

Nonsomatotopic Organization of the Higher Motor Centers in Octopus

Letizia Zullo,^{1,2,*} German Sumbre,^{1,5} Claudio Agnisola,³ Tamar Flash,⁴ and Binyamin Hochner¹

¹Department of Neurobiology, Institute of Life Sciences and Interdisciplinary Center for Neuronal Computation, Hebrew University, Jerusalem 91904, Israel

²Department of Neuroscience and Brain Technologies, The Italian Institute of Technology, 16163 Genova, Italy

³Department of Biological Sciences, University of Naples Federico II, 80134 Napoli, Italy

⁴Department of Computer Science and Applied Mathematics, Weizmann Institute of Science, Rehovot 76100, Israel

Summary

Hyperredundant limbs with a virtually unlimited number of degrees of freedom (DOFs) pose a challenge for both biological and computational systems of motor control. In the flexible arms of the octopus, simplification strategies have evolved to reduce the number of controlled DOFs [1–3]. Motor control in the octopus nervous system is hierarchically organized [4, 5]. A relatively small central brain integrates a huge amount of visual and tactile information from the large optic lobes and the peripheral nervous system of the arms [6–9] and issues commands to lower motor centers controlling the elaborated neuromuscular system of the arms. This unique organization raises new questions on the organization of the octopus brain and whether and how it represents the rich movement repertoire. We developed a method of brain microstimulation in freely behaving animals and stimulated the higher motor centers—the basal lobes—thus inducing discrete and complex sets of movements. As stimulation strength increased, complex movements were recruited from basic components shared by different types of movement. We found no stimulation site where movements of a single arm or body part could be elicited. Discrete and complex components have no central topographical organization but are distributed over wide regions.

Results

Type of Evoked Movements

Thirty-five sites in the supraesophageal mass (SeM) of 20 animals were examined for their response to electrical stimulation (see Figures S1A and S1B available online). At each site, we investigated the threshold for eliciting different behaviors (Figure 1). Ten identified patterns of motor behavior were commonly evoked; we have broadly divided these into “discrete responses” and “complex responses” (Figure S1).

The discrete responses show shorter response-onset latency than the complex responses, possibly due to the longer time needed to recruit more distributed circuits. In the

different experiments, stimulation at threshold, ranging from 3V to 40V (~0.4 s at 100 Hz), elicited changes in skin color or texture over part of the skin or over the entire body, as well as weak motor responses (e.g., small movement of eyelids, neck, etc.). We labeled these motor responses “discrete responses” and subdivided them into four different response types (Figure S1): change in color, retroflex posture (anterior arms covering the head as if for protection), contraction of eye muscles, and eye and head rotation. These responses had a latency of 0.08 to 0.240 s after stimulus onset and ceased with cessation of the stimulation. Because duration of the discrete response correlated well with stimulus duration, these responses apparently need to be sustained by continuous stimulation.

Increasing stimulus voltage led to a gradual recruitment of more complex movements (Figure 2; Movie S1). The sequence of recruitment of the discrete movements into the final complex behavior was relatively constant among different animals and experiments (Figure 2). Complex behaviors were induced at 3V to 80V (~0.4 s at 100 Hz) in the various region stimulated.

These “complex responses” appeared to be built up of sets of basic behavioral components. The initiation of these complex movements depended on stimulus duration: at least 100 ms was required to recruit complex movements. Once the movement was elicited, it continued during the stimulus duration. No recruitment of different behavior was observed by increasing the stimulus duration. We identified four main complex responses: arm extension displays [1], crawling [10], jet-propelled swimming (described as “jet” in [10]), and inking [11]. We failed to evoke certain movements of the animal’s natural behavioral repertoire, for instance, the stereotypic fetching movement [2, 3].

Topographic Organization of Movements

To investigate the representation of movements in the higher motor centers, we mapped postmortem the region within the SeM where each movement was elicited (for details, see Supplemental Experimental Procedures). The sites in the SeM where electrical stimulation evoked discrete and complex responses are shown in Figure S1B and Figure 1. Microstimulation at many sites induced both discrete and complex behaviors at low and high stimulus amplitudes, respectively. As shown in Figure 1, not all of the elicited behaviors could be evoked equally within the SeM. We found that the only systematic dependence on the area stimulated was for the lifting/standing movement, which showed a type of a “clustering” in the lower posterior part of the SeM (see Figure S1B). Stereotypic arm extension could be evoked by stimulating the basal lobe system (Figure 1A; see lobe location in Figure S1A). Crawling movements were elicited in the basal and the subvertical areas (Figure 1B), as were jet-propelled swimming (Figure 1C) and ink ejection (Figure 1D). Responses tended to either be ipsilateral to the site of stimulation (in more than 60% of the cases) or have no significant laterality (in less than 40% of the cases), whereas stimulation in the midline tended to evoke responses not specific to either side (in 86% of the cases). This is consistent with retrograde labeling results showing that, although the organization of efferent

*Correspondence: letizia.zullo@iit.it

⁵Present address: Department de Neurobiologie, École Normale Supérieure, 75005 Paris, France

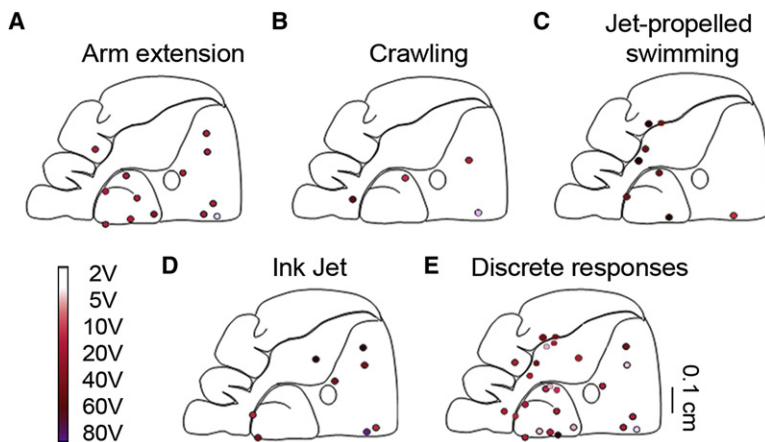


Figure 1. Microstimulation Maps

Stimulated loci are mapped as colored spots projected onto parasagittal sections of the supraesophageal mass (SeM). Stimulus threshold is marked by colors as shown in the color bar at the lower left.

- (A) Distribution of loci evoking arm extension display movements.
- (B) Distribution of loci evoking crawling movements.
- (C) Distribution of loci evoking jet-propelled swimming.
- (D) Distribution of loci evoking ink ejection behaviors.
- (E) Distribution of loci evoking discrete responses.

cell bodies innervating the arms is not entirely ipsilateral, their density is higher on the side of the innervated arm [12].

Evoked Complex Movements

Arm Extension Display

The sequence of movements involved in this evoked behavior is described in Figure 2. The voltage threshold for evoking this movement ranged from 3V to 20V for different regions within the central nervous system (CNS) (Figure 1A). Once the threshold for evoking an arm extension for a given site had been established, repeated stimulation reliably evoked the same movement (Figure 3A).

In all cases (7 animals, number of repetitions [n] = 40), evoked arm extensions kinematically resembled natural arm extensions (Figure 3B). The bend moved along a single linear plane in a simple, slightly curved path (data not shown) with a typical bell-shaped velocity profile (peak velocity from ~11 to 40 cm/s; Figures 3A and 3C). The evoked movements showed the typical three phases of arm extension: generation of a bend (phase I), often missing in the evoked movements; an accelerating phase (phase II); and a deceleration phase (phase III) [1, 13]. There was a direct correlation between stimulus and movement duration (Figure 4B).

Stimulus trains of ~0.4–0.5 s evoked bend propagation, but the bend did not propagate to the tip of the arm (Figure 3C,

blue and cyan traces). With longer stimulus durations (>0.6 s), the bend did reach the end of the arm, and the decelerating phase occurred during the stimulation (Figure 3C, red and orange traces).

The stimulus parameters for evoking arm extension via the SeM differed from those required to evoke arm extension by peripheral nervous system (PNS) stimulation. Even short trains of stimuli to the PNS evoked full arm extension [14]. In contrast, when stimulating the SeM, the train needed to last at least 75% of the duration of the acceleration phase (>0.6 s), and the movement continued for the duration of the stimulus. It seems, therefore, that a continuous central stimulation is needed for gating the arm extension command.

Evoked arm extensions were usually accompanied by forward or lateral movement of the animal. In addition, single stimulus trains always elicited extensions of several arms, either together or consecutively within 0.02 s and with comparable kinematics for different arms (Figure 3A). Similar patterns can be seen in naturally behaving octopuses, which extend two or more arms with similar velocity profiles simultaneously or with a delay of 0.4–1.3 s [1]. These results suggest that, in both normal [1] and centrally evoked behavior, the higher motor center generates a single extension command that is distributed to several arms. Issuing one motor command to several arms at once may simplify the central motor control of the eight arms. Although the octopus frequently uses a few arms together rather than a single arm for most tasks [10, 15], it can use sensory inputs, i.e., tactile or visual information, to guide a single arm toward a stimulus [2, 3, 16–18].

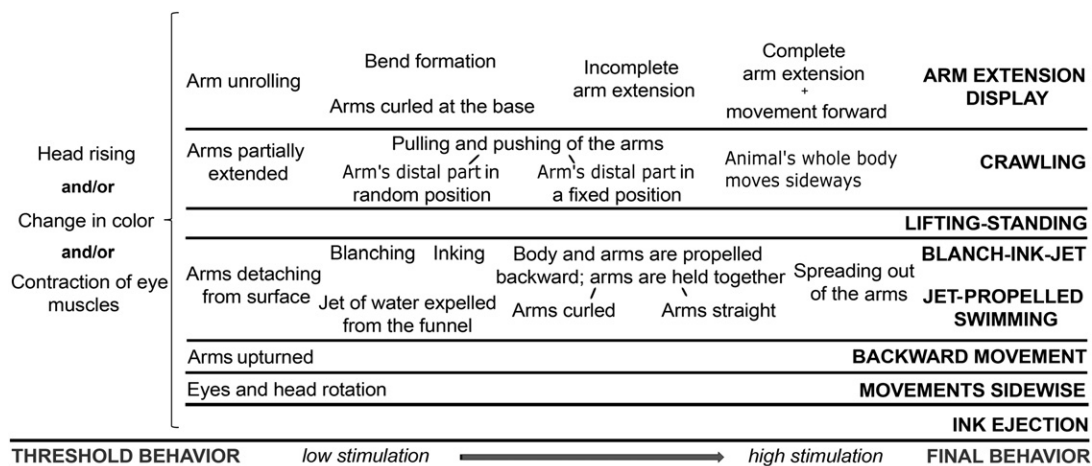


Figure 2. Description of the Four Complex Movements Elicited by Brain Microstimulation

Movements are identified by the sequence of their behavioral components. Discrete components occur at low stimulus strengths, whereas higher stimulus strengths are required to evoke a complete complex behavior.

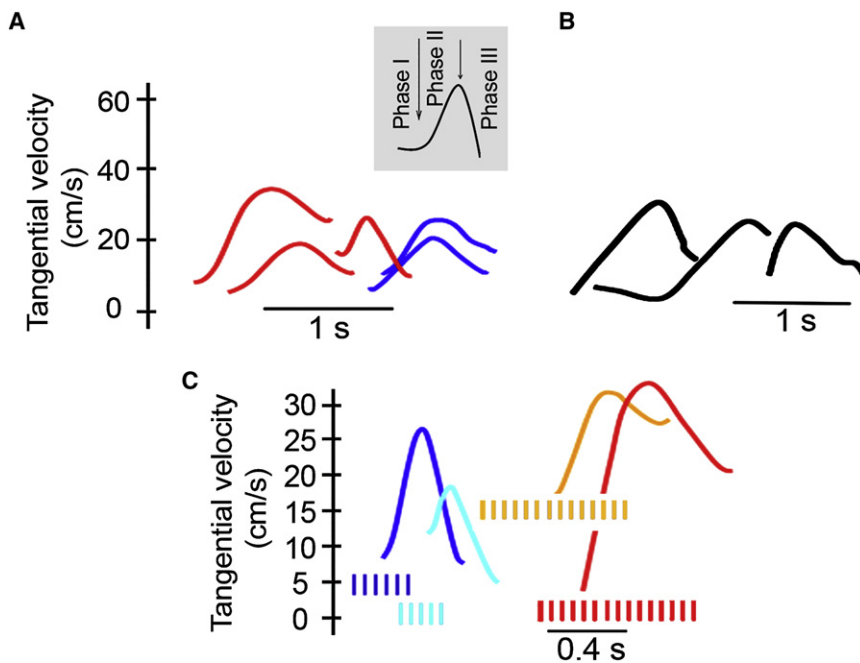


Figure 3. Velocity Profiles of Spontaneous and Electrically Induced Arm Extension

(A) Velocity profiles of evoked extension (one animal, one stimulation session). Velocity profiles of arms L1 and R1 evoked by the same stimulus are shown in blue (stimulation parameters 1.5V, 0.6 s, 100 Hz). Inset: velocity profiles are described by three phases; the transition between the different stages is indicated by the two arrows. The velocity profiles of the different arms are similar. (B) Velocity profiles of spontaneous extensions. (C) Arm extension velocity profiles elicited in various arms with short (~0.5 s, blue and cyan traces) and long (>0.6 s, red and orange traces) stimulus trains. Timing of stimulus pulses is marked schematically.

Sensory information might act as a gate to direct this global command to a specific arm, possibly at the level of lower motor control centers.

Crawling

The crawling movements evoked by central stimulation closely resembled natural crawling movements with their alternating rhythmical movements of the arms on both sides [10, 15]. The recruitment sequence of components involved is described in Figure 2. The evoked movement was always directed toward the left or right, with a clear correlation between the direction of movement and the side of the implanted electrode. The threshold for evoking this behavior varied from 2V to 20V for different regions within the CNS (Figure 1B), and there was a direct correlation between stimulus and movement duration (Figure 4B). Stimulation repeated at least ten times at each site reliably evoked a similar crawling pattern, suggesting that the behavior is a primary and not a secondary response to stimulation. The stimulation may have activated a central pattern generator inducing the rhythmic movements.

Jet-Propelled Swimming and Blanch-Ink-Jet Behavior

The sequence of movements involved in this evoked behavior is described in Figure 2. The same sequence has been described for jetting in freely behaving octopuses [10, 15]. The stimulus threshold for obtaining this behavior varied from 8V to 60V for different regions within the CNS (Figure 1C). Stimulation was repeated between two and fifteen times to ensure reproducibility of the behavior at each site. Once activated, the behavior was evoked consistently by each stimulation. Unlike arm extension and crawling, the duration of jet-propelled swimming was not linearly correlated with stimulus duration (Figure 4B). Thus, in this case, and in contrast to arm extension, the behavior is probably triggered by the central stimulation (see below), with the execution of the whole program lasting longer than the stimulus (Movie S1). On the other hand, we cannot exclude that the active phase of the behavior, i.e., water expulsion through the funnel, is correlated with the stimulus duration. This movement phase is difficult to discriminate from the rest of the motion.

The blanch-ink-jet behavior [11] was evoked at three different loci in three different (3 animals, n = 30) (Movie S1). The latency from stimulus onset to the appearance of the ink jet was relatively consistent ($\sim 1.9 \pm 0.4$ s), suggesting that the elicited behavior is a primary response to the stimulation. Ink ejection behavior could also be evoked without any swimming component. The stimulus threshold for obtaining this behavior varied from 20V to 80V for different regions within the CNS (Figure 1D).

Relationship between Stimulation and Movement Parameters

The high reproducibility of each response, lack of habituation, absence of clear poststimulation distress responses, and relatively constant onset latency (Figure 4A) indicate that the observed behaviors were directly evoked by the electrical stimulation rather than being secondary responses. These properties resemble data obtained in monkeys [19, 20] and insects [21, 22].

Arm extension, crawling, and jet-propelled swimming showed similar onset latencies (Figure 4A). A positive correlation between stimulus and movement durations (Figure 4B) was found for arm extension ($R^2 = 0.67$, $p < 0.0001$) and crawling ($R^2 = 0.69$, $p < 0.0001$), indicating that stimulation sustained the generation of these movements. No correlation was found for jet-propelled swimming ($R^2 = 0.02$, $p = 0.3185$). Thus, it appears that more complex movements are triggered by stimulation, whereas more stereotypic behaviors, like arm extension, need continuous stimulation to sustain the movement.

Discussion

We have shown here that microstimulation of the higher motor centers in the octopus brain can evoke movements and behavioral responses characteristic of the animal's behavioral repertoire. We found no evidence of somatotopic motor representation; the same behavior could be induced by local stimulation throughout the basal lobe system. Increasing stimulus strength gradually recruited the various movement components to achieve complex behavioral responses. We therefore suggest that movements are represented in the higher motor centers by a number of overlapping circuits that are not somatotopically organized. Increasing the stimulus voltage above threshold possibly increases the areas of the brain affected

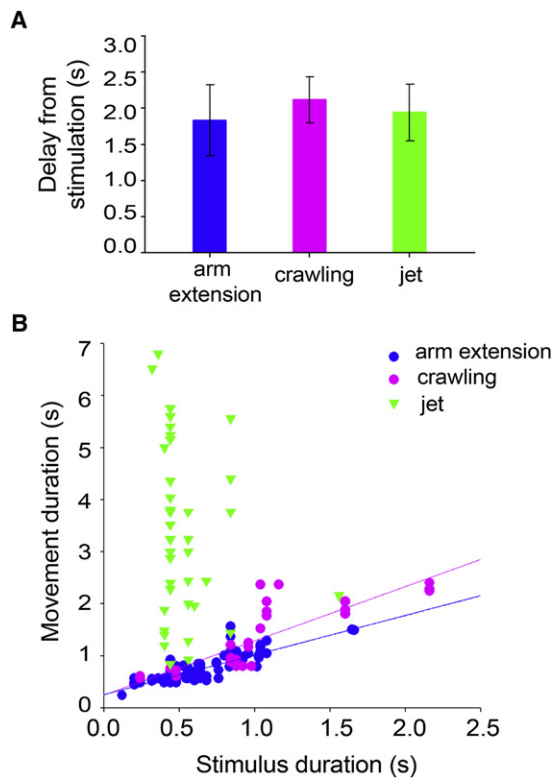


Figure 4. Relation between Stimulus and Movement Parameters for Arm Extension, Crawling, and Jet-Propelled Swimming

(A) Latency from stimulus onset to the start of movement. The three complex movements show relatively constant latencies (mean \pm standard error of the mean).

(B) Correlation between stimulus train and movement duration. Arm extension and crawling show a correlation between stimulus and movement duration, whereas jet-propelled swimming (jet) does not.

by the stimulation, resulting in sequential recruitment of movement components in the overlapping circuits (Figure 2).

Our physiological results fit morphological data [6] that also suggest a lack of a somatotopic organization in the higher motor centers (the basal lobes; Figure S1A, AB and PB). Morphological studies have shown that the higher motor centers receive input from several major pathways from the optic lobes and other sensory centers that project to both intermediate and higher motor centers [8]. Based on these findings, it has been hypothesized that the basal lobes, together with the peduncle lobes, are a major area for the integration of visual, vestibular, and proprioceptive inputs, but this issue requires further experimental clarification [5, 23–26].

Our results may be comparable to brain microstimulation studies in vertebrates that have shown that complex movements can be elicited by stimulation of the parietal cortex association area, an important multisensory integrative center [20], whereas multijoint movements are evoked by microstimulation of motor cortex [27, 28], where movements and body parts are coarsely represented somatotopically [19]. In vertebrates, unlike in the octopus, the areas devoted to motor control and to integrative processes tend to be morphologically distinct [19]. Our study shows that the response elicited from stimulation of the octopus higher motor center is similar to the response obtained by stimulating vertebrate integrative areas.

In contrast to vertebrate, insect, and even molluscan brains, centripetal cobalt filling in the cephalopod brain has shown

that there is no obvious somatotopic arrangement of motor and premotor neurons. The octopus thus represents a unique case among other mollusks and invertebrates. These animals show a morphological somatotopy in the distribution of sensory and mechanosensory projections to the CNS [29, 30].

We hypothesize that the absence of motor somatotopic representations in the octopus CNS might have evolved together with its unique body plan of an active body with eight long and highly flexible arms. We previously showed in the octopus that there is a division of labor between the CNS and the PNS: a relatively small central brain (\sim 50 million neurons out of a total of \sim 500 million neurons) controls the large, complex, and highly autonomous PNS of the arms (\sim 300 million neurons), as well as integrating processed information from the huge visual system (\sim 120 million neurons). The intermingled and distributed neural networks suggested by our results might point to a unique organization wherein single cells or groups of cells are dynamically recruited into several different higher control networks.

This raises the question of whether integration of multimodal sensory information [31] is achieved by this special nonsomatopic organization of the higher motor centers. Preliminary recordings in higher motor areas in freely behaving octopuses (L.Z. and B.H., unpublished data) found cells responding to different modalities (i.e., visual, tactile) within the same discrete area. This finding might support the hypothesis that crossmodal integration is indeed achieved in the octopus higher motor centers.

Experimental Procedures

Twenty adult *Octopus vulgaris* from the Mediterranean Sea were used for experiments. For electrode implantation, animals were deeply anaesthetized in artificial sea water supplemented with 3.5% MgCl₂. A single micro-wire electrode [32] made from Teflon-coated stainless steel wire was manually inserted into the supraesophageal mass (SeM). Following surgery, the animals were placed in the experimental tank and electrical stimulation protocols were applied. The stimulation signals and the elicited behaviors were video recorded together for offline analysis. Video sequences of interest were selected for frame-by-frame analysis with custom-built software (MATLAB). Movements, behaviors, and changes in skin color and texture were described as in previous studies [1, 10, 11, 13, 15, 33–35]. The electrode location was identified via the electrocoagulation method as in [36, 37]. The size of the lesion varied, usually extending 0.02–0.1 cm (for details, see Supplemental Experimental Procedures).

Supplemental Data

Supplemental Data include Supplemental Introduction, Supplemental Results, Supplemental Experimental Procedures, one figure, and one movie and can be found with this article online at [http://www.cell.com/current-biology/supplemental/S0960-9822\(09\)01546-2](http://www.cell.com/current-biology/supplemental/S0960-9822(09)01546-2).

Acknowledgments

This work was supported by US Defense Advanced Research Projects Agency grant N66001-03-R-8043, Israel Science Foundation grant 580/02, and the Interdisciplinary Center for Neural Computation exchange program in Computational Neuroscience. We thank H. Meiri and N. Feinstein for excellent technical assistance, J. Kien for suggestions and editorial assistance, M. Kuba for critical assistance in behavioral descriptions, and Y. Yekutieli and G. Fiorito for valuable discussions.

Received: May 5, 2009

Revised: July 27, 2009

Accepted: July 29, 2009

Published online: September 17, 2009

References

- Gutfreund, Y., Flash, T., Yarom, Y., Fiorito, G., Segev, I., and Hochner, B. (1996). Organization of octopus arm movements: A model system for studying the control of flexible arms. *J. Neurosci.* **16**, 7297–7307.
- Sumbre, G., Fiorito, G., Flash, T., and Hochner, B. (2005). Neurobiology: Motor control of flexible octopus arms. *Nature* **433**, 595–596.
- Sumbre, G., Fiorito, G., Flash, T., and Hochner, B. (2006). Octopuses use a human-like strategy to control precise point-to-point arm movements. *Curr. Biol.* **16**, 767–772.
- Wells, M.J. (1978). *Octopus: Physiology and Behaviour of an Advanced Invertebrate* (London: Chapman and Hall).
- Messenger, J.B. (1983). Multimodal convergence and the regulation of motor programs in cephalopods. *Fortschritte der Zoologie* **28**, 77–98.
- Budelmann, B.U., and Young, J.Z. (1985). Central pathways of the nerves of the arms and mantle of octopus. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **310**, 109–122.
- Budelmann, B.U. (1995). The cephalopods nervous system: What evolution has made of the molluscan design. In *The Nervous System of Invertebrates: An Evolutionary and Comparative Approach*, O. Breidbach and W. Kutsch, eds. (Basel: Birkhauser Verlag), pp. 115–138.
- Young, J.Z. (1971). *The Anatomy of the Nervous System of Octopus vulgaris* (Oxford: Clarendon Press).
- Hochner, B. (2008). Octopuses. *Curr. Biol.* **18**, R897–R898.
- Huffard, C.L. (2006). Locomotion by *Abdopus aculeatus* (Cephalopoda: Octopodidae): Walking the line between primary and secondary defenses. *J. Exp. Biol.* **209**, 3697–3707.
- Hanlon, R.T., and Messenger, J.B. (1996). *Cephalopod Behaviour* (New York: Cambridge University Press).
- Robertson, J.D., Schwartz, O.M., and Lee, P. (1993). Carbocyanine dye labeling reveals a new motor nucleus in octopus brain. *J. Comp. Neurol.* **328**, 485–500.
- Gutfreund, Y., Flash, T., Fiorito, G., and Hochner, B. (1998). Patterns of arm muscle activation involved in octopus reaching movements. *J. Neurosci.* **18**, 5976–5987.
- Sumbre, G., Gutfreund, Y., Fiorito, G., Flash, T., and Hochner, B. (2001). Control of octopus arm extension by a peripheral motor program. *Science* **293**, 1845–1848.
- Sinn, D.L., Perrin, N.A., Mather, J.A., and Anderson, R.C. (2001). Early temperamental traits in an octopus (*Octopus bimaculoides*). *J. Comp. Psychol.* **115**, 351–364.
- Mather, J.A. (1998). How do octopuses use their arms? *J. Comp. Psychol.* **112**, 306–316.
- Byrne, R.A., Kuba, M.J., Meisel, D.V., Griebel, U., and Mather, J.A. (2006a). Does *Octopus vulgaris* have preferred arms? *J. Comp. Psychol.* **120**, 198–204.
- Byrne, R.A., Kuba, M.J., Meisel, D.V., Griebel, U., and Mather, J.A. (2006b). Octopus arm choice is strongly influenced by eye use. *Behav. Brain Res.* **172**, 195–201.
- Graziano, M.S., and Aflalo, T.N. (2007). Rethinking cortical organization: Moving away from discrete areas arranged in hierarchies. *Neuroscientist* **13**, 138–147.
- Cooke, D.F., Taylor, C.S., Moore, T., and Graziano, M.S. (2003). Complex movements evoked by microstimulation of the ventral intraparietal area. *Proc. Natl. Acad. Sci. USA* **100**, 6163–6168.
- Huber, F. (1960). Untersuchungen über die Funktion des Zentralnervensystems und insbesondere des Gehirns bei der Fortbewegung und der Lauterzeugung der Grillen. *Z. Vergl. Physiol.* **44**, 60–132.
- Kien, J. (1983). The initiation and maintenance of walking in the locust: An alternative to the command concept. *Proc. R. Soc. Lond. B Biol. Sci.* **219**, 137–174.
- Messenger, J.B., and Woodhams, P.L. (1976). A molluscan ‘cerebellum’: Parallel fibers and visuo-motor control. *J. Physiol.* **259**, 20–21.
- Messenger, J.B. (1967). The effects on locomotion of lesions to the visuo-motor system in octopus. *Proc R Soc Lond B Biol Sci.* **167**, 252–281.
- Hobbs, M.J., and Young, J.Z. (1973). A cephalopod cerebellum. *Brain Res.* **55**, 424–430.
- Nixon, M., and Young, J.Z. (2003). *The Brains and Lives of Cephalopods* (New York: Oxford University Press).
- Graziano, M.S., Aflalo, T.N., and Cooke, D.F. (2005). Arm movements evoked by electrical stimulation in the motor cortex of monkeys. *J. Neurophysiol.* **94**, 4209–4223.
- Haiss, F., and Schwarz, C. (2005). Spatial segregation of different modes of movement control in the whisker representation of rat primary motor cortex. *J. Neurosci.* **25**, 1579–1587.
- Vitzthum, H., Müller, M., and Homberg, U. (2002). Neurons of the central complex of the locust *Schistocerca gregaria* are sensitive to polarized light. *J. Neurosci.* **22**, 1114–1125.
- Walters, E.T., Bodnarova, M., Billy, A.J., Dulin, M.F., Diaz-Rios, M., Miller, M.W., and Moroz, L.L. (2004). Somatotopic organization and functional properties of mechanosensory neurons expressing sensorin-A mRNA in *Aplysia californica*. *J. Comp. Neurol.* **471**, 219–240.
- Frye, M.A., and Dickinson, M.H. (2004). Motor output reflects the linear superposition of visual and olfactory inputs in *Drosophila*. *J. Exp. Biol.* **207**, 123–131.
- Kralik, J.D., Dimitrov, D.F., Krupa, D.J., Katz, D.B., Cohen, D., and Nicoletti, M.A. (2001). Techniques for long-term multisite neuronal ensemble recordings in behaving animals. *Methods* **25**, 121–150.
- Trueman, E.R., and Packard, A. (1968). Motor performances of some cephalopods. *J. Exp. Biol.* **49**, 495–507.
- Packard, A., and Sanders, G.D. (1971). Body patterns of *Octopus vulgaris* and maturation of the response to disturbance. *Anim. Behav.* **19**, 780–790.
- Borrelli, L., Gherardi, F., and Fiorito, G. (2006). *A Catalogue of Body Patterning in Cephalopoda* (Florence, Italy: Firenze University Press).
- Chichery, R., and Chanelet, J. (1976). Motor and behavioral responses obtained by stimulation with chronic electrodes of the optic lobe of *Sepia officinalis*. *Brain Res.* **105**, 525–532.
- Chichery, R., and Chanelet, J. (1978). Motor responses obtained by stimulation of the peduncle lobe of *Sepia officinalis* in chronic experiments. *Brain Res.* **150**, 188–193.