Control of Octopus Arm Extension by a Peripheral Motor Program

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genitors of the 1918 virus probably switched hosts from birds to mammals sometime after 1900 (*1, 6*), it is likely that the 1918 HA gene changed at a rate of 0.4 to 8.0% per year after it was generated. Thus, using the predicted sequence difference of 0.4% and the likely range of rates, we estimate that the recombination-to-preservation time was less than 1 year.

The victims from whom the 1918 influenza sequences were obtained died in the major "second wave" of the pandemic in late September and October 1918 (*2, 3*); thus, the 1918 HA gene was probably generated in late 1917 or early 1918. The "first wave" of the pandemic was in early 1918 (*2*), but the first outbreaks may have been in late 1917. Hence, the start of the pandemic coincided with a recombination event that might produce the phenotypic novelty required to trigger a pandemic. This coincidence suggests a causal link.

Recombination, like point mutation and reassortment, produces novel virus variants and can result in increased virulence (*13–15*). Because the HA gene is the major virulence determinant (*3–11*), recombination in this gene may have similarly altered the 1918 virus. The parental H1 HA genes would have been progressively altered by point mutation after their divergence; we estimate that they differed at up to 30 amino acid positions at the time of the recombination, and that the 1918 HA differed from each of its parents at about half as many positions. Recombination may have altered the antigenicity of the HA so that the immunity of those who had survived earlier infections was ineffective. Similarly, the membrane-fusion or receptor-binding function of the HA protein may have changed (*3, 31*), and this may have given the 1918 virus an unusual tissue specificity, such that it spread from the upper respiratory tract to the lungs. Experiments comparing reconstructed 1918 and parental HA proteins may distinguish between these possibilities.

Our analysis suggests that the two parental lineages were probably mammal-adapted and capable of mammal-to-mammal transmission, and yet they did not generate a pandemic. It is possible that the recombination event triggered the pandemic not only by altering HA structure or function, but also by permitting the virus to outcompete these parents or to be the first of these H1-subtype influenzas to switch hosts from some other mammal into humans.

References and Notes

1. J. K. Taubenberger, A. H. Reid, T. G. Fanning, *Virology* **274**, 241 (2000).
2. A. W. Crosby, *America's Forgotten Plague: The Influenza of 1918* (Cambridge Univ. Press, New York, 1989).
3. A. H. Reid, T. G. Fanning, J. V. Hultin, J. K. Taubenberger, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1651 (1999).
4. A. H. Reid, T. G. Fanning, T. A. Janczewski, J. K. Taubenberger, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6786 (2000).
5. C. F. Basler *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 2746 (2001).
6. R. G. Webster, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1164 (1999).
7. J. Lederberg, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 2115 (2001).
8. D. Khatchikian, M. Orlich, R. Rott, *Nature* **340**, 156 (1989).
9. K. Subbarao *et al.*, *Science* **279**, 393 (1998).
10. E. Kilbourne, *J. Am. Med. Assoc.* **237**, 1225 (1977).
11. R. G. Webster, W. J. Bean, O. T. Gorman, T. M. Chambers, Y. Kawaoka, *Microbiol. Rev.* **56**, 152 (1992).
12. O. T. Gorman *et al.*, *J. Virol.* **65**, 3704 (1991).
13. J. P. Anderson *et al.*, *J. Virol.* **74**, 10752 (2000).
14. M. Worobey, A Rambaut, E. C. Holmes, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7352 (1999).
15. J. S. Pita *et al.*, *J. Gen. Virol.* **82**, 655 (2001).
16. S. Fields, G. Winter, *Cell* **28**, 303 (1982).
17. M. Bergmann, A. Garcia-Sastre, P. Palese, *J. Virol.* **66**, 7576 (1992).
18. J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, D. G. Higgins, *Nucleic Acids Res.* **24**, 4876 (1997).
19. M. J. Gibbs, J. S. Armstrong, A. J. Gibbs, *Bioinformatics* **16**, 573 (2000).
20. E. C. Holmes, M. Worobey, A. Rambaut, *Mol. Biol. Evol.* **16**, 405 (1999).
21. D. H. Huson, *Bioinformatics* **14**, 68 (1998).
22. Supplemental material is available at *Science* Online (www.sciencemag.org/cgi/content/full/293/5536/1842/DC1).
23. D. C. Wiley, J. J. Skehel, *Annu. Rev. Biochem.* **56**, 365 (1987).
24. R. E. Shope, *J. Exp. Med.* **63**, 669 (1936).
25. A. B. Beklemishev *et al.*, *Mol. Gen. Mikrobiol. Virusol.* **1**, 24 (1993).
26. M. H. Bikour, E. H. Frost, S. Deslandes, B. Talbot, Y. Elazhary, *J. Gen. Virol.* **76**, 2539 (1995).
27. K. Strimmer, A. von Haeseler, *Mol. Biol. Evol.* **13**, 964 (1996).
28. M. J. Gibbs, G. F. Weiller, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 8022 (1999).
29. C. Scholtissek, S. Ludwig, W. M. Fitch, *Arch. Virol.* **131**, 237 (1993).
30. M. Garcia, J. M. Crawford, J. W. Latimer, E. Rivera-Cruz, M. L. Perdue, *J. Gen. Virol.* **77**, 1493 (1996).
31. J. J. Skehel, D. C. Wiley, *Annu. Rev. Biochem.* **69**, 531 (2000).
32. K. Tamura, M. Nei, *Mol. Biol. Evol.* **10**, 512 (1993).

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Control of Octopus Arm Extension by a Peripheral Motor Program

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For goal-directed arm movements, the nervous system generates a sequence of motor commands that bring the arm toward the target. Control of the octopus arm is especially complex because the arm can be moved in any direction, with a virtually infinite number of degrees of freedom. Here we show that arm extensions can be evoked mechanically or electrically in arms whose connection with the brain has been severed. These extensions show kinematic features that are almost identical to normal behavior, suggesting that the basic motor program for voluntary movement is embedded within the neural circuitry of the arm itself. Such peripheral motor programs represent considerable simplification in the motor control of this highly redundant appendage.

In directed voluntary movements, the nervous system generates a sequence of motor commands producing the forces and velocities that efficiently bring the limb to the target (*1*). In articulated appendages, the control of goal-directed movements appears to be simplified by the planning of optimal trajectories (*2*, *3*), by vectorial summation and superposition of basic movement primitives (*4*, *5*), and by the use of a flexible combination of muscle synergies (*6*). However, flexible structures introduce a further dimension of complexity.

The octopus arm can move in any direction, using a virtually infinite number of degrees of freedom. This high maneuverability results from octopus arms behaving like a muscular hydrostat, because they are almost entirely constructed of densely packed muscle fibers along their transverse, longitudinal, and oblique axes (*7*). These flexible arms are controlled by an elaborate peripheral nervous system containing $\sim 5 \times 10^7$ neurons distributed along each arm. Only $\sim 4 \times 10^5$ of these are motor neurons (*8*), which innervate the intrinsic

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muscles of the arm and locally control muscle action (*9*). This peripheral nervous system is organized as an axial nerve cord composed of ~300 interconnected ganglia and two cerebrobrachial (axonal) tracts of ~30,000 nerve fibers running dorsally to the ganglia (Fig. 1B). The axons in the tracts carry sensory and motor information to and from the highly developed centralized brain (*10*).

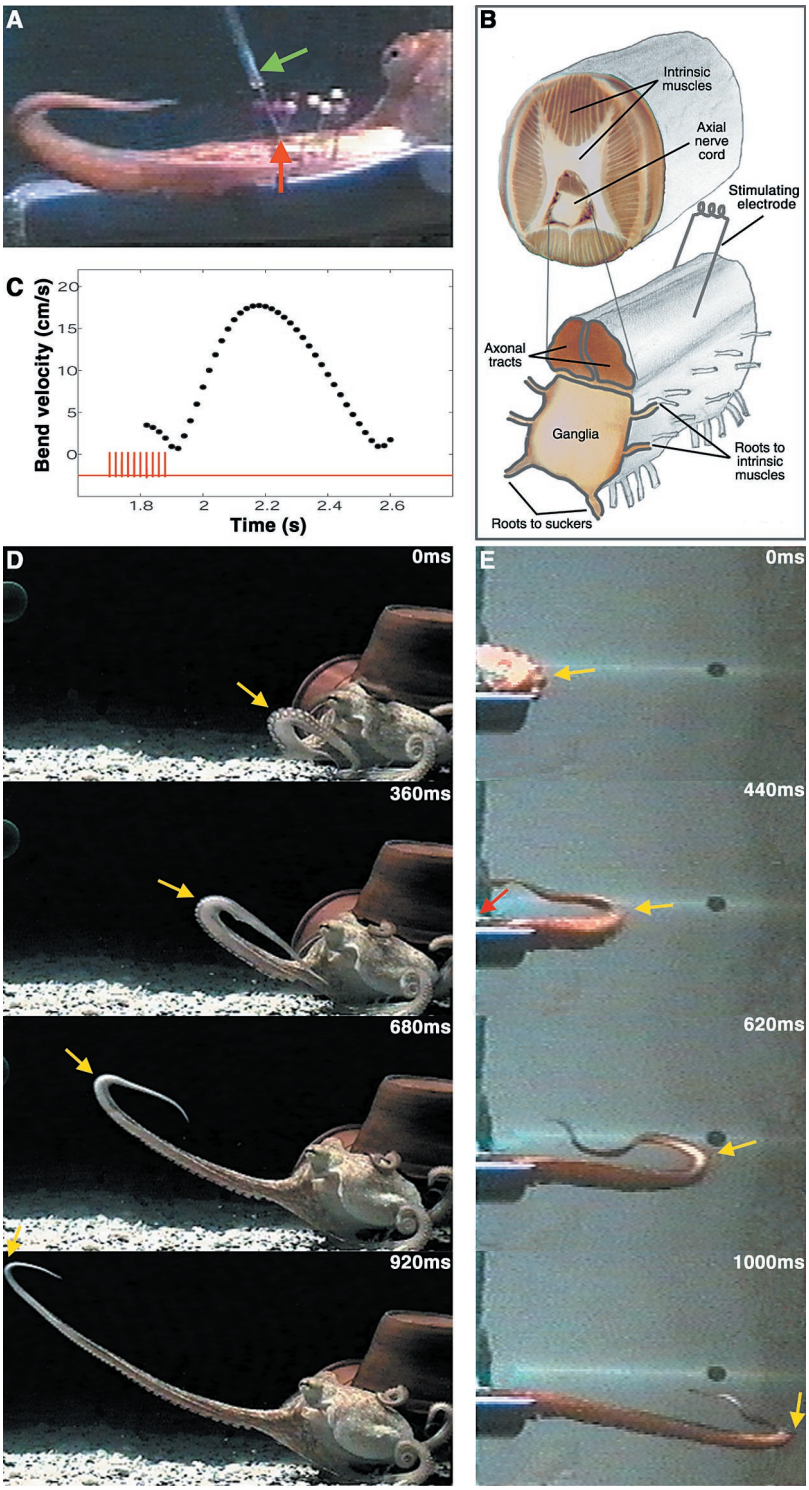

Fig. 1. Natural and evoked arm extensions are qualitatively similar. (**A**) A decerebrated preparation was fixed to the experimental platform (green arrow, stimulating electrode; red arrow, area where the axial nerve cord was transacted and stimulated). (**B**) The arrangement of the intrinsic arm muscles and an enlarged portion of the axial nerve cord showing the stimulating site. (**C**) A stimulus train (red trace) is superimposed on the velocity profile of an evoked arm extension (black dots). The acceleration phase begins after the stimulus ends. (**D**) A freely behaving octopus reaching toward a target. (**E**) Electrically evoked bend propagation in the denervated arm of a decerebrated animal (yellow arrows indicate the bend point).

The octopus reduces the complexity of controlling this flexible appendage by using highly stereotypical movements. Reaching movements consist of a bend propagating along the arm toward the tip (Fig. 1D) in a highly stereotypical and invariant way (*11*). The level of muscle activity [measured with an electromyogram (EMG)] shows a positive correlation with kinematic variables, and the EMG level measured during the initial stages of the movement predicts the bend's peak propagation velocity attained later during the movement. These predictive relations suggest that feed-forward motor commands play an important role in the motor program for arm extension (*12*).

Here we show that arm extensions can be elicited in denervated arms by electrical stimulation of the arm axial nerve cord or by tactile stimulation of the skin, suggesting that a major part of this voluntary movement is controlled by a pattern generator that is confined to the arm's neuromuscular system.

A short train of electrical stimulation to the dorsal part of the denervated nerve cord (*13*) evoked movements that involved the entire arm. Forty-six percent of these movements were characterized by a bend traveling forward along the arm (*14*). In 20 of these extensions, the movement occurred after the termination of the stimuli, indicating that the movement was indeed triggered by the stimulation and is not directly driven by the stimuli (Fig. 1C). In the remaining 30 movements, the movement started before the end of the stimulation train. The average overlap between the stimulation train and the acceleration phase of the movements was only 19%. Because kinematic analysis showed no differences between the two types of movements, both were combined for further analysis. Arm extensions were evoked by stimulation of the dorsal part of the axial nerve cord that contains the axonal tracts from the brain (Fig. 1B). In contrast, stimulation of the muscles within the same area or the ganglionic part of the cord (Fig. 1B) (*10*) evoked only local muscular contractions.

The evoked bend propagation resembled stereotypical arm extensions in freely behaving animals (*11*) (Fig. 1, D and E). As in natural behavior, a dorsally oriented bend propagated along the arm, causing the suckers to point in the direction of the movement. As the bend propagated, the part of the arm proximal to the bend remained extended. Movements resembling normal arm extensions could also be initiated in amputated

arms by electrical stimulation of the nerve cord or by tactile stimulation of the skin or suckers.

Bend propagations were more readily initiated when a bend was manually created before stimulation (n = 50 movements, 16 arms). However, such bend propagation could also be initiated in fully relaxed arms (n = 6, four arms). Here the stimuli triggered the initial phase of bend formation, followed by the arm extension. All these observations show that the nervous system of the arm does not just drive local reflexes (*15*–*17*) but controls complex movements involving the entire arm.

Kinematic analysis allowed quantitative comparison between the evoked and natural movements (*14*). In both tactile (n = 6, four arms) and electrically evoked movements (n = 50, 16 arms), the bend point (the point of maximal curvature) propagated within a single plane (*18*), generally along a lightly curved path (Fig. 2, A and B). The coefficient of determination for movement within a single plane was highly significant (average $R^2 = 0.96 \pm 0.06$); the P value was always smaller than 10^{-8} (F test). The average SE = 0.34 ± 0.39 cm, and the average distance traveled by the bend point was 12.5 ± 4.7 cm. The low SE, in comparison to the distance traveled, confirms that the bends propagate within a single plane. This result is similar to that obtained in freely behaving octopuses (*11*).

Velocity profiles were derived by calculating the tangential velocities of the bend point and plotting these against time (*19*). The velocity profiles of the evoked movements closely resembled those of arm extensions in freely behaving animals (*11*) (Fig. 2, C and D), both having bell-shaped velocity profiles and a similar maximal velocity range (7 to 60 cm/s and 9 to 61 cm/s, respectively).

Normalizing and superimposing the velocity profiles according to their maximal speed and duration and the bend propagation distance (*11*) revealed a robust invariance (Fig. 2, E and F) and showed that the evoked movements were almost kinematically identical to the movements of freely behaving octopuses (Fig. 2G). The variances of the velocity profiles of the evoked and natural movements were also similar, with lower variance during the acceleration phase of the movements (Fig. 2H).

To determine whether the evoked extension is generated passively by a whiplike moving wave or by an active propagation of muscle contraction, we analyzed the kinematics of passive bend propagations and recorded muscle activities (recorded with an EMG) during the evoked movements. Passive bend propagations were produced by dragging a dead arm behind a stick and then letting the stick collide with a solid barrier. A bend was formed and propagated along the arm (Fig. 3A), emphasizing the role of passive interactions with the water in shaping the movement (*12*). However, the velocity profiles were monotonically decelerating (Fig. 3B), unlike the bell-shaped velocity profiles of evoked or natural arm extensions (Fig. 2, C and D). Furthermore, EMGs recorded during the evoked movements (Fig. 3, C and D; n = 7, three arms) reveal an active propagation of muscle activity as in natural arm extensions (*12*).

In 23 experiments in which extensions could be repeatedly evoked, the initial posture of the arm was changed manually while the electrode location and stimulus parameters remained constant. Movements evoked from similar initial arm postures tended to have similar paths (Fig. 4). Likewise, different starting postures resulted in different final paths (15 experiments). Aiming in arm extension thus appears to involve adjusting the initial posture of the arm before, or together with, the command for arm extension.

Because the extensions evoked in denervated octopus arms were qualitatively and kinematically identical to natural arm extensions, there appears to be an underlying motor program embedded in the neuromuscular system of the arm, which does not require continuous central control. This finding is consistent with the remarkable autonomy of the arm local reflexes (*15*–*17*) and with the elaborate nervous system in each arm, which is connected to the brain by a relatively small number of nerve fibers (*8*, *10*).

The division between the central and peripheral levels of the octopus motor control system resembles the hierarchical organization of motor control systems in other invertebrates and vertebrates, even though in the octopus it uniquely serves as an important component in a goal-directed voluntary movement rather than in rhythmical or reflexive behaviors (*20*–*22*). We do not yet know whether this is a predom-

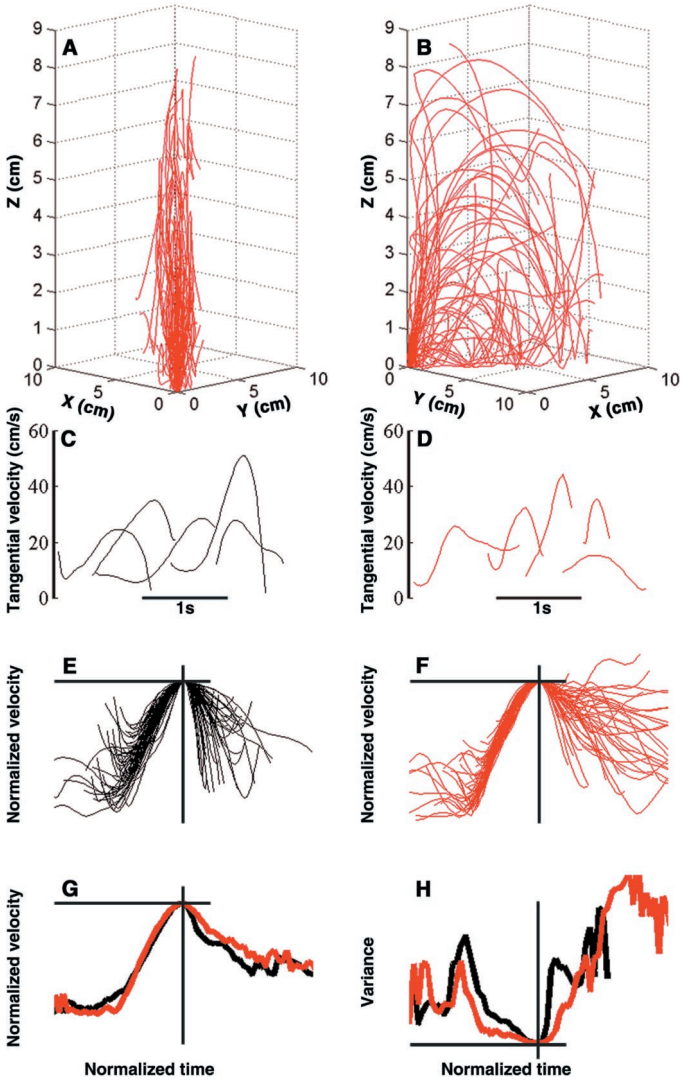

Fig. 2. Comparison between the velocity profiles of natural arm extensions (black) and evoked movements in the denervated arms (red). (**A** and **B**) The evoked arm extensions lie within a single plane. (A) The paths of 56 evoked extensions mapped into the best-fit plane. (B) The same arm extension paths rotated by 90°. Note the difference in path variability between (A) and (B). (**C** and **D**) Velocity profiles of natural and evoked movements. (**E** and **F**) Velocity profiles normalized according to their peak velocity, duration, and propagated distance and aligned at the peak (n = 56 in both cases). (**G**) The average normalized velocity profiles [calculated from (E) and (F)] of the natural and evoked movements are similar. (**H**) Superposition of the variances calculated from (E) and (F) reveals similar behavior.

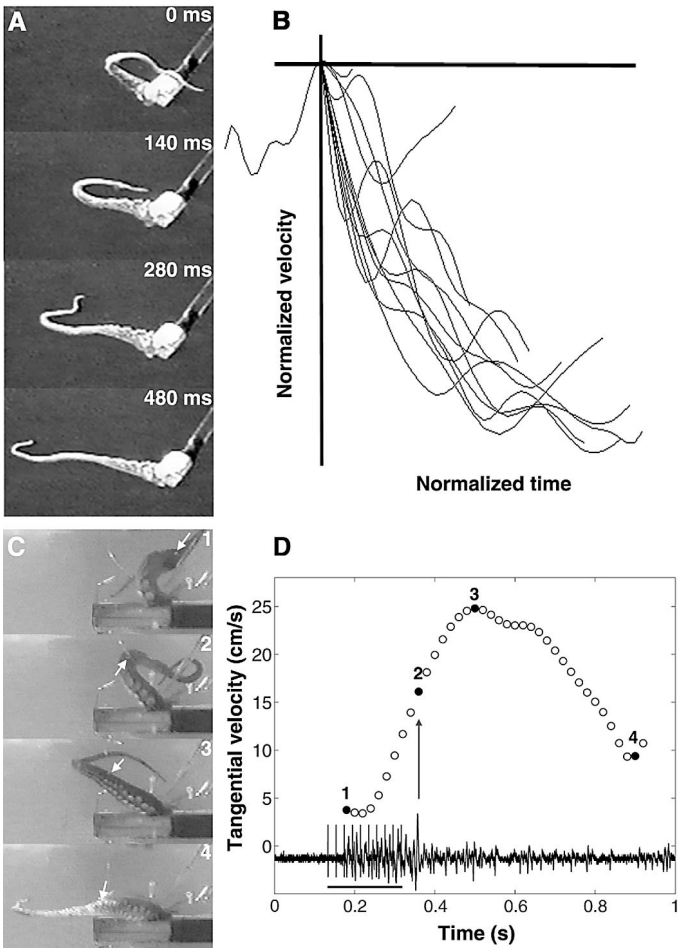

Fig. 3. Evoked movements differ from passive extensionlike movements. (**A**) A sequence showing an extensionlike movement generated by whipping a dead octopus arm. (**B**) Velocity profiles of 12 passive extensions showing decelerating velocity profiles, in marked contrast to the evoked or natural arm extensions (Fig. 2, C and D). (**C**) A sequence showing evoked extension in an amputated arm during which an EMG was recorded with a stainless steel electrode inserted though the dorsal muscles and held in place with a bead glued on each side of the arm (arrow) [see (*12*) for details]. (**D**) Velocity profile and EMG of the movement in (C). The numbers mark when the images in (C) were taken. Activity reaching the recording electrode during the stimulation train (horizontal line) probably results from fast-propagating activity along the axonal tracts. There is a burst of activity just before the bend point reaches the electrode (arrow), followed by tonic activity when the arm is extended.

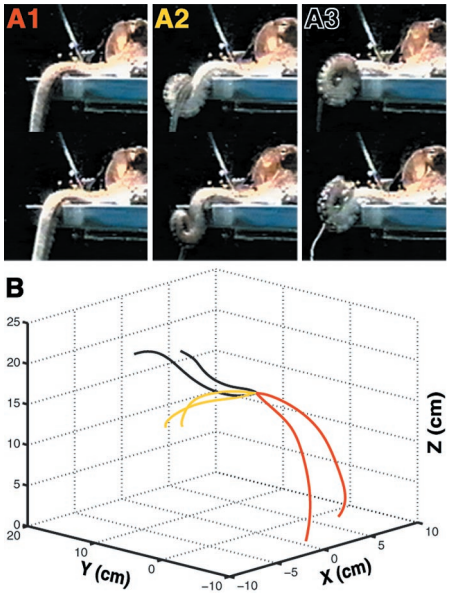

Fig. 4. Similar initial postures generate similar bend paths (when the electrode position and stimulus parameters are kept constant). (**A1** through **A3**) Three pairs of similar starting postures, just before stimulation. (**B**) The evoked paths are shown in pairs in different colors as in (A1) through (A3). The 3D paths are shifted to the same starting coordinates.

inantly feed-forward control mechanism (*12*) or involves a distributed control system based on local reflexes, as in other animals (*23*, *24*).

In this control scheme, the arm neuronal networks produce the neuronal activation patterns prescribing all of the spatiotemporal details of the basic movement patterns, suggesting that the higher central levels (that is, the brain) send global commands to the arm neuronal network to activate and scale the program variables. This division of labor between the central and peripheral nervous systems and the use of a propagating wave with a limited number of degrees of freedom greatly simplify the movement control of flexible arms.

References and Notes

1. J. M. Hollerbach, in *Visual Cognition and Action*, D. N. Osherson, S. M. Kosslyn, J. M. Hollerbach, Eds. (MIT Press, Cambridge, MA, 1990), pp. 151–182.
2. T. Flash, N. Hogan, *J. Neurosci.* **5**, 1688 (1985).
3. C. Harris, D. Wolpert, *Nature* **20**, 780 (1998).
4. T. Flash, E. Henis, *J. Cogn. Neurosci.* **3**, 220 (1991).
5. F. A. Mussa-Ivaldi, *Curr. Opin. Neurobiol.* **9**, 713 (1999).
6. E. Bizzi, M. C. Tresch, P. Saltiel, A. d'Avella, *Nature Rev. Neurosci.* **1**, 101 (2000).
7. W. Kier, K. Smith, *Zool. J. Linn. Soc.* **83**, 307 (1985).
8. J. Z. Young, *Proc. R. Soc. London Ser. B* **162**, 47 (1965).
9. H. Matzner, Y. Gutfreund, B. Hochner, *J. Neurophysiol.* **83**, 1315 (2000).
10. P. Graziadei, in *The Anatomy of the Nervous System of* Octopus vulgaris, J. Z. Young, Ed. (Clarendon, Oxford, 1971), pp. 45–59.
11. Y. Gutfreund *et al.*, *J. Neurosci.* **16**, 7297 (1996).
12. Y. Gutfreund, T. Flash, G. Fiorito, B. Hochner, *J. Neurosci.* **18**, 5976 (1998).
13. To obtain the denervated arm preparation, *Octopus vulgaris* from the Bay of Naples were anesthetized in a mixture of 2% ethanol in seawater (as was also done before each operation) and then "decerebrated" by removal of the supraesophageal brain mass [B. B. Boycott, J. Z. Young, *Symp. Soc. Exp. Biol.* **4**, 432 (1950)] to produce a subdued preparation (Fig. 1A). After a recovery period of ~24 hours, the nerve cord of one of the arms was exposed and transected. We also used acutely amputated arms. The preparations were submerged in circulating aerated seawater at ~20°C. The proximal part of the denervated arm was fixed to a platform; the distal part hung freely. Tactile stimulation was applied to the suckers or skin of the arm. Electrical stimulation (at 40 to 90 Hz for 200 to 500 ms) was applied through a Teflon-coated silver bipolar electrode placed on the dorsal part of the exposed axial nerve cord (Fig. 1B).
14. The evoked arm responses were filmed with two video cameras set ~90° apart and were recorded simultaneously with the stimulus train on a video recorder. The three-dimensional (3D) spatial position of the bend point (the point of maximum curvature) was determined with the direct linear transformation method [see (*11*)]. Out of the many evoked movements (such as local contraction, stiffening, waving, etc.), only in ~10% (n = 116) was a bend observed and its movement analyzed. Only those movements in which the bend was found to propagate were studied (n = 63). Fifty-six movements (89%) had a bell-shaped velocity profile and were compared to natural movements.
15. M. J. Wells, *J. Exp. Biol.* **36**, 590 (1959).
16. C. H. F. Rowell, *J. Exp. Biol.* **40**, 257 (1963).
17. J. S. Altman, *Nature* **229**, 204 (1971).
18. The plane of motion was determined by rotating the 3D coordinates of the paths in space and subjectively determining the best plane containing the bend point movement. The path was then transformed into a coordinate system where the selected plane lay at 45° to the *zy* plane. The fit to a single plane was estimated by the coefficient of determination (R^2) of the regression analysis.
19. Time derivatives of the 3D position coordinates of the bend point were calculated in order to obtain the tangential velocity in the direction of motion (*11*).
20. M. L. Shick, G. N. Orlovsky, *Physiol. Rev.* **56**, 465 (1976).
21. Y. I. Arshavsky, T. G. Deliagina, G. N. Orlovsky, *Curr. Opin. Neurobiol.* **7**, 781 (1997).
22. F. Delcomyn, *Science* **210**, 492 (1980).
23. W. B. Kristan *et al.*, in *Biomechanics and Neural Control of Posture and Movement*, J. Winters, P. E. Crago, Eds. (Springer-Verlag, New York, 2000), pp. 206–218.
24. H. J. Chiel, R. D. Beer, *Trends Neurosci.* **20**, 553 (1997).

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