Report

Self-Recognition Mechanism between Skin and Suckers Prevents Octopus Arms from Interfering with Each Other

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Summary

Controlling movements of flexible arms is a challenging task for the octopus because of the virtually infinite number of degrees of freedom (DOFs) [1, 2]. Octopuses simplify this control by using stereotypical motion patterns that reduce the DOFs, in the control space, to a workable few [2]. These movements are triggered by the brain and are generated by motor programs embedded in the peripheral neuromuscular system of the arm [3–5]. The hundreds of suckers along each arm have a tendency to stick to almost any object they contact [6–9]. The existence of this reflex could pose significant problems with unplanned interactions between the arms if not appropriately managed. This problem is likely to be accentuated because it is accepted that octopuses are "not aware of their arms" [10–14]. Here we report of a self-recognition mechanism that has a novel role in motor control, restraining the arms from interfering with each other. We show that the suckers of amputated arms never attach to octopus skin because a chemical in the skin inhibits the attachment reflex of the suckers. The peripheral mechanism appears to be overridden by central control because, in contrast to amputated arms, behaving octopuses sometime grab amputated arms. Surprisingly, octopuses seem to identify their own amputated arms, as they treat arms of other octopuses like food more often than their own. This self-recognition mechanism is a novel peripheral component in the *embodied organization* of the adaptive interactions between the octopus's brain, body, and environment [15, 16].

Results

Freshly amputated octopus arms are highly active for more than 1 hr after amputation; the arms make movements similar to those seen in the intact animal, and the suckers reflexively grasp, attach, and hold the objects that they touch. However,

in more than 30 trials during the preliminary observations, we never saw the suckers of amputated arms attach to the arm itself or to another arm covered with skin, whether they originated from the same animal or from different animals. The suckers did grab skinned arm parts of freshly amputated arms (Movie S1 available online) and even the bare flesh at the site of amputation of the arm itself. They also avoided grabbing plastic Petri dishes covered with octopus skin taken from the same or another octopus (n = 13). Remarkably, when only part of the dish was covered with skin, the amputated arms firmly held the exposed plastic but avoided grabbing the part covered by the skin (n = 11). Movie S2 demonstrates how adjacent suckers follow this rule. These observations suggested that the arm skin was involved in the mechanism preventing suckers from grabbing their own arm.

We quantified these observations by measuring the grasping forces that arms applied to intact and skinned arm parts and expressing the force relative to the force applied to plastic Petri dish (see the Supplemental Experimental Procedures). Amputated arms never applied any grasping force to other intact amputated arms (arm with skin, n = 21), but did grasp skinned arms, applying 27% ± 19% (mean ± SD) of the force relative to the reference (n = 20; Figure 1A; t(19) = 40.25 p < 0.001, one-tailed, single-sample t test against 0 mean).

Interestingly, not only did we confirm our initial observations, but we also found that the suckers applied significantly different forces to different types of objects that are associated with natural food (Figure 1A). For example, the force applied to hold a piece of fish was always the strongest of all items in each trial (67% ± 28%, n = 9), but boiled and peeled shrimps they held with a relatively moderate force (16% ± 12%, n = 4).
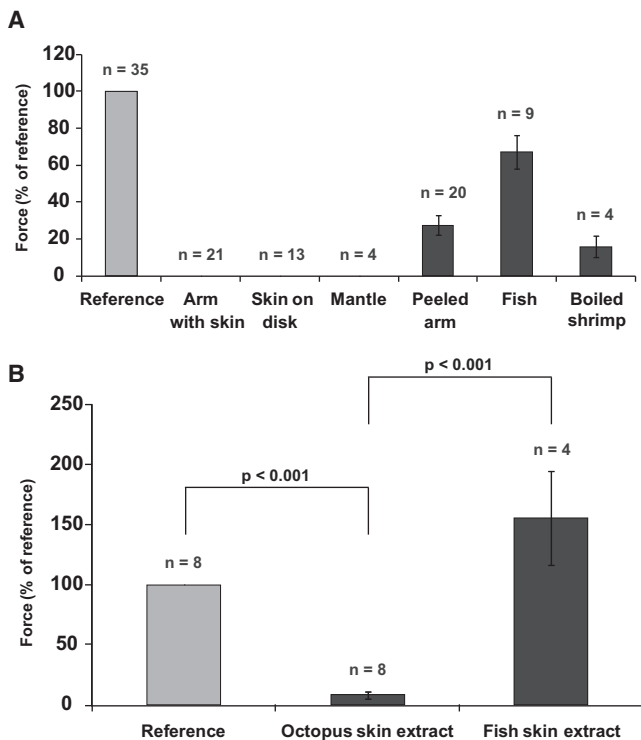
These results led us to hypothesize that the skin triggers a local inhibition that prevents the suckers from activating their attachment mechanism. This mechanism could rely on chemical and/or tactile information. Octopus skin is a complex structure that contains chromatophores, iridophores, and subcutaneous neuromuscular systems that make it chemically and mechanically heterogeneous [17–21]. In addition, each arm is a huge sensory organ containing about 40 million tactile and chemical receptors. The receptors are spread all over the arm, but with greater concentration in the suckers, especially on the rim of the sucker [10, 11].

To test whether substances in the skin are involved in the apparent inhibition, we coated plastic Petri dishes with gel soaked in skin crude extract and then dried them (see the Supplemental Experimental Procedures). The coated dishes were introduced to the amputated arms as in the force measurement experiments to examine the response of amputated arms to skin crude extracts in various solvents (see the Supplemental Experimental Procedures). The most consistent and reproducible results were obtained with hexane extract. Grabbing forces applied to dishes coated with gel containing hexane extract of octopus skin were approximately 10-fold weaker than those applied to the reference Petri dishes coated with gel containing pure hexane (8% ± 8%, n = 8) and approximately 20-fold weaker than those applied to dishes coated

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Figure 1. Forces that Freshly Amputated Arms Applied on Different Objects

Each value is given as the percentage of the force that the specific arm applied to the reference item, given separately for each subpanel.

(A) The force that was necessary to pull an item free from the sucker grasp of a freshly amputated octopus arm. The items were as follows: another freshly amputated arm from the same or from another conspecific octopus (arm with skin, 12 trials with arms from the same animal and nine trials with arms from two different animals), a section of skin from a freshly amputated arm from the same or from another conspecific octopus glued to a plastic Petri dish (skin on disk, five trials with arms from the same animal and seven trials with arms from two different animals), a section of fresh octopus skin from mantle (mantle), a skinless fresh arm from the same or from another conspecific octopus (peeled arm), defrosted fish (fish), defrosted boiled shrimp (boiled shrimp), and plastic Petri dish as a reference (reference).

(B) The forces required to pull free from the sucker grasp. Petri dishes were coated with (1) octopus skin extract (as we found no significant differences between data originating from the extraction of skin from the same animal [n = 5] or from another conspecific animal [n = 3], so we pooled the data of all these experiments to one group), (2) fish skin extract, and (3) hexane soaked gel as reference (reference). The significant p values were obtained by a two-tailed unpaired t test.

All force measurements both in (A) and in (B) are expressed as the rounded percentage of the force that was needed to free the reference item from the sucker grasp. Each value is the mean ± SEM, and n is given above the corresponding bar. See also Movies S1 and S2.

with gel containing hexane extract of fish skin (which were 156% ± 78% of the reference, n = 4). These results confirm that substances in the skin are involved in the inhibition of the attachment reflex. The strong and significant attachment of the sucker to Petri dishes coated with gel containing pure hexane or fish extract confirm that the procedure itself does not induce repellency (unpaired t test, p < 0.001 for both procedures; Figure 1B).

The results so far show, for the first time, that the skin of the octopus prevents octopus arms from attaching to each other or to themselves in a reflexive manner. The drastic reduction in the response to the skin crude extract suggests that a

Figure 2. Octopus Holding the Cut End of an Amputated Arm in Its Mouth Only by the Beak, or "Spaghetti Holding"

An image showing the unusual way octopuses sometimes hold amputated arms. The arm (marked with an arrow) is seen hanging freely from the mouth, rather than held by the arms and the interbrachial web as a food item.

specific chemical signal in the skin mediates the inhibition of sucker grabbing.

To test whether and how this self-avoidance mechanism is involved in the behavior of the animal, we studied the response of behaving octopuses to amputated arms presented to them as food. *Octopus vulgaris* is known to be cannibalistic [22]. Octopuses use their arms to seize, manipulate, and bring food to the mouth and hold it tightly with the arms and within the interbrachial web of skin that stretches between the proximal parts of the arms.

In contrast to amputated arms, the response of behaving animals toward amputated arms was equivocal: in some of the cases, octopuses showed the usual "prey capture" behavior described above, but in others, octopuses showed a behavior uncharacteristic of feeding. When presented with amputated arms, the octopus would repeatedly rub its arms over the amputated arm, touching it, but not attaching to or grabbing it (see Movie S3). In some of these cases, the octopus did grab the amputated arm, but only at the amputation site (where the flesh is exposed), brought it to the mouth, and then held it using only the beak, rather than the interbrachial web or the proximal parts of the arms as it would when handling food items. In these cases, the amputated arm hung from the beak untouched by the other arms of the octopus (Figure 2 and Movie S3). We named this odd holding "spaghetti holding." This behavior was also displayed by intact octopuses (data not shown) that were offered amputated arms, and therefore it is unlikely that this unusual behavior is due to either anesthesia or amputation.

While amputated arms consistently refrained from grabbing octopus skin, live octopuses were less consistent. They did occasionally grab amputated arms even by the skin (Movie S4), suggesting that the arm avoidance mechanism can be overridden by higher levels of the nervous system. However, we could not initially identify a consistent pattern in the choosing between grabbing and not grabbing of the skin. To
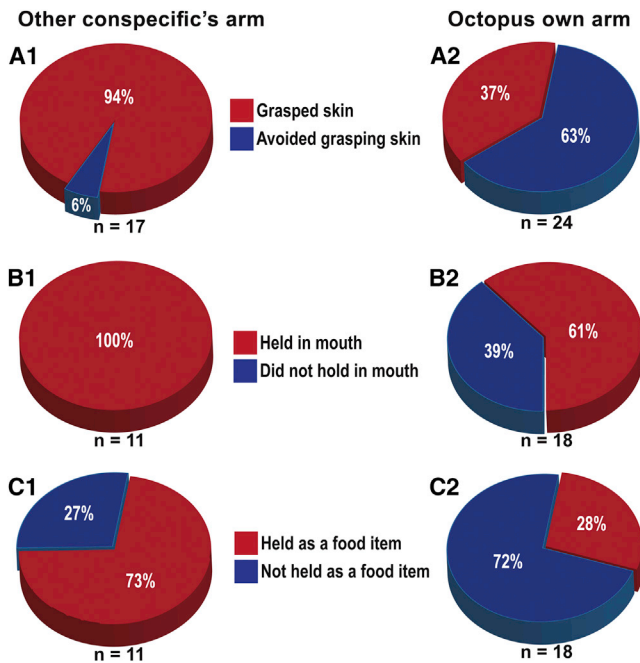
Figure 3. Octopuses Can Distinguish between Their Own and a Conspecific's Amputated Arms

The number of positive trials for the indicated action presented as percentage from the total number of trials in a specific experiment. Right, octopuses' response to their own arms; left, octopuses' response to another conspecific's arms.

(A) Attachment or nonattachment of suckers to the skin of "intact" (not skinned) amputated arms (n = 24 for octopuses' own arms and n = 17 for a conspecific's).

(B) Holding or not holding of amputated arms in the mouth.

(C) Holding of amputated arms in the mouth as food items or not (n = 18 for octopuses' own arms and n = 11 for a conspecific's).
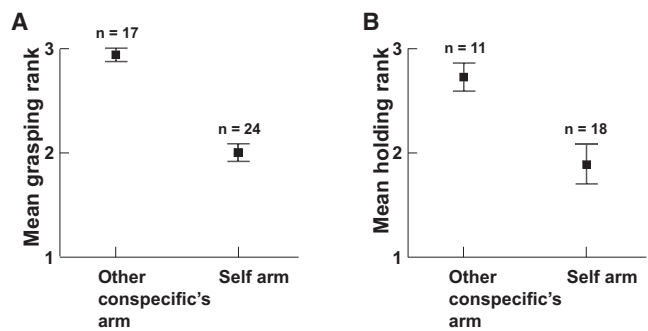
See also Movies S3 and S4.

Figure 4. Significant Differences in Preference of Octopuses toward Their Own and a Conspecific's Amputated Arms

Arms that originated from the tested animal or from another conspecific animal. Rectangles mark the weighted average rank of the categories according to the rate of recurrence of each rank, with the rank scale of 1–3, as assigned by the trained observer. Error bars indicate ± 1 SE. The vertical axis depicts (A) the grasping amputated arms category (1, not grasping at all; 2, grasping only the flesh at the amputation site; and 3, grasping also the skin) and (B) the holding in mouth category (1, not holding at all; 2, spaghetti holding; and 3, holding as a typical food item). The intensity of both grasping [$U'(24,17) = 84$, $p < 0.001$] and holding [$U'(18,11) = 44$, $p < 0.01$] is significantly lower for the self-arm condition.

help resolve this issue, we had a neutral ("blind") observer evaluate the behaviors according to a predefined set of criteria (see the Supplemental Experimental Procedures).

The results show that octopuses can distinguish between their own and another conspecific's amputated arms. Our first criterion was whether the octopus attached its suckers to the skin of the amputated arm. In about 94% of the trials (16 out of 17), octopuses attached their suckers to the skin of amputated arms taken from a conspecific (Figure 3A1). Alternatively, octopuses attached their suckers to the skin of their own amputated arms in less than 40% of the trials (9 out of 24; Figure 3A2). A Mann-Whitney U test showed a significant difference in the degree of grasping of their own versus another conspecific's arms [$U'(24,17) = 84$, $p < 0.001$], as summarized in Figure 4A.

To characterize the behavior of octopuses toward amputated arms, we asked two behavioral questions that are related to manipulation of food: (1) did the octopuses hold the amputated arm in their mouth (no matter in what way) or not and (2) did they hold the arm as a food item (i.e., tightly using their web and arms) or not (i.e., spaghetti holding or not holding at all)? All of the octopuses (11 animals) that were offered a conspecific's arm brought the arm to the mouth and held the arm in the mouth (i.e., either as food or as spaghetti holding; Figure 3B1). On the other hand, almost 39% of the octopuses that were offered their own amputated arm (7 out of 18)

avoided holding it in the mouth at all (Figure 3B2). The results show a significant difference between the two groups (p < 0.03, two-tailed Fisher's exact test). Also, more than 72% of the octopuses (8 out of 11) held conspecific's amputated arms as food items (Figure 3C1), while less than 28% of the octopuses (5 out of 18) treated their own amputated arms as food (Figure 3C2). A Mann-Whitney U test showed a significant difference in the degree of holding their own versus conspecific's arms [$U'(18,11) = 44$, $p < 0.01$)], as summarized in Figure 4B. When the offered arm was skinned, it was always treated as a typical food item in all criteria, regardless of its origin, self or another conspecific.

Discussion

In this study, we found that isolated octopus arms unequivocally avoided grabbing octopus skin. The amputated octopus arms also avoided attaching to Petri dishes that were coated with gel soaked with octopus skin crude extract, suggesting that avoidance was elicited by molecules in the skin that possibly inhibit the activation of suckers' reflexive attachment mechanism. These molecules are probably hydrophobic as they are extracted and dissolved almost exclusively in hexane.

We can't indicate which receptors on the arm can mediate self-recognition mechanisms. However, the density of receptors is much greater in the suckers then in the rest of the arm [11], and the suckers are the part of the arm that comes into contact with the substrate and behave individually according to the substrate they contact (e.g., a sucker refrained from attaching to the skin, while its next neighbor attached firmly to the plastic). Therefore, this mechanism is most likely mediated by the chemoreceptors in the suckers at the level of an individual sucker.

In contrast to isolated arms, freely behaving octopuses occasionally attached to octopus skin, suggesting that central brain control can override (veto) the inhibition of the reflexive attachment behavior. This mechanism is most likely related to the central control of sucker release mechanisms and supports the mechanism proposed to explain the "sticky suckers"

phenomenon in "decerebrated" animals [6, 7]. Octopuses could differentiate between their own and others' amputated arms, suggesting that the skin recognition mechanism is more elaborate than a skin-sucker interaction and may involve higher brain circuits, integrating additional sensory inputs and even cognitive functions.

Self-recognition mechanisms are involved in diverse biological processes, for example in defense and immune systems [23–25] and in development, such as in the setting of dendritic tree morphological organization [26]. Here we describe a self-recognition mechanism that is involved in motor control.

In goal-directed arm movements, like reaching and fetching, the octopus simplifies the control of its highly redundant arms by using stereotypical motor programs that reduce the number of degrees of freedom to only a few (three) and by using predefined motor programs that are embedded in the neuromuscular system of the arms [3–5]. The mechanism reported here demonstrates yet another simplification strategy to the potentially very complicated problem of controlling the autonomous arm behaviors like searching, grasping, collecting gravel, etc. [8]. This is due to the removal of the need to represent and compute where and when the arms touch each other. This is another example in which simplification is achieved by moving part of the control labor to the peripheral neuromuscular system of the arm, facilitating the local control.

It is likely that the constraining effect of the self-avoidance mechanism led the octopuses to adopt the "spaghetti holding" behavior as the beak is the only organ that allows holding when the suckers action is inhibited. This is a vivid demonstration of how peripheral interactions can shape behavior in an embodied fashion (see below), rather than by top-down control.

Finally, the mechanism of self-recognition fits well with the embodiment concept that explains why this and previous studies have revealed so many "surprises" in the octopus motor control system [15, 16]. The concept of embodied organization, proposed as a tool for designing autonomous robots [27], is that adaptive behavior emerges from the reciprocal and dynamic interactions of sensory and physical information among body, controller, and environment. In this form of organization, in contrast to hierarchical organization, the system functions as a whole, as it allows *self-organizational* processes to set the relevant dynamic properties of the elements building up the system—the embodiment. In robotics, this approach has led to the *emergence* of adaptive behavior by a robot in a specific environment (see [27]).

As explained previously [15, 16], studies on octopus motor control suggest that the concept of embodied organization may also be useful for biological systems. Crucial attributes of octopus embodiment are the *morphology* and the *physical properties* with which the octopus interacts with its environment. The morphology and flexibility of the octopus body are so unusual that almost every level of their motor system organization, from the higher motor control centers [13], to the autonomy of the elaborated peripheral nervous system of the arms [4], down to the neuromuscular system of the arms [28], have evolved special properties. This multilevel uniqueness is best explained by embodied organization—coevolution of all the octopus systems to cope collectively with the complex motor control and behavior of this morphologically special animal [15, 16].

The results of the current study introduce a novel player into these dynamic interactions—a peripheral self-recognition mechanism that constrains interactions between arms. Such a mechanism is mostly advantageous in the control of flexible embodiments, where body parts can easily interfere with one another. Peripheral self-avoidance is a striking addition to the list of surprises in the motor system of this uniquely embodied animal.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and four movies and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.04.024.

Author Contributions

N.N., G.L., and B.H. designed the experiments. N.N. and G.L. performed the experiments. N.N. and G.L. and B.H. and F.W.G. analyzed the results and wrote the paper.

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