.I.

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Data availability

Video recordings of the experiments and micro-CT scans of select specimens are available from the Dryad Digital Repository (Clark et al., 2018).

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.192104.supplemental>

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