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SUPPLEMENTARY MATERIALS

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ANIMAL PHYSIOLOGY

The shocking predatory strike of the electric eel

Kenneth Catania

Electric eels can incapacitate prey with an electric discharge, but the mechanism of the eel's attack is unknown. Through a series of experiments, I show that eel high-voltage discharges can activate prey motor neurons, and hence muscles, allowing eels to remotely control their target. Eels prevent escape in free-swimming prey using high-frequency volleys to induce immobilizing whole-body muscle contraction (tetanus). Further, when prey are hidden, eels can emit periodic volleys of two or three discharges that cause massive involuntary twitch, revealing the prey's location and eliciting the full, tetanus-inducing volley. The temporal patterns of eel electrical discharges resemble motor neuron activity that induces fast muscle contraction, suggesting that eel high-voltage volleys have been selected to most efficiently induce involuntary muscle contraction in nearby animals.

The electric eel (*Electrophorus electricus*) is one of just a few species that uses electrical discharges to capture prey and defend against predators. It is the most powerful electrogenic fish, with most of its body composed of electrocytes (muscle-derived biological batteries), providing a combined discharge of up to 600 V (*1*). Early attempts to understand electricity made use of electric eels (*2*), and more recently, eels were important for identifying acetylcholine receptors (*3*) and for providing insights into the evolution of electric organs (*4*), but little is known about how the eel's electrical discharge affects prey. In this study, I designed a set of experiments to explore the impacts of the electric eel discharges on potential prey and the mechanism that operates during such attacks.

Electric eels emit three distinct types of electric organ discharges: (i) low-voltage pulses for sensing their environment, (ii) pairs and triplets of high-voltage pulses given off periodically while hunting in complex environments, and (iii) high-frequency volleys of high-voltage pulses during prey capture or defense (movie S1) (*5*–*9*). Under most conditions, eels attack free-swimming prey with the latter strategy, using high-voltage volleys combined with a suction-feeding strike. To explore this more common behavior, I simultaneously recorded eel behavior and electric organ discharges in a naturalistic experimental environment (*10*). Eels began their attack with a high-frequency (~400 Hz) volley of high-voltage pulses 10 to 15 ms before their predatory strike. In response to these volleys, prey voluntary movement was completely arrested 3 to 4 ms after the first strong discharge (Fig. 1 and movie S2). Fish that were not successfully captured during this period of immobility were often able to

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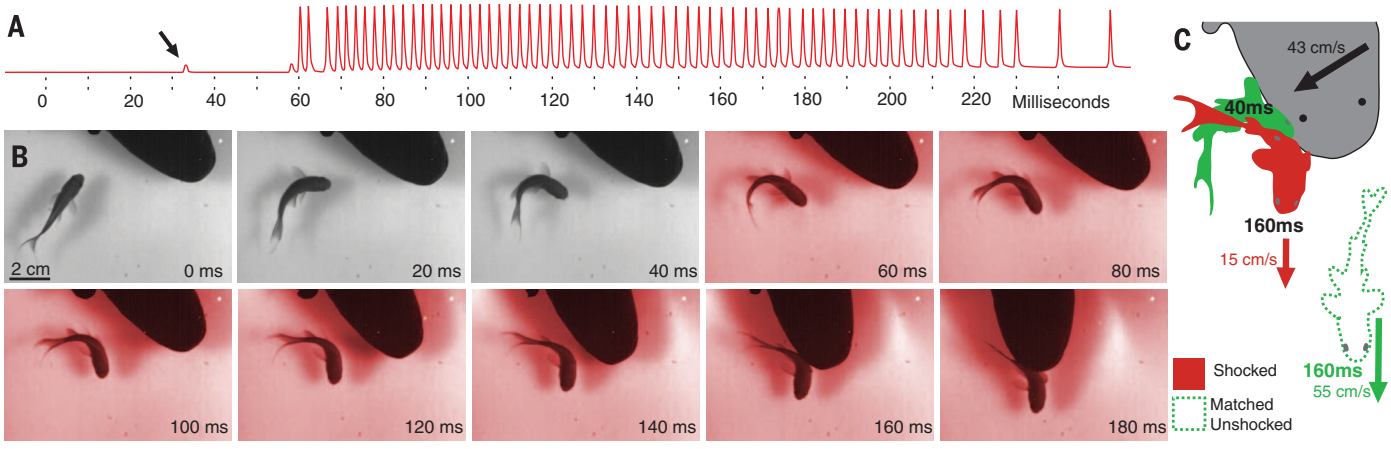

Fig. 1. Eel's discharge and strike. (**A**) Electric organ discharge corresponding to plates below. Arrow indicates low-amplitude discharge. (**B**) Video frames showing that fish movement is arrested by discharge. Red frames indicate electric organ discharge (movie S1). (**C**) The utility of the discharge illustrated. Shown are the prey fish at 40 ms (green) and later, the position and velocity of the eel and fish at 160 ms (red fish). Green dotted fish outline shows velocity and location of uninterrupted escaping fish matched in time, size, and position from 40 ms, suggesting that the eel would have missed without the discharge.

return to previous movement patterns and escape (movie S2).

To characterize the mechanism by which high-voltage volleys cause this remote immobilization of prey (*10*), anesthetized fish were pithed (to destroy the brain), the hole was sealed with cyanoacrylate, and the fish was attached to a force transducer. An eel in the aquarium was separated from the fish by an electrically permeable agar barrier (Fig. 2A) (*11*) and fed earthworms, which it attacked with volleys of its high-voltage discharge. The discharge directed at the earthworms induced strong muscular contractions in the fish preparation, precisely correlated in time with the volley (no tension developed during the weak discharge). A steep rise in fish tension occurred with a mean latency of 3.4 ms (n = 20 trials) after the first strong pulse (Fig. 2B), which is similar to the 2.9-ms mean immobilization latency (n = 20 trials) observed in free-swimming fish. Tension induced by the eel in the fish preparation was similar to the maximum that could be induced experimentally (fig. S1) (*10*). This result indicates that fish are immobilized by massive, involuntary muscle contraction.

To further investigate the fidelity of prey muscle contractions relative to the electric organ

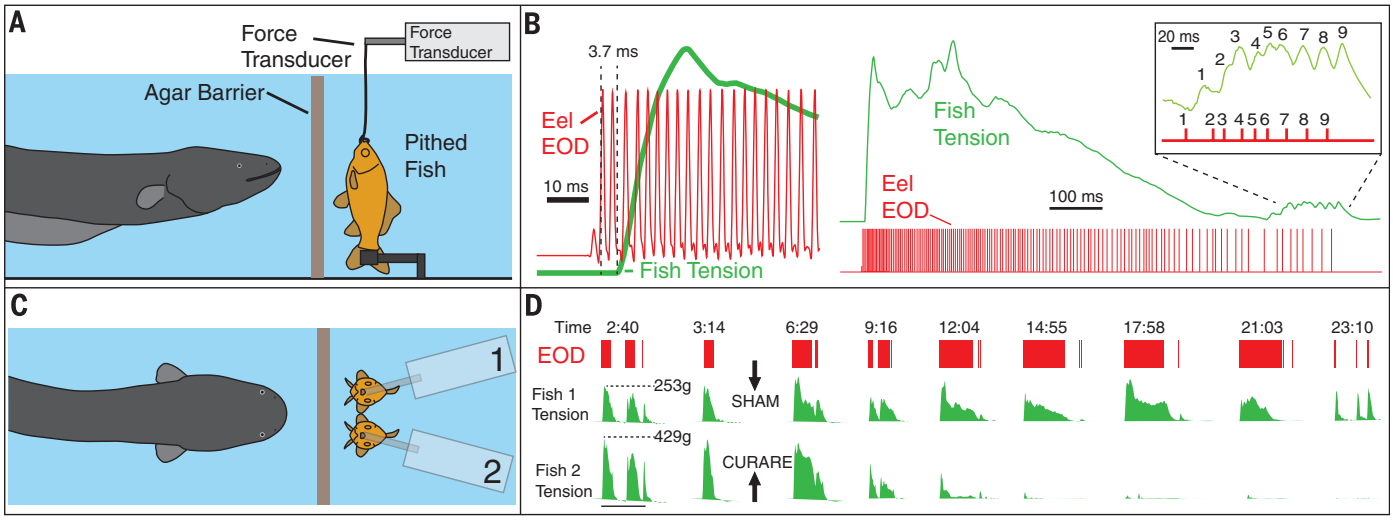

Fig. 2. Paradigm for investigating strong electric organ discharge. (**A**) An agar barrier separated eels from pithed fish. Eels shocked earthworms while fish tension was recorded. (**B**) All eels induced whole-body tension, occurring 2 to 4 ms after strong discharge onset. No tension was developed from weak discharge. At low frequencies, individual twitches emerged for each discharge (top right) (fig. S2). (**C**) Two pithed fish (fish 1, 19 g; fish 2, 21 g) preparation. (**D**) Effect of curare. Red trace indicates strong electric organ discharge matched in time to unnormalized fish tension (green). Arrows indicate time of injections (fig. S3). Bar in (D) = 500 ms.

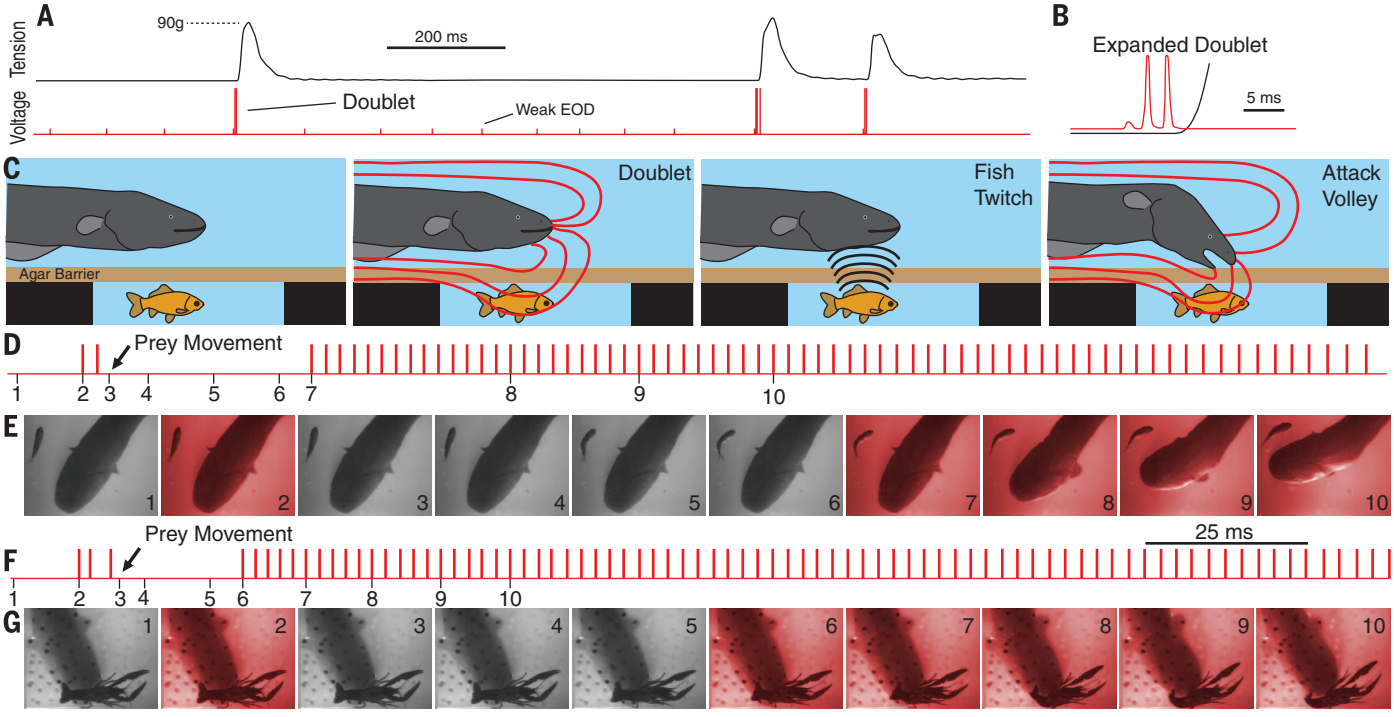

Fig. 3. Doublets during hunting. (**A**) Examples of doublets and corresponding tension responses. (**B**) Expansion of the first doublet and corresponding tension trace (off-scale peaks were estimated). (**C**) Schematic of attack sequence. (**D**) Example of high-voltage electric organ discharge for an attack preceded by a doublet. (**E**) Video frames from volley shown in (D). Numbers correspond to numbers in (D). (**F**) Timing of the high-voltage discharge for attack preceded by a triplet. (**G**) Video frames for volley shown in (F).

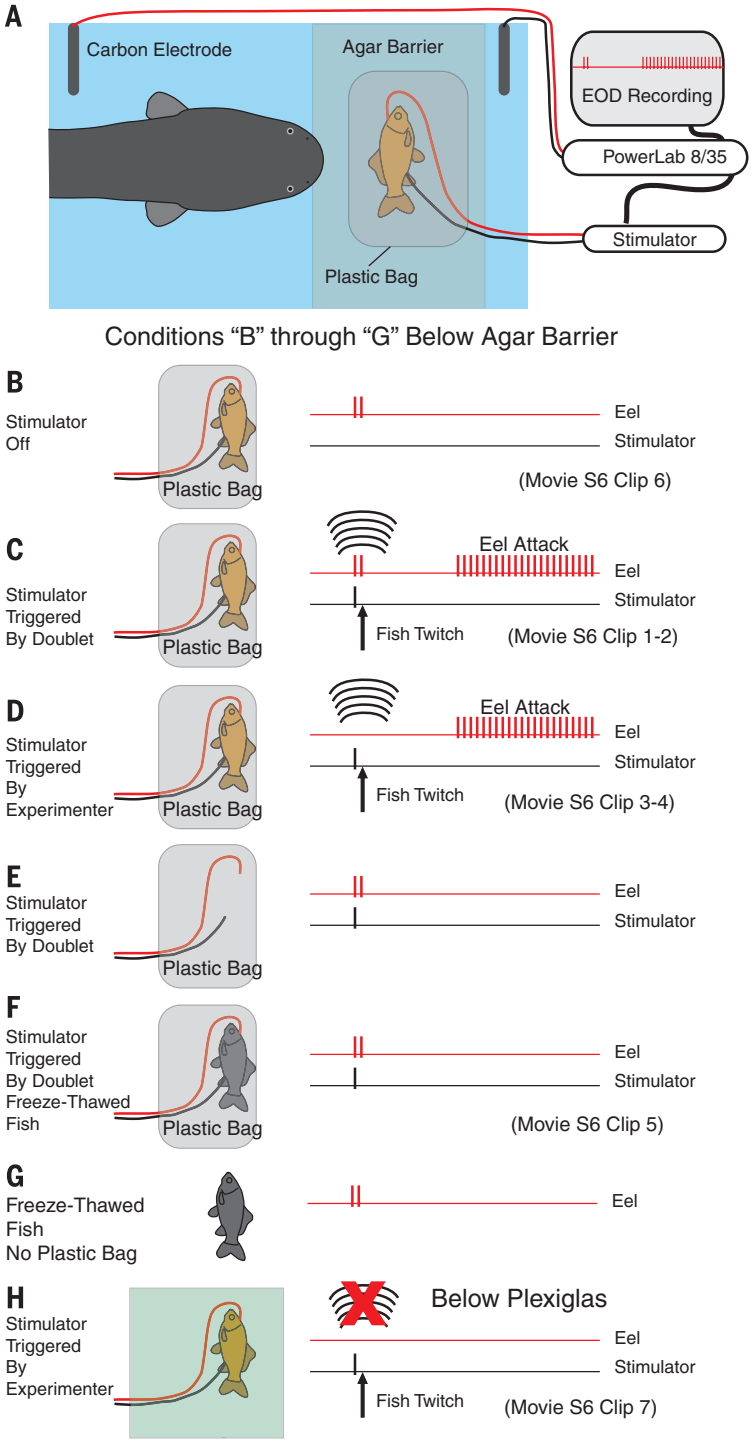

Fig. 4. Paradigm and controls showing eels attack doublet-generated movements. (**A**) Movement in electrically isolated pithed fish (below agar) was generated through stimulator. (**B**) Without fish twitch, eels did not follow doublets with attack (10 trials each for two eels). (**C**) When stimulator triggered fish twitch after doublets, eels attacked (10 trials each for two eels). (**D**) Without doublets, fish twitches also elicited attack volleys (10 trials each of two eels). (**E**) Doublets that triggered stimulator leads in bag did not elicit attack (10 trials for each of two eels). (**F**) Likewise, no attack volleys were elicited after stimulation of a freeze-thawed fish (10 trials each of two eels). (**G**) Doublets directed at a freeze-thawed fish under agar without the plastic bag or stimulator did not elicit eel attack volleys or strikes (10 trials each of two eels). These latter conditions, along with (**H**) trials with Plexiglas barrier, show that visual cues did not generate eel attacks. Examples are provided in movie S6 and (*10*).

discharge, and the mechanism of the contractions' induction, two pithed-fish preparations were stationed side by side (Fig. 2C). The high-voltage discharge reliably created muscle tension with similar form and time course in both fish (fig. S2). As the discharge frequency decreased, individual fish twitches often emerged on the tension trace, each corresponding to a single discharge (Fig. 2B and fig. S2). To determine whether the discharge induced muscle contractions by initiating action potentials directly in prey muscles or through activation of some portion of fish motor neurons, one of two similarly sized fish was injected with curare (an acetylcholine antagonist) so as to block the acetylcholine gated ion channels at the neuromuscular junction, whereas the other fish was sham-injected (Fig. 2D). In each of four cases, tension responses in the curarized fish dropped to near zero, whereas the sham-injected fish continued to respond (fig. S3). These findings indicate that fish motor neuron activation is required to induce tetanus in prey. To determine whether this activation of prey motor neurons was the result of central nervous system (spinal) activity or activity in efferent branches of motor neurons, the dual tension experiment was repeated twice with extensively double-pithed fish (in which both the brain and spinal cord were destroyed, but the branches of motor efferents were left intact within the fish body) and compared with a brain-pithed fish. No diminution in contractile response, or difference in contractile response latency, was observed for the double-pithed fish relative to the brain-pithed fish (fig. S2). These experiments suggest that the electric eel's strong electric organ discharge remotely activates motor neuron efferents of its prey, although this activation could occur anywhere between the spinal cord and the presynaptic side of the neuromuscular junction. Given that the eel's strong electric organ discharge remotely activates prey motor neurons, it was useful to consider the form of this pulse train in the context of prey muscle activation. Analysis of the first 11 impulses from strong discharge volleys from each of four eels showed that each begins with a doublet—two pulses with a shorter interpulse interval (fig. S4). Doublets at the onset of motor neuron trains have been shown to induce high rates of muscle tension (*12*–*15*). Moreover, the overall distribution of pulses in the eel's strong discharge resembles motor neuron trains found to be near optimal for muscle tension development (*16*, *17*). These observations raise the possibility that eel volleys have been selected to efficiently induce rapid muscle tension.

As described above, hunting eels often pause and give off isolated high-voltage doublets (*9*), particularly in complex environments, when seeking hidden prey or when exploring conductors (movie S3). In the course of the present study, eels stationed behind the agar barrier in the fish tension experiments occasionally emitted such isolated doublets or triplets and then attempted to break through the barrier to reach the fish preparation (movie S4). This suggested that eels were able to detect fish movements through the thin agar barrier, which was not designed to mask

mechanosensory cues. To identify the function of this additional behavior, eels were presented with prey hidden below a thin agar barrier (Fig. 3C). In some cases, eels detected prey through the barrier and attacked directly, but in other cases, the eel investigated the agar surface with a low-amplitude electric organ discharge and then produced a high-voltage doublet. The doublet invariably caused prey movement. Stimulated prey movement was closely followed (in 20 to 40 ms) by a full predatory strike consisting of a strong electric discharge volley and directed attack (Fig. 3 and movie S5), as characterized in the first experiments. The distinct form of the discharge trace in these trials consisted of a doublet (or triplet) followed by a 20- to 40-ms pause (during which prey moved) and then a full discharge volley (Fig. 3, D and F).

The results of the doublet experiment suggest that the eels may use doublet and triplet discharges to detect cryptic prey by inducing movement. To test this hypothesis, a pithed fish was placed in a thin plastic bag to isolate it from the eel's discharge. The electrically isolated fish was positioned below an agar barrier, with electrical leads embedded in the head and tail region (*10*) that allowed production of artificial fish twitch by the experimenter. Artificial fish twitch was triggered remotely through a stimulator (Fig. 4A), allowing control over its timing and occurrence. When the stimulating electrodes were inactive, eel doublets caused no response in the pithed fish and eels did not attack the preparation (Fig. 4B and movie S6). However, when the stimulator was configured to trigger fish twitch when the eel produced a doublet, the eel's full "doublet attack" behavior was replicated (Fig. 4C and movie S6). The attack pattern consisted of a doublet, followed by a short pause, during which the prey moved (resulting from the triggered stimulator), followed by a high-voltage volley and strike. This key experiment showed that eels never (10 of 10 trials for each of two eels) followed a doublet with an attack volley without a "mechanosensory echo" from the prey, but attacked in response to the stimulator-generated fish twitch (10 of 10 trials for each of two eels; $P < 0.0001$, binomial test). Experimenter-triggered twitches, in the absence of eel hunting doublets, also generated attacks (movie S6) with the time course observed above (Fig. 4D and supplementary materials). Thus, prey movement, whether doublet-generated or independently generated, elicited short latency (20 to 40 ms) attacks. Eels also appeared to use either active or passive electrolocation to detect live prey under agar and often attacked without a preceding doublet. But in no case did an attack volley follow a doublet in the absence of prey response. Thus, the doublet appears to answer the question, "Are you living prey?" when information is limited. Preliminary observations suggest that "doublet hunting" is most common in complex environments (movie S7). A range of controls confirmed that eels were responding to twitch-generated mechanosensory cues in this paradigm (Fig. 4 and movie S6).

Together, the results of these experiments show that high-voltage discharges of electric eels remotely activate motor neuron efferents in nearby animals. Prey that have been detected can be immobilized and captured. Hidden prey can be induced to twitch, revealing their location. The latter strategy, which often triggers an escape response, depends on the eel's short reaction time. An eel can discharge its high-voltage train 20 ms after a mechanosensory stimulus, allowing it to cancel the very escape response it has generated. Overall, this study reveals that the electric eel has evolved a precise remote control mechanism for prey capture, one that takes advantage of an organisms' own nervous system.

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SUPPLEMENTARY MATERIALS

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INFLAMMATION

Neutrophils scan for activated platelets to initiate inflammation

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Immune and inflammatory responses require leukocytes to migrate within and through the vasculature, a process that is facilitated by their capacity to switch to a polarized morphology with an asymmetric distribution of receptors. We report that neutrophil polarization within activated venules served to organize a protruding domain that engaged activated platelets present in the bloodstream. The selectin ligand PSGL-1 transduced signals emanating from these interactions, resulting in the redistribution of receptors that drive neutrophil migration. Consequently, neutrophils unable to polarize or to transduce signals through PSGL-1 displayed aberrant crawling, and blockade of this domain protected mice against thromboinflammatory injury. These results reveal that recruited neutrophils scan for activated platelets, and they suggest that the neutrophils' bipolarity allows the integration of signals present at both the endothelium and the circulation before inflammation proceeds.

Neutrophils are primary effectors of the immune response against invading pathogens but are also central mediators of inflammatory injury (*1*). Both functions rely on their remarkable ability to migrate within and through blood vessels. The migration of neutrophils is initiated by tethering and rolling on inflamed venules, a process mediated by endothelial selectins (*2*). Selectin- and chemokine-triggered activation of integrins then allows firm adhesion, after which leukocytes actively crawl on the endothelium before they extravasate or return to the circulation (*3*). A distinct feature of leukocytes recruited to inflamed vessels is the rapid shift from a symmetric morphology into a polarized form, in which intracellular proteins and receptors rapidly segregate (*4*). In this way, neutrophils generate a moving front or leading edge where the constant formation of lamellipodia (actin projections) guides movement, and a uropod or trailing edge where highly glycosylated receptors accumulate (*5*, *6*). We deemed it unlikely that this dramatic reorganization served to exclusively generate a front-to-back axis for directional movement, and we explored the possibility that neutrophil polarization functions as an additional checkpoint during inflammation.

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