

Current Biology

Use of Peripheral Sensory Information for Central Nervous Control of Arm Movement by *Octopus vulgaris*

Highlights

- Octopuses learn to direct arm movement on the basis of arm position
- Octopuses learn to direct arm movement on the basis of tactile clues
- The behaviors are not specific to a particular arm
- Octopus brains receive and use proprioceptive and tactile input from the periphery

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In Brief

Gutnick et al. show that octopuses use proprioceptive and tactile information from the periphery to accomplish learning tasks that entail directed control of arm movement. Using two-choice, single-arm mazes, they show that octopuses learn operant motor tasks that require the brain to use focal sensory information from a single arm.



Report

Use of Peripheral Sensory Information for Central Nervous Control of Arm Movement by *Octopus vulgaris*

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Octopuses are active predators with highly flexible bodies and rich behavioral repertoires [1–3]. They display advanced cognitive abilities, and the size of their large nervous system rivals that of many mammals. However, only one third of the neurons constitute the CNS, while the rest are located in an elaborate PNS, including eight arms, each containing myriad sensory receptors of various modalities [2–4]. This led early workers to question the extent to which the CNS is privy to non-visual sensory input from the periphery and to suggest that it has limited capacity to finely control arm movement [3–5]. This conclusion seemed reasonable considering the size of the PNS and the results of early behavioral tests [3, 6–8]. We recently demonstrated that octopuses use visual information to control goal-directed complex single arm movements [9]. However, that study did not establish whether animals use information from the arm itself [9–12]. We here report on development of two-choice, single-arm mazes that test the ability of octopuses to perform operant learning tasks that mimic normal tactile exploration behavior and require the non-peripheral neural circuitry to use focal sensory information originating in single arms [1, 10]. We show that the CNS of the octopus uses peripheral information about arm motion as well as tactile input to accomplish learning tasks that entail directed control of movement. We conclude that although octopus arms have a great capacity to act independently, they are also subject to central control, allowing well-organized, purposeful behavior of the organism as a whole.

RESULTS**Proprioceptive Learning**

Proprioception, the sense of one's posture and movement, is essential for the generation of coordinated motor behaviors, and defects in proprioception can result in uncontrolled movements and impaired locomotion [13].

We designed an operant task to test the availability to the CNS of self-motion or location information of single arms. Using food as a reward, we used a two-choice Perspex maze, opaque to the octopus, which allowed the animal to insert only a single arm and was based on the natural probing movements of octopus arms when exploring or hunting in small crevices (Figure 1; see STAR Methods). We trained six octopuses to repeatedly insert a single arm to the same side of the maze. In every trial, the octopus needed to insert a single arm through the central stem into the goal box of one of the maze sides. Although both goal boxes contained a piece of food, on the incorrect side the food was placed behind a net and could not be retrieved. Correct choices resulted in a food reward, and incorrect choices resulted

in the immediate removal of the maze. Animals completed ten trials a day until the criterion for learning (80% correct trials within a single training day) was reached (Figures 1A–1C; for details, see STAR Methods).

Five of six naive octopuses reached the criterion for learning within 20–90 trials (Figure 2A). Of the five animals, three were trained to side 1 correct, side 2 incorrect, and two were trained to side 2 correct, side 1 incorrect. In order to complete the task, animals had to associate their own voluntary motor action with the subsequent food reward, without visual indication of either the location of the food or the location of the arm. Whereas performance of the animals in the first half of the experiment was at chance level ($\chi^2 = 2.531$, $n = 128$, $p = \text{NS}$, trials pooled over five animals, excluding 20 trials in which the maze was approached but no choice was made), performance in the last half of the experiment was significantly above chance ($\chi^2 = 9.709$, $n = 141$, $p < 0.005$, trials pooled over five animals, excluding 7 trials in which the maze was approached but no choice was made).

Throughout the training, octopuses used two different arm movement strategies in the maze: straight and search (Figure 2B;

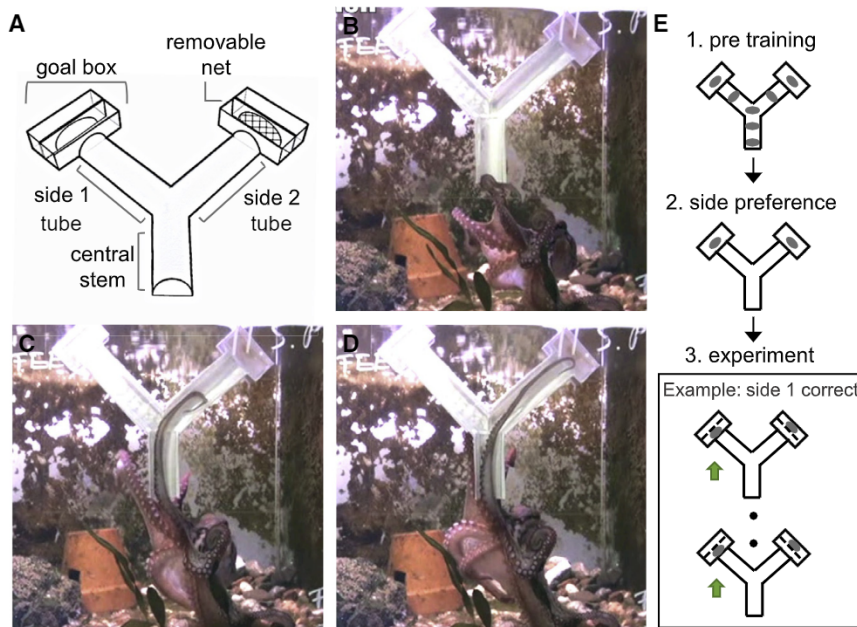


Figure 1. Proprioceptive Learning

The octopus had to insert a single arm into the central stem, through one of the side tubes, and into the goal compartment.

(A) Diagram illustrating the two-choice single-arm maze. To control for chemical cues, food is presented on both sides but is behind a net in the incorrect goal box. The maze is fixed to the front glass of the tank and is opaque toward the animal. (B–D) An octopus inserts a single arm into the central stem (B), into one of the sides (C), and to the correct goal box (D). See also [Video S1](#).

(E) Schematic illustrating the order of training and experiment. After pretraining to use the maze (1), octopuses completed 20 side preference trials (2) and were then trained to retrieve a food reward from the correct goal box (green arrow) that was always on the same side of the maze (3).

Tactile Learning

Most invertebrates use antennae or similar structures [14–17] for tactile exploration of their environment, and

[Video S1](#)). Straight movements involve the unrolling or pushing upward of a bend through the central stem and side tube directly into the goal box. Search movements involve a combination of unrolling or pushing a bend, with probing and crawling in the central stem and side tubes before reaching the goal box. We found that both on the first and on the last days, straight was used significantly more often than search (first ten trials: 41 straight versus 9 search, binomial $p < 0.001$; last ten trials: 46 straight versus 4 search, binomial $p < 0.001$). These movements proved to be of different durations from arm insertion into the central tube until choice. Straight movements took a median of 3.4 s (95% confidence interval [CI] [3.14, 3.84], $n = 87$), while search movements took a median of 6.14 s (95% CI [3.95, 10.41], $n = 13$), which was significantly longer (Mann-Whitney $n = 100$, $p < 0.001$, $z = 3.746$). Upon reaching criterion, trials in which straight movements were used were significantly more successful than not ($\chi^2 = 2.531$, $n = 46$, $p < 0.001$). It is important to note that in all interactions with the maze, animals maintained a similar orientation relative to the maze and never faced in the opposite direction.

Analysis of which arm entered the maze in each trial revealed that each octopus used seven or eight different arms throughout the experiment ([Figure S1](#); [Table S1](#)). On the last day of the experiment, animals used between three and five different arms to complete correct trials ([Figure 2D](#); [Figure S1](#); [Table S1](#)). In all animals the most commonly used arms were front arms; one animal had a significant preference for an arm (animal P3, arm R1; $\chi^2 = 18.14$, $n = 56$, $df = 3$, $p < 0.01$, calculated among the four most frequently used arms; [Table S2](#)). Because animals used multiple arms throughout the experiment, we compared the arms used for choices on all days prior to the day of criterion with those used on the day of criterion. We found that in one animal (P4), two arms that were used correctly in five trials on the last day had never been used before. Moreover, in animals P4 and P5, arms that were used correctly on the last day had never before been used in a correct trial ([Figure S1](#)).

many mammals rely on vibrissae [18]. In contrast, octopuses actively use the suckers to explore objects as well as to grasp them [1–3, 19].

To test the ability of octopuses to use tactile information collected by a single arm to direct the movement of any arm, we used an adapted version of the two-choice Y maze. In this maze, the surface of one of the side tubes was rough, while the other side tube was smooth ([Figure 3A](#)). We trained six octopuses to associate either rough ($n = 3$) or smooth ($n = 3$) with a food reward. In every trial, the octopus needed to insert a single arm through the central stem into a side tube, determine whether it was rough or smooth, and then either continue to the goal box or remove the arm and try again ([Figures 3B and 3C](#)), as appropriate. Reaching the correct goal box resulted in a food reward, and reaching the incorrect goal box resulted in the immediate removal of the maze. Although both goal boxes contained a piece of food, on the incorrect side the food could not be retrieved, as it was behind a net. A restricted randomness sequence decided which side had the rough stimulus. Animals completed ten trials a day until the criterion for learning (80% correct trials within a single training day) was reached ([Figures 3A–3C](#); for details see [STAR Methods](#)).

Five of six naive octopuses reached the criterion for learning in 20–120 trials ([Figure 4A](#)). Of the five animals, three were trained to rough correct, smooth incorrect, and two were trained to smooth correct, rough incorrect. Whereas performance of the animals in the first half of the experiment was at chance level ($n = 134$, $\chi^2 = 1.91$, $df = 1$, $p = \text{NS}$, trials pooled over five animals, excluding 18 trials in which the maze was approached but no choice was made), performance in the last half of the experiment was significantly above chance ($n = 145$, $\chi^2 = 7.51$, $df = 1$, $p < 0.01$, trials pooled over five animals, excluding 7 trials in which the maze was approached but no choice was made). Importantly, when reaching criterion, the numbers of choices of side 1 and side 2 were not significantly different, ruling out the use

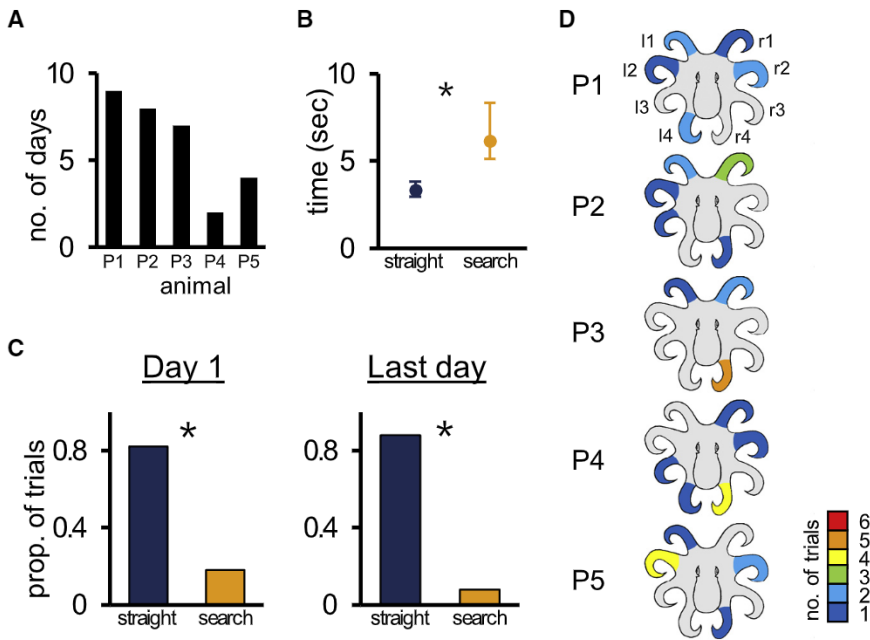


Figure 2. Proprioceptive Learning, Arm Movement Strategies, and Use of Multiple Arms

(A) Number of days to reach criterion for learning. (B) Octopuses used two arm movement strategies in the maze, straight ($n = 87$) and search ($n = 13$), which differed in duration from arm insertion into the maze till choice. Median duration of movements (median \pm 95% CI). Mann-Whitney $n = 100$, $p < 0.001$, $z = 3.839$. See also [Video S1](#).

(C) Proportion of trials using the two movement strategies on day 1 (binomial $p < 0.001$) and the last day of the experiment (binomial $p < 0.001$).

(D) Arms used for making correct choices on the last day of the experiment.

See also [Figure S1](#); [Tables S1](#) and [S2](#).

Analysis of which arm entered the maze in each trial revealed that octopuses used between five and eight different arms throughout the experiment ([Figure S2](#); [Table S1](#)). On the last day of the experiment animals used four or five different arms to complete correct trials

of a positional strategy instead of the tactile association task (22 side 1 and 28 side 2 choices).

Here too octopuses used similar arm movements to those defined in the proprioception experiment: straight and search ([Figures 4B](#) and [4C](#); [Video S2](#)). These movements were different in the duration from the time of arm insertion into the central stem until a choice was made, with straight taking a median of 3.08 s (95% CI [2.72, 3.68], $n = 32$) and search taking a significantly longer median of 4.6 s (95% CI [4.00, 5.92], $n = 67$) (Mann-Whitney $n = 99$, $p < 0.001$, $z = 4.339$). On the first day of the experiment, straight and search movements were used with the same frequency (first 10 trials: 21 straight versus 29 search, binomial $p = \text{NS}$). However, after learning, the slower search movement was used significantly more often (last 10 trials: 11 straight versus 39 search, binomial $p < 0.001$), and only search movements were significantly more successful (82% correct trials, binomial $n = 39$, $p < 0.001$).

According to the task design, when an arm entered the maze and encountered the stimulus, the octopus had to decide whether the stimulus was correct, in which case it would continue to the goal box, or it was incorrect, in which case it would change to the other side ([Video S2](#)). Changing sides was achieved by either removing the arm from the maze and reinserting the same or a different arm or by redirecting the arm inside the maze. On the first day of the experiment, 45% of trials in which the arm entered the incorrect side first were redirected or reinserted, and 50% of trials in which the arm entered the correct side first were redirected or reinserted. However, on the final day of the experiment, only 34% of trials in which the correct side was entered first were redirected, while 68% of trials in which the arm entered the incorrect side first were redirected. Importantly, correct trials were not restricted to those in which the arm went directly to the correct side (21 correct to 6 incorrect), but were also achieved when the arm was redirected (19 correct to 4 incorrect).

([Figure 4D](#); [Figure S2](#); [Table S2](#)). Unlike in the proprioceptive experiment, here we found that for two of the octopuses (T2 and T3), the most frequently used arms were hind arms (R4 and L4, respectively). However, only one animal showed a significant preference for a particular arm (animal T5, arm L2; $\chi^2 = 13.16$, $n = 75$, $df = 3$, $p < 0.01$, calculated among the four most frequently used arms; [Figure S2](#); [Table S2](#)); interestingly, this arm was not used at all on the last day of the experiment. Because animals used multiple arms throughout the experiment, we compared the arms used for choices on all days before the day of criterion with those used on the day of criterion. Although for all animals, arms used on the last day had been used during learning, we found that in two animals, T2 and T4, arms that were used correctly on the last day had been used before but never correctly ([Figure S2](#)).

DISCUSSION

Our study demonstrates that octopuses use sensory information gathered by single arms for CNS-mediated learning. This is the first study to establish the use of proprioceptive information for learning in octopuses [1, 3, 7]. Using the opaque maze as a means of isolating a single arm, we could exclude visual information on the location or action of the arm in intact animals, without surgical intervention.

Successful performance in the task requires the learning of the association between interacting with the maze and receiving a food reward. The octopus never sees the location of the reward, nor does it see its arm. Yet it learns to insert an arm through the maze to a goal box to retrieve it, thus associating the action of the arm with the subsequent reward. It has been shown that in cephalopods, positive reinforcement learning using food as a reward is processed in the brain [1, 7]. This leads us to conclude that learning of the tactile and proprioceptive tasks was mediated by the CNS and could not be solely achieved by the arms alone.

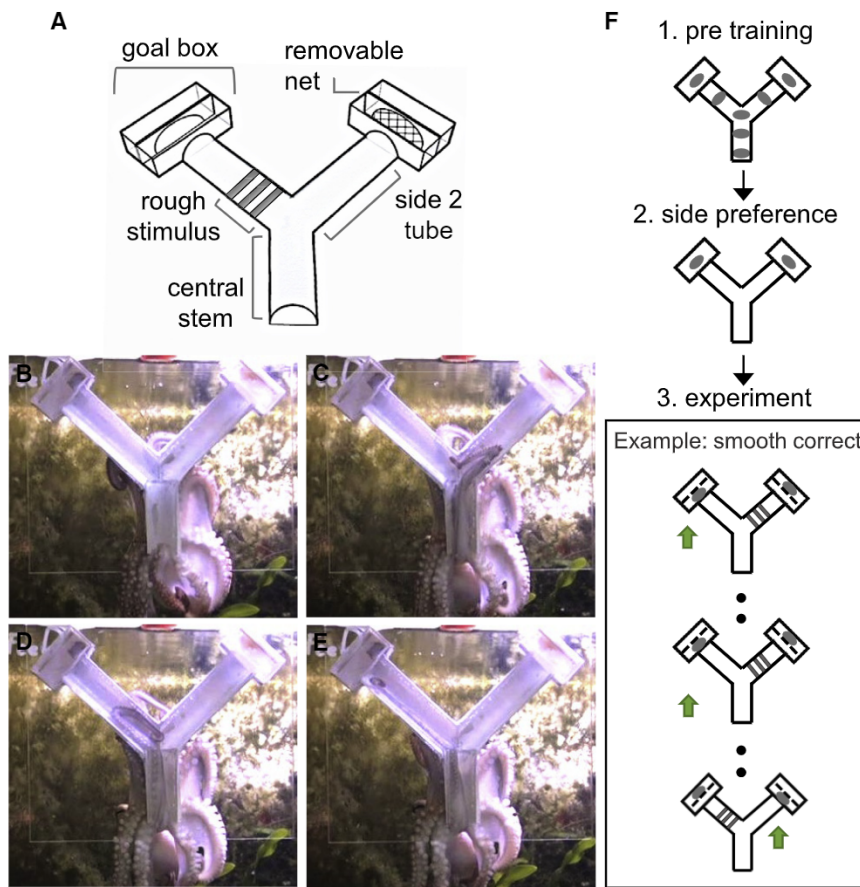


Figure 3. Tactile Learning

The octopus had to insert a single arm into the central stem, through one of the side tubes, and into the goal compartment.

(A) Diagram illustrating the tactile discrimination two-choice single-arm maze. Three grooves are cut out in the flat surface of the side arm. As in the proprioceptive maze, to control for chemical cues, food is presented on both sides but is behind a net in the incorrect goal box. The maze is fixed to the front glass of the tank and is opaque toward the animal.

(B–D) An octopus inserts a single arm into the central stem (B), enters one of the sides and touches the rough stimulus (C), and the arm moves to the opposite side (D) and continues to the correct goal box (E). See also [Video S2](#).

(F) Schematic illustrating the order of training and experiment. After pretraining to use the maze (1), octopuses completed 20 side preference trials (2) and were then trained to associate a tactile cue with the correct goal box (green arrow) to retrieve a food reward (3). The side of the tactile stimulus was alternated following a restricted randomness sequence.

frequency before the task was learned, most octopuses ultimately chose slower search movements, which involved exploration of the interior of the maze. This movement choice allowed tactile exploration of the stimulus. Our results regarding the speed of learning are not

The two experiments suggest that different sensory modalities were used in the learning process. In the first experiment, proprioceptive learning, octopuses learned to direct an arm inserted into the maze repeatedly to the same side. The information about the motion or location of the arm was associated with the food reward.

There are several possible strategies an animal might adopt to solve this type of proprioceptive task [1, 20–22]. One way would be to examine the interior of the maze, to determine the location of the reward, and to create a mental “image” of the maze and the location of the arm within it [23]. However, there is no evidence that octopuses can create mental images of objects without the involvement of vision. The consistent arm movement strategy, straight, which is the faster of the two, suggests a simpler solution. By repeating a directed fast arm movement in the confined space of the maze, similar to the presumably feed-forward controlled bend propagation [11], the same side can be reached over and over again. The information provided to the brain might not give a precise location or an online account of motion, but it would require knowledge of a general direction of movement of the arm in the maze that would result in a positive outcome in order to repeat it, in other words, a sense of self-motion [13].

Unlike the proprioceptive experiment, learning the tactile task required making a decision about where or how to move the arm only after the arm was already in the maze. In this experiment, although both straight and search were used with equal

markedly different from those of others on the ability of octopuses to learn using tactile information [1–3, 7]. Early researchers suggested a transfer of a learned tactile discrimination between arms within 30 min of the discrimination being taught to a single arm [24]. However, the present study is unique in that, upon encountering the tactile stimulus, the animal was required to make an immediate decision as to whether to continue the movement, to withdraw from the maze, or to redirect within the maze. Octopuses clearly learned to correctly make these decisions, as correct choices were made both in trials in which the correct side was entered first and in trials in which the incorrect side was entered first and the animal had to change or restart the movement.

The question remains whether this learning is entirely a CNS phenomenon or whether individual arms learn the task through a mechanism that is independent of the CNS. Clear involvement of the CNS is shown by learning of the food association without visual input; however, details of what makes a specific side correct or incorrect might still be processed by each individual arm. It is noteworthy that in the proprioceptive learning experiments, in which the octopuses learned to direct an arm repeatedly to the same side, they never explored the interior of the maze during the learning process. Rather, they used the rapid, straight arm movement, and proceeded directly to the goal box as soon as they entered the maze. This strongly suggests that the direction of arm movement is dictated from the CNS and not learned by the individual arms. As to the tactile learning

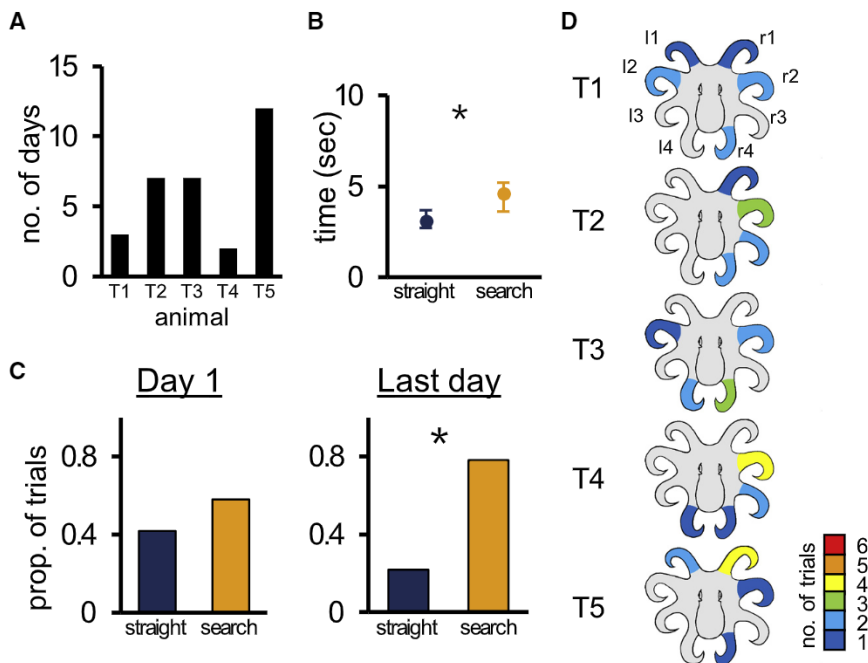


Figure 4. Tactile Learning, Arm Movement Strategies, and Use of Multiple Arms

(A) Number of days to reach criterion for learning. (B) Octopuses used two arm movement strategies in the maze, straight ($n = 32$) and search ($n = 67$), which differed in duration from arm insertion into the maze till choice. Median duration of movements (median \pm 95% CI). Mann-Whitney $n = 99$, $p < 0.001$, $z = 4.339$. See also [Video S2](#).

(C) Proportion of trials using the two movement strategies on day 1 (binomial $p = ns$) and the last day of the experiment (binomial $p < 0.001$).

(D) Arms used for making correct choices on the last day of the experiment.

See also [Figure S2](#); [Tables S1](#) and [S2](#).

experiment, in which the decision was made after an arm had already entered the maze, it is more difficult to determine whether the tactile information is processed at the level of the arm, the CNS, or both. However, in order for an arm that reached criterion to be the arm that learned the task independently, it must have at least been both successful and unsuccessful during the training process. Thus, arms that had never been used before, arms that had been used before but had never been correct, and arms that had only ever been used correctly before could not have learned independently. A definitive solution to the question of central versus peripheral learning is hampered by the relatively small number of animals and trials. On one hand, two animals did reach criteria using arms that had never been used correctly during the training; these arms could not have independently learned the task. One of these animals, used two naive arms a total of five times, each correctly. On the other hand, many of the other animals did make both correct and incorrect choices during the course of learning using arms that were then used once criterion was reached and therefore might have learned independently. It thus remains possible that the CNS-based, goal-directed behavior is accomplished in part through interaction between central and peripheral learning mechanisms.

Researchers have never found a vertebrate-like mototopic representation of the octopus's body and arms in the higher brain centers [8, 25]. This, combined with the potentially huge amount of information generated in the peripheral nervous system (PNS) raises interesting questions about how this invertebrate's CNS might deal with input from the periphery [1, 21, 22, 26]. The octopus's arms are replete with local reflexes. Indeed, some octopus species actually discard arms as a protective mechanism, and the disconnected arm continues to move in a seemingly coordinated fashion for a prolonged period. Nevertheless, despite the abundant and complex information processing at the peripheral level, our findings make it clear

that the activities of an octopus's arms are not totally independent of the CNS. Indeed, the arms are integral parts of the entire organism's ability to understand and interact with its surroundings. Rather than considering the octopus to be a nine-brained animal, it is probably more accurate to deem it an invertebrate animal with one elaborate brain and eight very clever appendages.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.08.037>.

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AUTHOR CONTRIBUTIONS

Conceptualization and Methodology, T.G., L.Z., B.H., and M.J.K.; Investigation, T.G. and M.J.K.; Writing, T.G. and M.J.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Wild type <i>Octopus vulgaris</i> collected by local fishermen	Local fishermen	Wild type
Software and Algorithms		
MS Office	HUJI software	N/A
Final Cut	HUJI software	N/A
The Observer XT, Noldus	HUJI software	N/A
SPSS	HUJI software	RRID:SCR_002865

RESOURCE AVAILABILITY**Lead Contact**

Further information or materials associated with this research will be made available upon reasonable request to lead contact, Michael Kuba (michi.kuba@mac.com).

Materials Availability

This study did not generate any new or unique reagents.

Data and Code Availability

This study did not generate any unique datasets or code.

EXPERIMENTAL MODEL AND SUBJECT

12 wild-caught *Octopus vulgaris* (Lamarck, 1798) (250–450 g bodyweight) were collected by fishermen from the Israeli coast of the Mediterranean Sea. The animals were housed individually in 100-l artificial sea water tanks within a closed circulation system, and held according to the guidelines for the EU Directive 2010/63/EU for cephalopod welfare [2]. Tanks were enriched with clay-pot dens, gravel, rocks and green algae (*Caulerpa prolifera*), temperature of the holding rooms was held constant at about 19°C. Day and night cycles were simulated by artificial illumination for 12 hours. Animals were fed every other day with either dead shrimps or pieces of fish. Once training began animals were fed through the maze.

Six octopuses were trained in a proprioceptive learning task and six were trained in a tactile learning task.

Ethical statement

All experimental animals were held according to the guidelines for the EU Directive 2010/63/EU for cephalopod welfare [27, 28]. For training, food was used as a positive reinforcement and no negative reinforcement was used. If during the training an animal did not receive enough food reward it was fed additional food 1–2 hours after end of training.

METHOD DETAILS**Study design****Experimental maze**

We designed a Plexiglas two-choice Y shaped maze that attached to the front glass of the home tank. The maze was transparent to the observer and opaque to the octopus (Figures 1A and 3A). The shape allowed octopuses to insert only a single arm. The central stem opened into two side tubes each ending in a goal box where a food reward could be placed. Goal boxes could be divided with a removable net, and food placed behind the net could not be pulled out by the octopus.

Pretraining

After initial acclimatization for 2–3 weeks, pretraining sessions began once every two days. For each trial the opaque Plexiglas maze was attached to the front glass of the tank. The maze was baited with food throughout the central stem, both side tubes and both goal boxes (Figures 1E and 3F). Animals were lured to approach it by placing a visible piece of food at the entrance to the central stem. When the octopus removed the food from one of the goal boxes the maze was removed from the tank and refilled with food to prevent an arm reaching a goal box without getting a food reward. Once an octopus approached and inserted an arm into the maze for several

trials, food was placed only in the side tubes and goal boxes. Animals were considered ready for training when they consistently recovered the food from the maze and entered the arm all the way into a goal box.

Side Preference

For side preference trials we used the opaque Plexiglas maze with available food in both goal boxes (Figures 1E and 3F). A choice was marked as the point at which any part of the arm entered into a goal box. Every choice was rewarded, and the next trial began only after the reward was eaten. Each trial lasted until a choice was made; or if the octopus did not make a choice within a maximum of 3 min the maze was removed. If within 1 min from the beginning of the trial the octopus did not make any attempt to approach the maze it was lured with movement outside the tank. If the octopus did not approach the maze in three consecutive trials training was ended for the day. Trials in which the animal approached the maze, but no arm reached a goal box were recorded as no choice. Sessions were conducted once every two days and consisted of 10 trials. Each animal completed a total of 20 side preference trials. At the end of the trial, the time, the choice made, whether the animal was lured and whether it took the food, were recorded.

Proprioception Experiment

For this test, the octopuses were trained to insert an arm into the maze and go to the same goal box on every trial (Figure 1). Three octopuses were assigned side 1 correct, and three were assigned side 2 correct. To prevent octopuses from using chemical information to find the correct side, both goal boxes contained food, but the food was blocked by a net on the incorrect side. Apart from the location the side tubes were identical, and the maze was opaque, preventing the octopus from using visual cues to locate the correct side or the location of its arm in the maze.

Tactile Experiment

For this test, the original Y maze design was modified to include tactile stimuli, three grooves on the flat surface inside one of the side tubes (Figure 3).

Three octopuses were assigned a correct stimulus “*rough+smooth-*” and three were assigned “*smooth+rough-*.” In order to randomize the position of the correct goal box, we used two mazes, with the stimulus on opposite sides, and presented them following a restricted randomness sequence [29]. Briefly, the Fellows restricted randomness sequence, in this case used as a list of right left positions, was designed to prevent the successful completion of the task by an animal following a non-learning rule. This prevents reaching criterion for learning based on: win-stay lose-stay; win-switch lose-switch; win-stay lose-switch; or win-switch lose-stay strategies, all of which require no learning of the task. To prevent octopuses from using chemical information to find the correct side, both goal boxes contained food, but the food was blocked by a net on the incorrect side. The maze was opaque to the animal, preventing the animal from using visual cues to locate the correct side or the location of its arm in the maze.

End of Trial

During each trial, the animals made only one choice and were not allowed to correct. If the arm entered the correct goal box, the animal was given time to retrieve the food reward before the maze was removed. If the arm entered the incorrect goal box, the maze was immediately removed.

At the end of the trial, the time, the choice made, correct or incorrect, whether the animal was lured and whether it took the food, were recorded.

QUANTIFICATION AND STATISTICAL ANALYSIS

Video recording

All side preference and experiment sessions were recorded using a digital video camera recording in HD format positioned in front of the housing tank giving a frontal view of the front glass and maze. All sessions were later imported to Observer XT, for frame-by-frame analysis.

Video analysis

In subsequent video analyses time points were recorded for: first touch of the maze; arm insertion into the central stem; arm at bifurcation point; arm entered side 1; arm enter side 2; and choice. Additionally, each trial was marked with the number of arm insertions into the maze; number of entries to each maze side, movement strategy of the arm inside the maze; the identity of the individual arm making the choice; position of the animal relative to maze (as marked by eye level and position of the mantle).

Statistical methods

Successes within each stage of the experiment were analyzed using χ^2 tests. Frequency of arm movements used were compared using binomial tests, and their durations were compared using Mann-Whitney U test. Arm preference was calculated on the 4 most frequently used arms using χ^2 tests, assuming an equal probability of use if no preference exists. In the case of animal P4, preference was calculated between all 7 arms used, as 4 of these were used with the same frequency.

For tactile experiment – 1 outlier was removed from statistical calculation of arm movement duration (animal T1, trial 5, 63.8sec). During this trial, the animal stopped reacting while his arm was already in the maze and had to be lured with movement outside the tank in order to complete the trial.

Observer reliability (Cohen's kappa for inter-rater reliability) was calculated between the initial recordings during the experiment and later video analysis, viewed by one experimenter who was not involved in filming (For tactile experiment - $\kappa = 0.949 \pm 0.025$, $p < 0.001$; For proprioception experiment - $\kappa = 0.977 \pm 0.013$, $p < 0.0001$). For arm entrances into the maze an additional observer analyzed 5% of all trials (For tactile experiment - insertions and redirection of arm $\kappa = 0.911 \pm 0.087$, $p < 0.0001$; first side entered $\kappa = 0.407 \pm 0.187$, $p < 0.05$; For proprioception experiment – arm and tilt direction $\kappa = 0.556 \pm 0.163$, $p < 0.005$.) All tests are 2 sided).