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Electric shock causes fear-like persistent behavioral response in the nematode Caenorhabditis elegans — Source link

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Published on: 07 Sep 2021 - bioRxiv (Cold Spring Harbor Laboratory)

Topics: Stimulus (physiology)

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ABSTRACT

Electricity is widely utilized as environmental stimulus to sense the world by many animal species. Despite its importance, however, molecular and physiological mechanisms for responding to electrical stimulus have been far less understood compared to other sensory stimuli. Here we report novel behavioral responses to electrical stimulus of the nematode *C. elegans*. When the animals on food are stimulated by alternating current, their movement speed suddenly increases more than 2-fold, which persists for a few minutes even after the electrical stimulation is terminated. Genetic analyses reveal that voltage-gated channels are required for the response, possibly as the sensors, and neuropeptide signaling suppresses the persistent response. Additional behavioral analysis reveals that, in addition to the persistence, the animal's response to electrical shock is scalable and has a negative valence, which are recently regarded as emotion primitives, suggesting that the response may reflect a primitive form of "fear" of animals.

INTRODUCTION

In order to survive and reproduce, animals respond to various environmental sensory stimuli by perceiving and processing these cues within the neural circuit, and convert them into behavioral outputs. In addition to the well-known stimuli cues, such as light, sound, chemical and temperature, animals also respond to other stimuli, such as magnetic field and electricity (Baker et al., 2013; Wiltschko & Wiltschko, 2005).

In neuroscience research, electricity is used as unconditioned stimulus with negative valence to cause associative learning in rodents and in flies (Aceves-Piña & Quinn, 1979; Blair et al., 2005; Tarpley et al., 2010; Tully et al., 1994). In nature, however, multiple animal species are known to respond to electricity for survival purposes, such as communication, navigation and/or prey detection (Clarke et al., 2013; Crampton, 2019; Pettigrew, 1999). For example, weakly electric African fish (*Gnathonemus petersii*) utilize their epidermal electroreceptors to receive self-produced electric signals, allowing the fish to identify, locate, and examine nearby objects (von der Emde et al., 1998). In addition, platypus (*Ornithorhynchus anatinus*), blind cave salamander (*Proteus anguinus*), and bumblebees (*Bombus terrestris*) are also known to sense electric signals for navigation and/or foraging (Istenič & Bulog, 1984; Roth & Schlegel, 1988; Scheich et al., 1986; Sutton et al., 2016). Such wide use of electrical signals in the animal kingdom suggests that the molecular mechanisms of electricity perception as well as neural circuits to utilize the perceived information have independently emerged or diverged during evolution. Despite their importance, the molecules required for responses to electrical signals have only been revealed in sharks and skates: Bellono et al. reported that electrosensory cells in little skate and chain catshark use L-type voltage-gated calcium channels (VGCC) (Bellono et al., 2017; 2018).

The nematode *Caenorhabditis elegans* has been widely used in neurobiological research because of the feasibility of molecular, physiological, and behavioral analyses of neural functions. The animals have been known to respond to direct current (DC), migrating along the electric field from the positive end to the negative end (Sukul & Croll, 1978), and a few classes of chemosensory neurons (ASH and ASJ) were found to be required for their ability to align themselves according to the DC field (Gabel et al., 2007). The animals are also reported to respond to strong alternating current (AC)—they exhibit a "convulsant" phenotype (paralysis and elongation) upon delivery of a brief electrical shock (200 Hz, 3.5 ms, 47 V) and recover rapidly after removal of the electrical shock (Risley et al., 2016). However, other behavioral responses as well as molecular mechanisms for electrical signals have not been revealed.

In this study, we report that *C. elegans* responds to AC electric stimulus by immediately increasing their speed. The speed increase lasts for minutes even after an electric stimulus of seconds is terminated, suggesting that the response is caused not by direct stimulation of the motor system for rapid movement but possibly by persistent activity of a specific set of neurons to generate the behavioral response. Further behavioral analyses suggest that, in addition to the persistence, the behavioral response is associated with valence and scalability, thus exhibiting at least 3 out of the 4 features of "emotion primitives" (Anderson & Adolphs, 2014). A series of candidate genetic analyses reveal that the response is not mediated by well-known chemo- or mechano-sensory mechanisms. Instead, it requires both L-type VGCC, as in the shark and skate, and N-type VGCC, which have not previously been implicated in animal electrical responses. Furthermore, we find that neuropeptide signaling is required to suppress the persistence. These results indicate that the animals' response to

electric shock can be a suitable paradigm to reveal molecular and physiological mechanisms of electrosensation as well as a primitive form of emotion, such as fear.

RESULTS

Worms' speed increases by AC stimulation

In order to study *C. elegans*' response to electric shock, we established a setup (Figure 1), where several adult wild-type animals were placed onto 9 cm NGM agar plates seeded with a small bacterial food patch and subjected to AC stimulation. The complete trajectories produced by the animals were video-recorded, and their speed was calculated based on the *x*-*y* coordinates of worms' centroids in each image frame.

We first studied the response to AC stimulation covering a range between 15 - 105 V at 60 Hz (the commercial power frequency in Japan), and found that the animals increased their average speed during electrical stimulation by varying amounts (Figure 2—figure supplement 1). We then conducted a series of systematic analyses with different voltages and frequencies at 30 – 75 V and 0.25–256 Hz (Figure 2—figure supplement 2). After the analysis, we noticed that an interesting characteristic of this behavioral phenotype is most apparent when using 4 Hz stimuli: When worms were stimulated with 30 V, their average speed of movement suddenly increased more than 2-fold, and this persisted during the electrical admission. We named this behavior the "ON response" (Figure 2A and C). During this running behavior, the worms engage in rapid body bends as well as rapid head movements (Figure 2—video 1). In the ON response, we did not detect a statistical bias in any direction (Figure 2—figure supplement 3). Moreover, when a stronger electric stimulus of 75 V was applied, it caused a significant increase in average speed not during but immediately after the stimulus, which we named the "OFF response" (Figure 2B). A fraction of the animals

responded during the stimulus in the OFF response condition, while, in the majority of the animals, the speed was suppressed during the stimulus and then increased immediately after the removal (Figure 2—figure supplement 4 and video 2). During application of voltage, those worms that are immobilized appear to be convulsing, with jerky, unproductive muscle contractions occurring throughout the body. With other frequencies, ON and OFF responses were also observed, but were less cleare compared to those with 4 Hz (Figure 2—figure supplement 1). We considered the ON and OFF responses at 4 Hz to be interesting because only 2.5-fold differences in the voltage at the same frequency caused completely different behavioral responses, which does not happen generally with other stimuli, such as odor, to the animals (Bargmann et al., 1993).

We then analyzed whether this response depends on voltage or current by manipulating the salt concentration in the assay plate: The higher salt concentration should result in a larger current when the same strength of voltage is applied. As shown in Figure 3, 30 V and 75 V stimulus caused ON and OFF responses, respectively, regardless of the current value, indicating that the behavioral response depends on voltage.

Speed increase lasted for several minutes

Next, we examined how long the increased speed persists during and after the stimulus. When the duration of applied electric shock was 1-2 minutes, significant speed increases were maintained during the stimulus, lasted for ~1 min after the stimulus, and went back to the baseline level (Figure 4A). Interestingly, when the animals were stimulated only for 5 sec, the speed increase still lasted for 1.5 min. When 4 min stimulus was applied, the increase was maintained during the stimulus but went back to the baseline level 30 sec after the stimulus. During 10 min stimulation, the significant speed increase was observed only for 5.5 min.

Thus, we concluded that the ON response caused by 30 V stimulation persists ~5 min at most.

This result suggested the possibility that the speed increase persisted for ~5 min because of fatigue in motor systems. However, animals stimulated intermittently 5 times for 30 seconds per stimulation maintained a speed increase for much longer time than those under the continuous stimulus (Figure 4B versus "10 minutes" in A). This result suggests that the decrease in speed during the long ON stimulation period is not caused by fatigue in the motor system, but possibly by sensory adaptation, which is widely known to adjust the animal's sensory response to new environments (Webster, 2012).

We then tested the persistence of speed increase in the OFF response with 75 V. Interestingly, 5 and 30 sec stimuli caused longer persistent responses after the stimulus than 30 V did (Figure 4C). 45 sec stimulus caused >2 min persistent response, which is the longest among the responses to 30 and 75 V stimuli after the stimulus. However, when animals were stimulated for 1 min, no ON or OFF responses was observed, possibly due to physical damage to the animals. The fact that the larger stimulus (75 V) caused longer persistent responses than the smaller one (30 V) suggests that the response to electric shock is "scalable", one of the critical "emotion primitives" together with persistence (Anderson & Adolphs, 2014).

We then tested the effect of food presence on the speed increase. *C. elegans* move slowly on the bacterial food lawn and faster out of the lawn (Sawin et al., 2000). As we used a small food lawn to localize the animal's initial positions in the center of the plate (Figures 1 and 2C), it was possible that the electrical stimulus caused the animals to move away from the

152 food lawn, which then caused increased speed due to the absence of food. If this is the case,
153 the animal's speed would be considerably lower with the electrical stimulus when the plates
154 were fully covered with a bacterial lawn. To test the hypothesis, we compared the time-
155 course of speed changes on plates with a small patch of food lawn and with a full food lawn.
156 As shown in Figure 4D and E (compare Figure 4A "4 minutes" and B " 30 seconds",
157 respectively), there was no substantial difference in the time course of speed change between
158 the behavioral responses on the small food and the full food plates, demonstrating that the
159 speed increase is not caused by the food absence but by the electrical stimulation itself.
160
161 To further confirm the result, we analyzed the animals' speed on a stripe-like food pattern
162 (Figure 4—figure supplement 1A). We did not observe a significant difference in the speeds
163 when the animals moved into or out of the food area (Figure 4—figure supplement 1B). This
164 result indicates that the animals' migratory speed is not affected by the presence or absence of
165 food, which is one the most influential environmental signals for the animals. It may further
166 suggest that animals prioritize moving away from a harmful condition, such as strong
167 electrical shock, to protect themselves.
168
169 **Two types of voltage-gated calcium channels, but not chemo- or mechano-sensory**
170 **molecules, are required for the sensation of electric shock.**
171 To identify gene(s) required for the response to electric shock, we analyzed a series of mutant
172 strains of candidate genes. We tested the mutants of genes involved in the animals' chemo- or
173 mechano-sensation, the homologues of genes involved in electroreception in shark and skate,
174 and genes involved in the biosynthesis of neuromodulators.
175

176 *C. elegans'* chemo-sensation is largely mediated by the 12 pairs of amphid sensory neurons in
177 the head, which are classified into the ones using TAX-2 and TAX-4 cyclic nucleotide-gated
178 channel (CNGC) subunits or the others using OSM-9 and OCR-2 transient receptor potential
179 (TRP) channel subunits for depolarization (Coburn & Bargmann, 1996; Colbert et al., 1997;
180 Komatsu et al., 1996; Tobin et al., 2002). In addition to loss-of-function mutants for the
181 above-mentioned genes, we tested mutants for *che-2*, a gene required for the proper
182 formation and function of the ciliated sensory neurons (Fujiwara et al., 1999). For mechano-
183 sensation, we analyzed loss- or reduction-of-function alleles of *mec-4, mec-10*, and *trp-4*.
184 *mec-4* and *mec-10* genes encode DEG/ENaC proteins and form a mechanosensory ion
185 channel complex for transduction of gentle touch (Chalfie & Sulston, 1981; Driscoll &
186 Chalfie, 1991; Huang & Chalfie, 1994), while *trp-4* encodes TRPN (NOMPC) for harsh
187 touch response (Kang et al., 2010; Li et al., 2011). All the mutant strains exhibited wild-type-
188 like responses in ON as well as OFF responses (panel A in Figures 5 and 6 for ON and OFF
189 responses, respectively). Some mutants (*osm-9;ocr-2*, *che-2*, *mec-10*, and *tph-1*) exhibited
190 statistical differences in the OFF response, suggesting the partial involvement of these genes,
191 although the defects in speed increase (i.e. ΔSpeed) were not as severe as the ones of VGCC
192 mutants (see below). The non-involvement of *tax-4* also indicates that temperature increase
193 caused by the electric stimulus or speed increase induced by high O_2 due to the *npr-1*
194 mutation is not responsible for the response (Coates & de Bono, 2002) (see Discussion for
195 details).
196
197 We then tested *egl-19*, the orthologue of the L-type VGCC alpha subunit, which functions in
198 the sensory organ for environmental electric signals for shark and skate (Bellono et al., 2017,
199 2018). We found that two reduction-of-function alleles of *egl-19* mutants exhibited strong

200 defects in ON and OFF responses (Figures 5 and 6, panels B and F). This result suggests that
201 the VGCC may be an evolutionarily conserved sensor for environmental electricity.
202
203 This finding further motivated us to test two other types of voltage-gated calcium channels,
204 i.e. N-type voltage-gated calcium channel (UNC-2) and T-type voltage-gated calcium
205 channel (CCA-1), although only L-type VGCC had been found to be involved in electrical
206 responses in other animals. Unexpectedly, mutants for two alleles of *unc-2* were defective in
207 both ON and OFF responses, while *cca-1(ad1650)* mutants behaved similar to the wild-type
208 controls (Figures 5 and 6, panels B and F). These results demonstrate that UNC-2, the N-type
209 VGCC, is also required for the electric sensation, and also suggest that the worms may utilize
210 similar but substantially different molecular mechanisms for electrical sensation than sharks
211 and skates.
212
213 Lastly, we analyzed the genes required for the biosynthesis of neuromodulators, such as
214 serotonin, dopamine, octopamine and tyramine. As shown in panel C in Figures 5 and 6,
215 these mutants also exhibited wild-type-like responses, indicating that these neuromodulators
216 are not involved either. Because dopamine and serotonin signaling are known to be required
217 for the feeding status-dependent modulation of migratory speed, these results are also
218 consistent with the fact that feeding status is not the causal reason for the speed increase
219 (Figure 4D and E, and Figure 4—figure supplement 1).
220
221 We also tested the involvement of neuropeptides by using loss- or reduction-of-function
222 mutations of *egl-3*, a gene required for maturation of pro-neuropeptides (Kass et al., 2001).
223 Unexpectedly, mutations in both alleles of *egl-3*, *n589* and *ok979*, caused much longer
224 persistence of the speed increase after the electric shock (Figures 5 and 6, panels D-F),

indicating that the persistent activity in the neural circuit for speed increase is down-regulated by neuropeptide signaling in the wild-type animals.

DISCUSSION

Response to AC stimulus and its molecular mechanism

Multiple vertebrate and invertebrate species are known to sense electric signals for navigation and/or foraging. For example, in addition to the electrical fish, platypus (*Ornithorhynchus anatinus*) detects electrical signals via their duck-like bills to locate and avoid objects when navigating in the water (Scheich et al., 1986). Blind cave salamander (*Proteus anguinus*) perceives a moving back-and-forth direct-current field and its polarity via ampullary organs to survive and navigate in their environment, which is in complete darkness as their eyes are undeveloped (Istenič & Bulog, 1984; Roth & Schlegel, 1988). And in invertebrates, bumblebees (*Bombus terrestris*) sense environmental electric fields via sensory hairs to make foraging decisions (Sutton et al., 2016). These results suggest that sensation of electrical signals are essential for survival and reproduction of animals in the wild.

In this study, we established an original experimental paradigm and found that *C. elegans* responds to AC electrical stimulus: The animals significantly increase their movement speed during and after the stimulus for minutes. Although the animals have also been reported to respond to DC (Gabel et al., 2007), we consider that the responses to AC and DC are different for the following reasons. (1) In the DC field, the animals moved at a certain angle (~4° per 1 V/cm), which was not observed in our AC stimulus (Figure 2—figure supplement 3). (2) Movement speed did not change with the DC stimulus.

In addition, five pairs of amphid sensory neurons were involved in the DC response (Gabel et al., 2007), while mutations is genes required for sensory signaling in the amphid sensory neurons (*tax-4*, *osm-9*, *ocr-2*, and *che-2*) did not affect the AC response in our study (Figures 5 and 6), indicating that DC and AC responses utilize different sensory mechanisms. Our result also rules out the possibility that the animals respond to increased agar temperature due to the AC stimulus, because the mutation in *tax-4*, the gene essential for temperature response (Komatsu et al., 1996) did not affect the response. In addition, the genes required for mechano-sensation (*mec-4*, *mec-10*, and *trp-4*) are not required for the AC response either.

We found that L-type as well as N-type VGCC, EGL-19 and UNC-2, respectively, are required for the AC response. L-type VGCC has been found to function in the electrosensory organs in the shark and skate, but not N-type, indicating that *C. elegans* utilizes similar but different molecular mechanisms. Since EGL-19 is expressed in most if not all the neurons (Lee et al., 1997), it will be interesting to identify the neurons in which the channel functions, whether they are the same or different from the neurons that utilize the N-type channels, and how they contribute to the increase in the movement speed. As mentioned above, various organs in different animal species are known to sense electrical stimuli. Therefore, it would be interesting to investigate whether L-type as well as N-type VGCCs also function in the organs of these animals to sense electrical signals.

Electric stimulus causes persistent behavioral response.

Persistent neural activity, a sustained neural activity caused by a short-term stimulus, plays critical roles in brain function, such as controlling the oculo-motor system, working memory, and decision making, although its detailed mechanisms have not been sufficiently elucidated (Curtis & Lee, 2010; Major & Tank, 2004). Persistent behavioral state is likely caused by

274 persistent neural activity, suggesting that genetic analysis of persistent behavioral state may
275 reveal molecular mechanism(s) of persistent neural activity.
276
277 We unexpectedly found that *C. elegans*' high speed response persists after electrical shock. In
278 the animals, two other types of persistent behavioral responses have been reported. The first
279 is that the animal's movement speed is elevated at high O_2 concentration in *npr-1(lf)* and in
280 the Hawaiian wild isolate CB4856, which has the same amino acid variation in *npr-1*
281 (Cheung et al., 2005). In this behavioral response, the elevated speed returns rapidly to the
282 basal speed when the high O_2 is terminated, the animals still recognize and aggregate at the
283 edge of a food lawn, and a mutation in the *tax-4* CNGC homolog for sensory depolarization
284 abolishes the response (Coates & de Bono, 2002). Another type of persistent behavioral
285 response is roaming (Flavell et al., 2020; Manabi Fujiwara et al., 2002). Roaming is a
286 behavioral state with high movement speed, although it is only exhibited when the animals
287 are on food and requires serotonin signaling. Because the behavioral response to electrical
288 shock persists more than 2 min after 30-45 sec stimulus with 75 V and more than 1.5 min
289 after only 5 sec stimulus, is not affected by food stimulus, and does not require CNGC
290 activity or serotonin signaling, the analysis of electrical shock response is likely different
291 from the above-mentioned two behavioral responses and may provide a unique opportunity
292 for genetic dissection of a persistent behavioral state.
293
294 **Response to the electric stimulus may reflect a primitive form of emotion.**
295 Anderson and Adolphs defined emotion as an internal state triggered by specific stimuli
296 likely rewarding or punishing and that persistence, scalability, valence, and generalization are
297 key characteristics for primitive forms of emotion in animals (Anderson & Adolphs, 2014).
298

In addition to persistence, we consider that the electrical stimulus has negative valence. This is because the animals ignore food during the electrical shock response, despite the fact that food is one of the most influential signals for *C. elegans*, affecting many aspects of their behavior. For example, during the high speed state caused by high O_2, animals still recognize and stay at the edge of a food lawn (Cheung et al., 2005; Coates & de Bono, 2002), suggesting that the electrical shock signal has a strong negative valence that overrides the strong positive valence of food. The third point is the scalability—stronger stimulus causes stronger behavioral response. Compared to the 30 V stimulus, the 75 V stimulus results in a larger number of immobile animals during the stimulus period (right panels in Figure 2A and B) as well as a longer high speed response after the stimulus (compare the panels for responses to 5 and 30 second stimulus in Figure 4A and C).

In summary, we found that *C. elegans* responds to electrical shock, which is regulated by VGCCs and neuropeptide signaling. Our findings may suggest the following model (Figure 7). When the animals sense 30 or 75V AC stimulus at 4 Hz, the stimulus is sensed with the L- and N-type VGCCs and their internal state transits from basal speed state to persistent high speed state. The persistent high speed state eventually returns to the basal speed state, which requires neuropeptide signaling. Thus, by studying the electrical responses of *C. elegans*, we will be able to investigate the mechanisms of animal electroception, persistent activity, and possibly a primitive form of emotion.

MATERIALS AND METHODS

C. elegans strains

C. elegans strains were maintained with standard procedures (Brenner, 1974). In brief, for regular cultivation, animals were grown on standard 6 cm nematode growth medium (NGM)

agar plates which had been spread with *E. coli* strain OP50 and incubated at 19.0-19.5 °C. Strains used were the wild-type strain Bristol N2 and mutant strains PR678 *tax-4(p678)*, CX4652 *osm-9(ky10);ocr-2(ak47)*, CB1033 *che-2(e1033)*, TU253 *mec-4(u253)*, ZB2551 *mec-10(tm1552)*, TQ296 *trp-4(sy695)*, MT1212 *egl-19(n582)*, DA995 *egl-19(ad995)*, VC39 *cca-1(ad1650)*, CB55 *unc-2(e55)*, VC854 *unc-2(gk366)*, KDK11 *cat-2(tm2261)*, MT7988 *bas-1(ad446)*, GR1321 *tph-1(mg280),* RB993 *tdc-1(ok914),* VC671 *egl-3(ok979)* and MT1219 *egl-3(n589)* .

C. elegans cultivation for electric shock behavioral assay

Before the behavioral assay, animals were cultivated as described previously (Kimura et al., 2010). In brief, four adult wild-type animals were placed onto NGM agar plates with OP50 and kept at 19.5°C for 7.5 hours before being removed. After removal, these plates were incubated at 19.5 °C for 3 days until the assay day. On the assay day, about 100 synchronized young adult animals were grown on each plate. As some mutant animals had slower growth or laid fewer eggs than wild-type animals did, the incubation temperature and number of these mutant animals were adjusted and increased accordingly in order to obtain a comparable developmental stage (i.e. young adult) with the wild-type animals. All behavioral assays were carried out with young adult hermaphrodites.

Experimental instruments for electric shock behavioral assay

The following electrical instruments (Figure 1) were utilized for the electric shock behavioral assay. A 50 MHz Arbitrary Waveform Generator (FGX-295, Texio Technology Corporation) was used to generate different types of electrical waveforms over a wide range of frequencies. However, this waveform generator has an output limit of 10 V. Thus, an AC Power Supply (PCR500MA, Kikusui Electronics Corp.) was used to amplify the voltage

supply. We also used an Digital Storage Oscilloscope (DCS-1054B, Texio Technology Corporation) in parallel to measure the voltage and observe the electrical waveforms produced as well as a Digital Multimeter (PC720M, Sanwa Electric Instrument Co., Ltd.) to measure current. A USB camera (DMK72AUC02, The Imaging Source Co., Ltd.) with a lens (LM16JC5M2, Kowa) was used to record trajectories produced by the animals.

Electric shock behavioral assay with small OP50 bacterial food patch

Most of the behavioral assays were conducted on 9 cm NGM agar plates seeded with a small food patch unless indicated otherwise: For the food patch, the bacteria OP50 was grown in 100 mL of LB culture overnight at 37°C, spun down and resuspended in 10 volumes of NGM buffer, and 5 μL of the suspension was applied at the center of the plate with 3 × 10 mm in size on the assay day. This is to minimize the thickness of food patch as it prevents clear images of worms in the patch. Four animals per plate were placed in the food patch one hour before the assay to accustom the animals to the environment and to reduce their movement speed to the basal level. The assay plates were then inverted and placed onto a custom-made copper plate bridge, whose distance is 6 cm (Figure 1), the images were acquired 2 frames per s, and electric shock was delivered with the conditions described in each figure. Move-tr/2D software (Library Inc., Japan) was used to calculate the *x*-*y* coordinates of the animal centroids in each image frame, which were then analyzed using Excel (Microsoft) or R (The R Project). Baseline speed was calculated from the average speed over 30 s before the stimulation, and ΔSpeed was calculated by subtracting the baseline value from each animal's speed during or after the stimulus.

Electric shock behavioral assay with full OP50 bacterial food lawn

For the assays conducted with full food lawn, the assay plates were seeded with OP50 and kept on the bench overnight until the assay began. Animals grown in regular cultivation plates were washed in two droplets of NGM buffer and then transferred to the center of the assay plate and left for 5 minutes. The rest of the procedures were the same as for assays conducted with small food patch.

Investigation of relationship between speed increase, current and voltage

Three different types of NGM agar plates were prepared with varying salt concentration and similar osmolarity: High-salt plates had 200 mM of sodium chloride; low-salt plates had 10 mM of sodium chloride and 380 mM of sucrose; control plates had 50 mM sodium chloride and 300 mM of sucrose. The purpose of adding sucrose into the plates was to adjust and balance the osmolarity. The final total osmolarity for sodium chloride (Na^+ and Cl^-) and sucrose, the osmo-regulator, for all the plates was 400 mOsm. The rest of the procedures were the same as for assays conducted with small food patch.

Data analysis and statistics

All the statistical analyses were performed in R (The R Project). Generally, data of 20 – 50 animals in total from 9 plates from 3 days of experiments for each condition were pooled and analyzed together. We chose this sample number based on a large scale behavioral analysis of *C. elegans* (Yemini et al., 2013). Data is presented as means ± SD unless otherwise specified.

FIGURE LEGENDS

Figure 1. Experimental setup for electrical shock experiment. This setup consists of an arbitrary waveform generator, amplifier, multimeter, camera, desktop computer and oscilloscope.

Figure 2. Animals' speed is increased by AC stimulation. **A,** (Left) Speed-time graph with 30 V stimulation at 4 Hz. Thin and thick lines are for individual and average values, respectively. Gray indicates the duration of electrical stimulation (0-30 s). (Right) Scatter plot showing average speed of individual animals before, during and after electrical stimulation. Each period is 30 s. n = 35. **B,** Speed-time graph (left) and scatter plot (right) with 75 V stimulation at 4 Hz. n = 36. **C,** Cartoons of worm's response to the electrical shock. (Left) When electrical stimulation is absent, the worms stay on food patch and maintain their speed at around 0.1 mm/s. (Right) When electrical stimulation is delivered, the worms increase speed to around 0.2 - 0.3 mm/s and leave the food patch. Statistical values were calculated using Friedman test with *post hoc* Wilcoxon test with Bonferroni correction. ** $p < 0.001$.

Figure 2—figure supplement 1. Speed-time graphs with different voltage stimulation at 60 Hz.

Figure 2—figure supplement 2. Speed-time graphs with different voltage stimulation at different frequencies.

Figure 2—figure supplement 3. Movement directions of animals during the response.

Figure 2—figure supplement 4. Low and high speed groups during 75 V stimulation.

Figure 3. Speed increase is dependent on voltage, not on current. **A,** Voltage-current graph with different salt concentrations (indicated by different symbols). Each dot represents the measured value on the day of the experiment. The final total osmolarity for sodium chloride (Na^+ and Cl^-) and sucrose for all the plates was 400 mOsm. **B–E,** Behavioral responses of animals assayed on high-salt plate with 30 V (**B**; n = 32), on control plate with 75 V (**C**; n = 35) or 30 V (**D**; n = 36), or on low-salt plate with 75 V (**E**; n = 34). Stimulation period is

423 indicated by a shaded grey box. **F–I,** Scatter plot showing average speed of individual
424 animals before, during and after electrical stimulation, corresponding to the panels **B–E**,
425 respectively. Statistical values were calculated using Friedman test with *post hoc* Wilcoxon
426 test with Bonferroni correction. ** $p < 0.001$.
427
428 **Figure 4.** Speed increase persisted for minutes even after the stimulation. **A,** Speed-time
429 graphs of ON response with 30 V stimulation of different time periods, ranging from 5
430 seconds to 10 minutes. **B,** Speed-time graph for intermittent electrical stimulation of 30
431 seconds, 5 times with 90 s–intervals. **C,** Speed-time graphs of OFF response with 75 V
432 stimulation of different time periods, ranging from 5 seconds to 1 minute. **D** and **E,** Speed-
433 time graphs for electrical stimulation of 30 V for 4 minutes (**D**) or 75 V for 30 s (**E**) with
434 worms placed on full food lawn. Shaded regions around the lines represent standard
435 deviation. Statistical values were calculated using Kruskal-Wallis test with *post hoc*
436 Wilcoxon test with Bonferroni correction. * $p < 0.01$, ** $p < 0.001$. Sample numbers were
437 32–46 per condition, and the details are described in Supplementary Table.
438
439 **Figure 4—figure supplement 1.** Worms' speed did not change when they move in or out of
440 food.
441
442 **Figure 5. ON response is dependent on VGCC, and the persistence is regulated by**
443 **neuropeptide signaling. A–D,** Speed-time graphs of ON response with 30 V stimulation of 4
444 min on mutants of sensory signaling (**A**), VGCC (**B**), biogenic amine biosynthesis (**C**), and
445 neuropeptide biosynthesis (**D**). **E**, Scatter plot showing Δspeed of individual animals during *t*
446 = 330-360 s in **D**. **F**, Scatter plot showing Δspeed of individual animals during the
447 stimulation. In a series of daily experiments, wild-type N2 and three to five mutant strains

448 were analyzed in parallel, and all the N2 data are combined in **F**. Statistical values were
449 calculated using Kruskal-Wallis test with *post hoc* Wilcoxon test with Bonferroni correction.
450 ** $p < 0.001$. Sample numbers were 30–36 per mutant strain, and the details are described in
451 the Supplementary Table.
452
453 **Figure 6. OFF response is dependent on VGCC, and the persistence is regulated by**
454 **neuropeptide signaling. A–D,** Speed-time graph of ON response with 75 V stimulation of
455 30 s on mutants of sensory signaling (**A**), VGCC (**B**), biogenic amine biosynthesis (**C**), and
456 neuropeptide biosynthesis (**D**). **E**, Scatter plot showing Δspeed of individual animals during *t*
457 = 180-210 s in **D**. **F**, Scatter plot showing Δspeed of individual animals during the
458 stimulation. In a set of daily experiments, wild-type N2 and three to four (???) mutant strains
459 were analyzed in parallel, and all the N2 data are combined in **F**. Statistical values were
460 calculated using Kruskal-Wallis test with *post hoc* Wilcoxon test with Bonferroni correction.
461 ** $p < 0.001$. Sample numbers were 30–36 per condition, and the details are described in the
462 Supplementary Table.
463
464 **Figure 7. Model for mechanism of speed increase caused by electrical shock.**
465
466 **SUPPLEMENTARY FIGURE LEGENDS**
467 **Figure 2—figure supplement 1**. Speed-time graphs with different voltage stimulation at 60
468 Hz. Gray indicates the duration of electrical stimulation (0-30 s). The thick line and the
469 shaded region indicate the average ± SD. Sample numbers were 57–58 per condition, and the
470 details are described in the Supplementary Table.
471

Figure 2—figure supplement 2. Speed-time graphs with different voltage stimulation at different frequencies. Gray indicates the duration of electrical stimulation (0-30 s). The thick line and the shaded region indicate the average ± SD. Sample numbers were 33–37 per condition, and the details are described in the Supplementary Table.

Figure 2—figure supplement 3. Movement directions of animals during the response. The angles of movement vectors from the beginning to the first 2 min of the stimulation were plotted. **A-C,** Rose plot for animals which were assayed on plate with small food patch (**A**, n = 35, 30 V at 4 Hz), full food lawn (**B**, n = 85, 30 V at 4 Hz), or small food patch (**C**, n = 36, without electrical stimulation). Bin number for each chart is set at 16 bins. Statistical analysis performed is Watson U2 test.

Figure 2—figure supplement 4. Low and high speed groups during 75 V stimulation. **A**, Histogram and its density (black line) indicates speed of each animal during the electrical shock. From the histogram, we set the threshold as 0.125 mm/s to separate the low- (**B**) and high-speed (**C**) groups. Sample numbers were 20 and 15 for lower and higher speed groups, and the details are described in Supplementary Table.

Figure 4—figure supplement 1. Worms' speed did not change when they move in or out of food. **A,** Illustration showing worms' movement across multiple food strips. When worms leave food strip and enter no food area, this movement is defined as "outward movement". When worms enter food strip from no food area, this movement is defined as "inward movement". **B,** Scatter plot showing average speed of individual animals with outward (left; n = 44) or inward (right; n = 32) movement during 30 V stimulation for 4 min. The average

speed was calculated 10 s before and after the food exit/entry. Statistical analysis was performed by Wilcoxon signed-rank test, and no significant difference was observed.

ACKNOWLEDGEMENT:

We thank Yuki Tanimoto and Yuka Tsuda for the initial phase of the electrical shock paradigm, Shinobu Aoyagi for setting up the system, and Young-Jay You, Aki Takahashi, and the Kimura lab members for their valuable advice, comments and technical assistance for the study. Nematode strains were provided by the Caenorhabditis Genetics Center (funded by the NIH Office of Research Infrastructure Programs P40 OD010440).

FUNDING:

This study was supported by Japan Society for the Promotion of Science (KAKENHI JP16H06545, 20H05700 and 21K19274 to K.D.K.), Grant-in-Aid for Research in Nagoya City University (48, 1912011, 1921102 and 2121101), the Joint Research by National Institutes of Natural Sciences (01112002), Toyoaki Scholarship Foundation, and RIKEN Center for Advanced Intelligence Project (to K.D.K).

COMPETING INTERESTS

The authors declare no competing interests.

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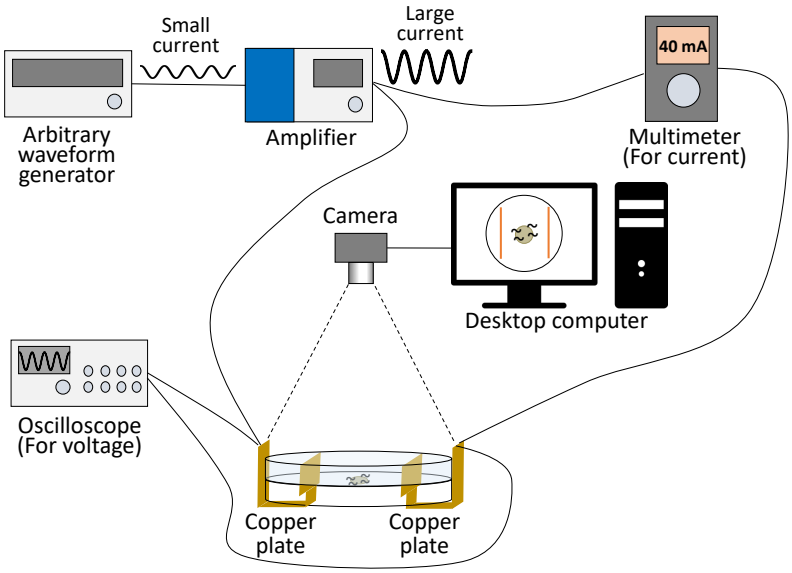
Figure 1



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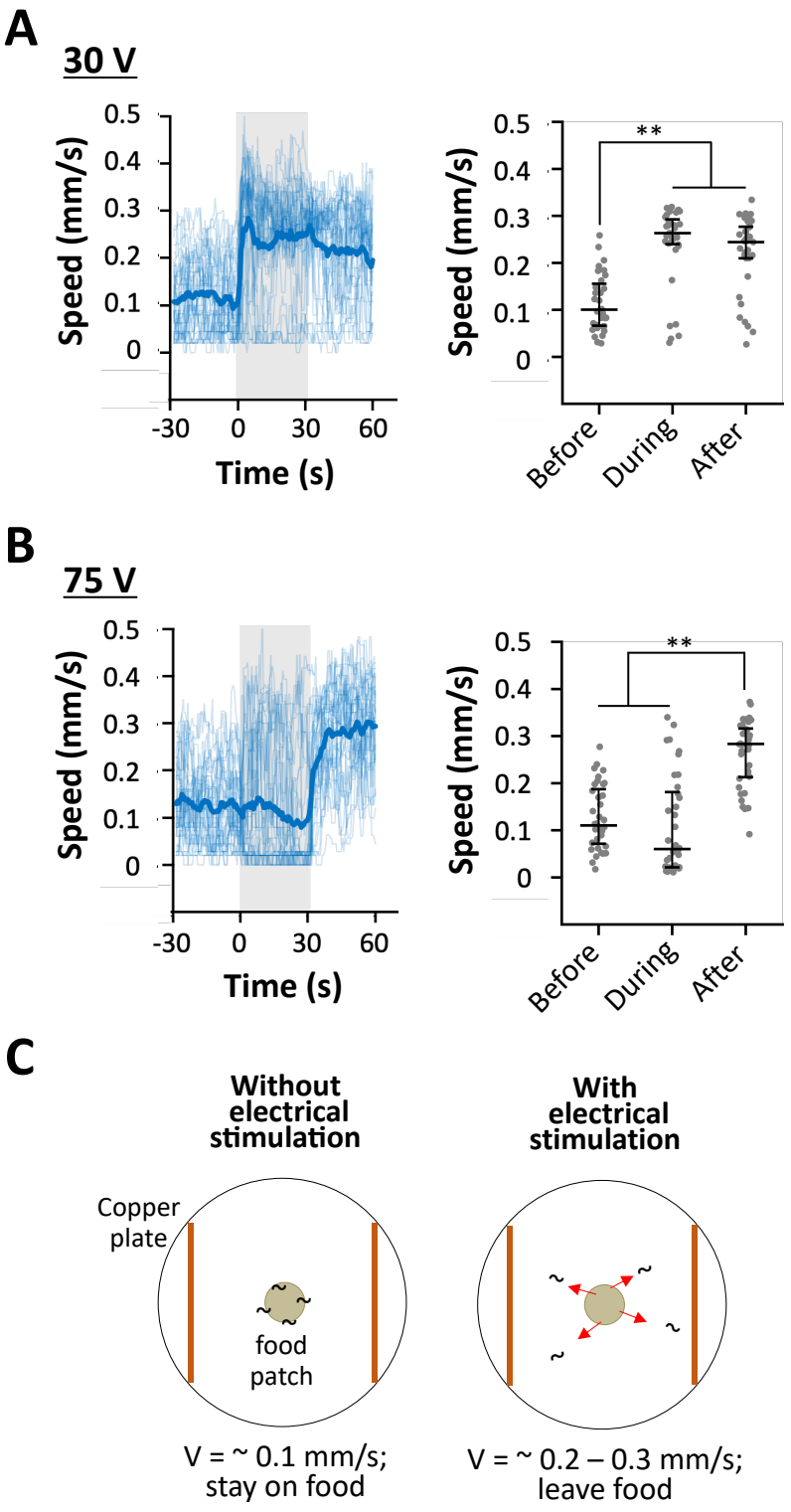

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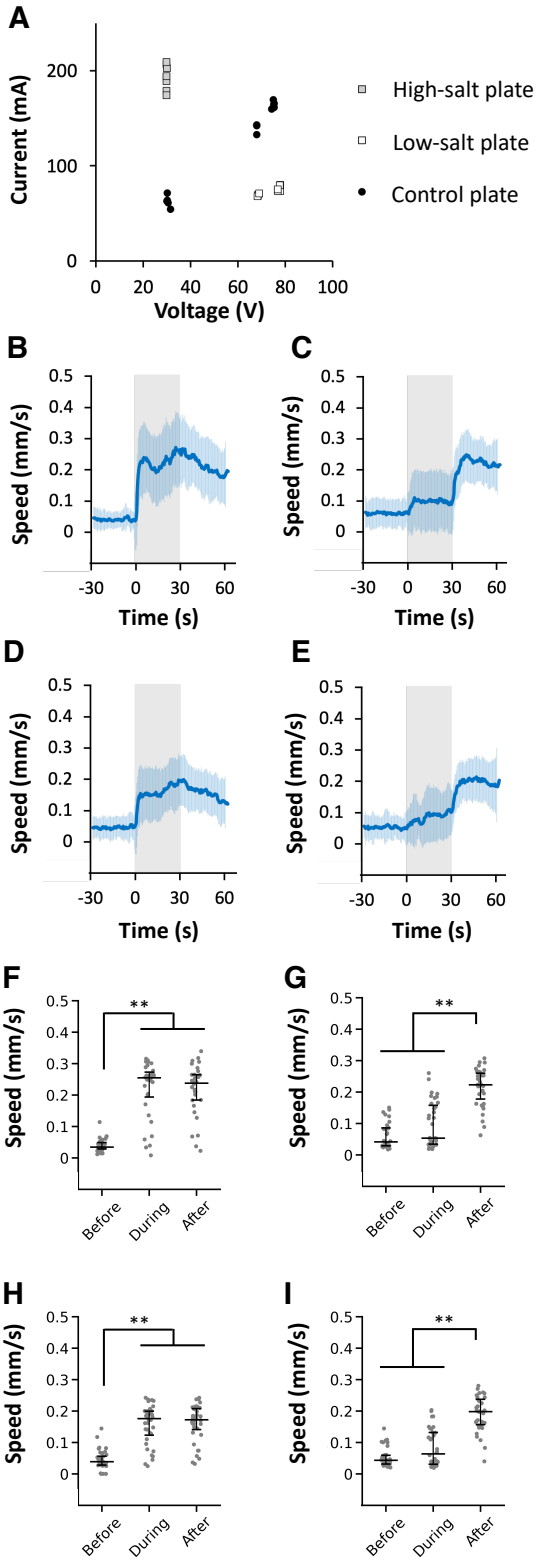

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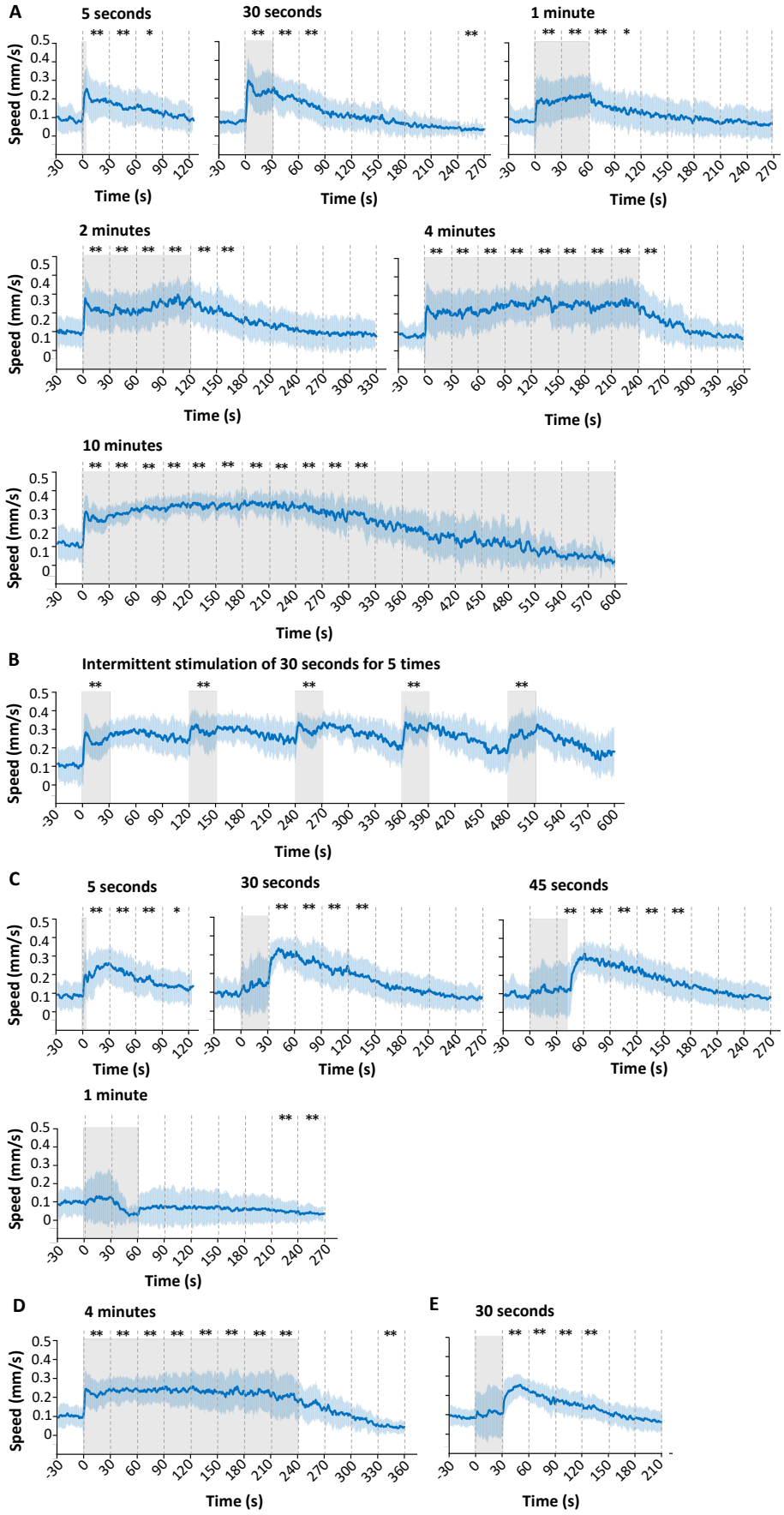

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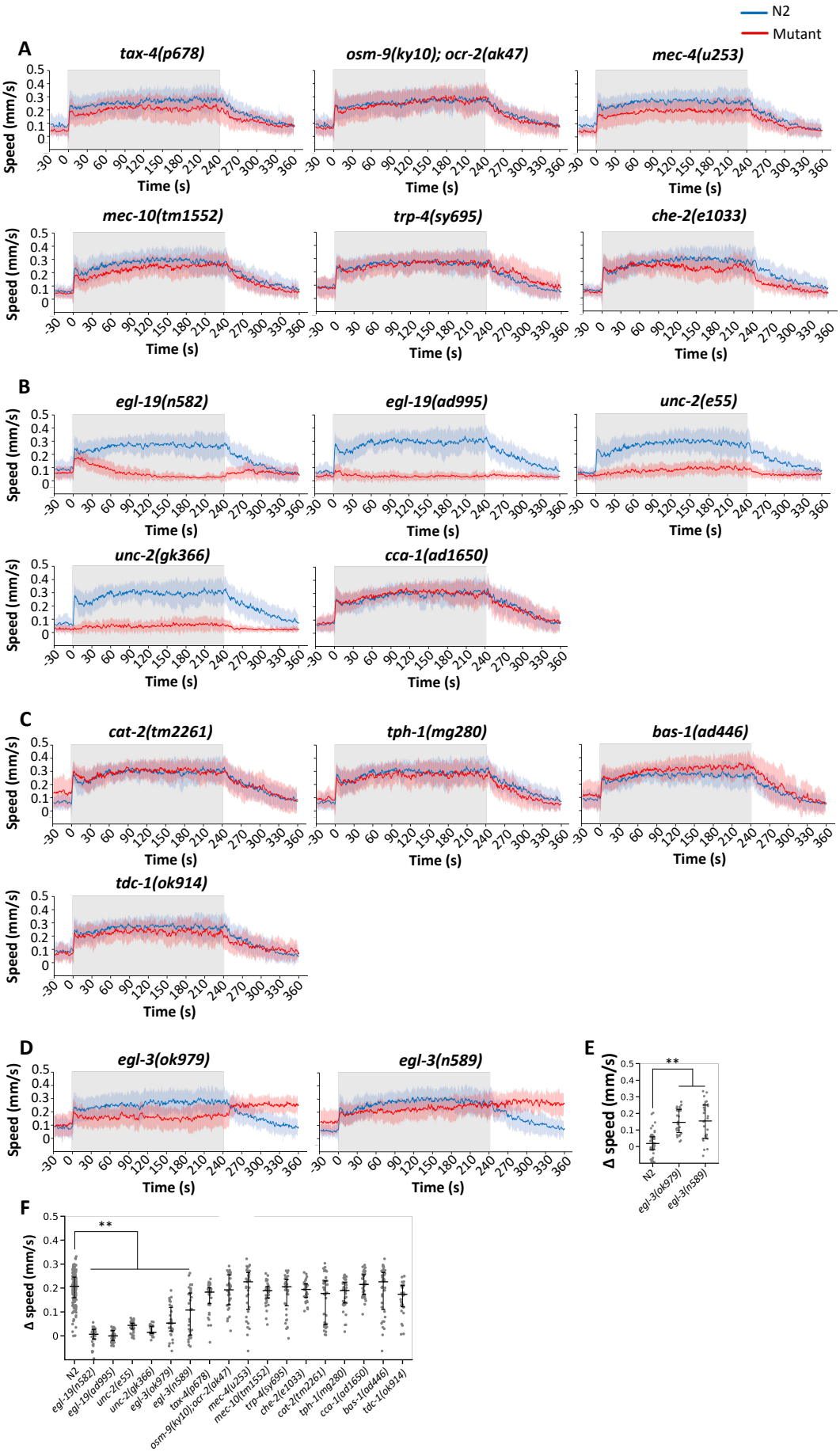

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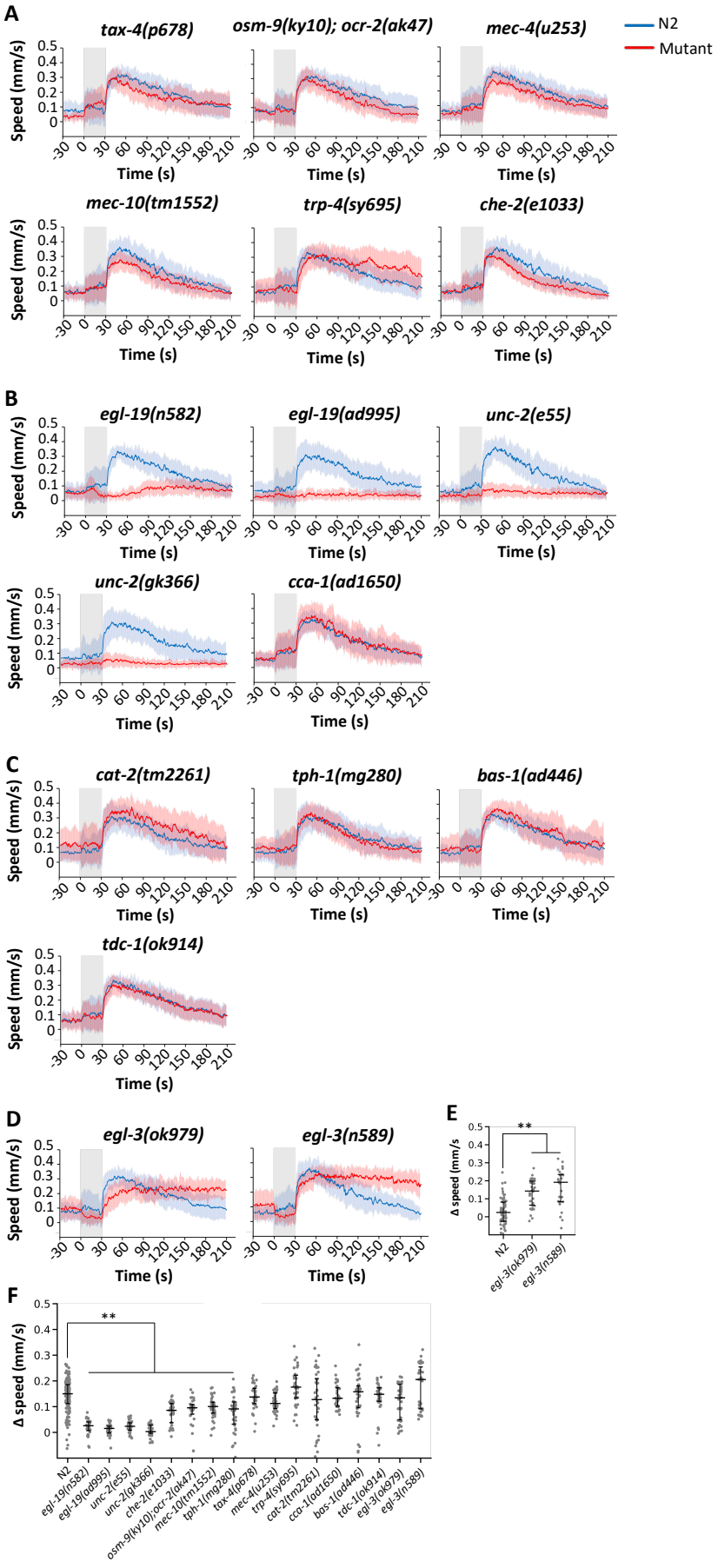

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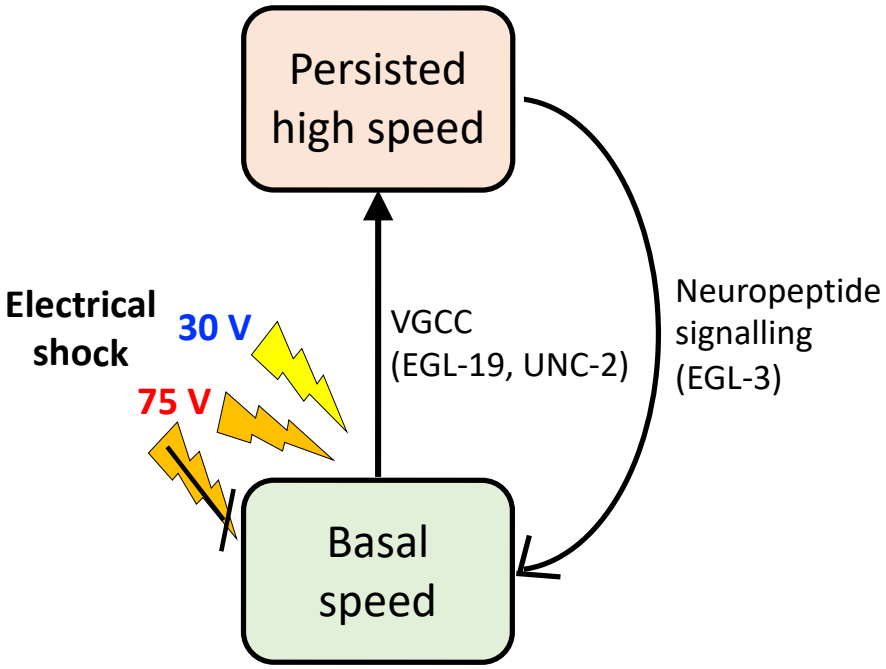

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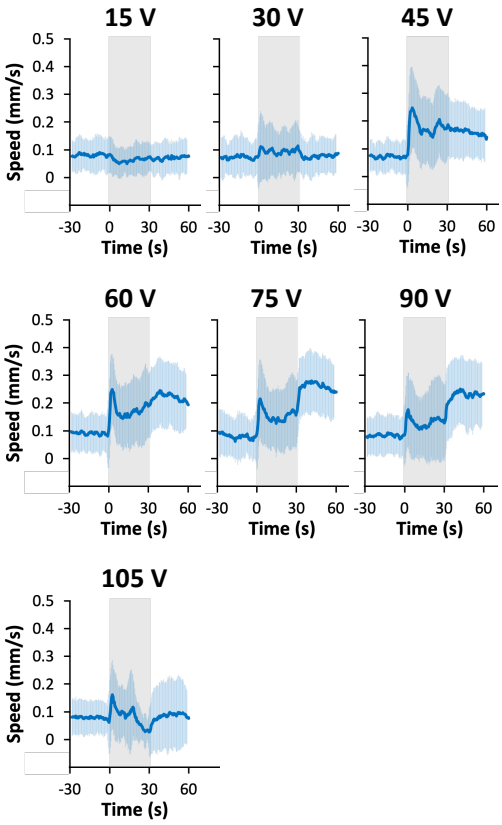

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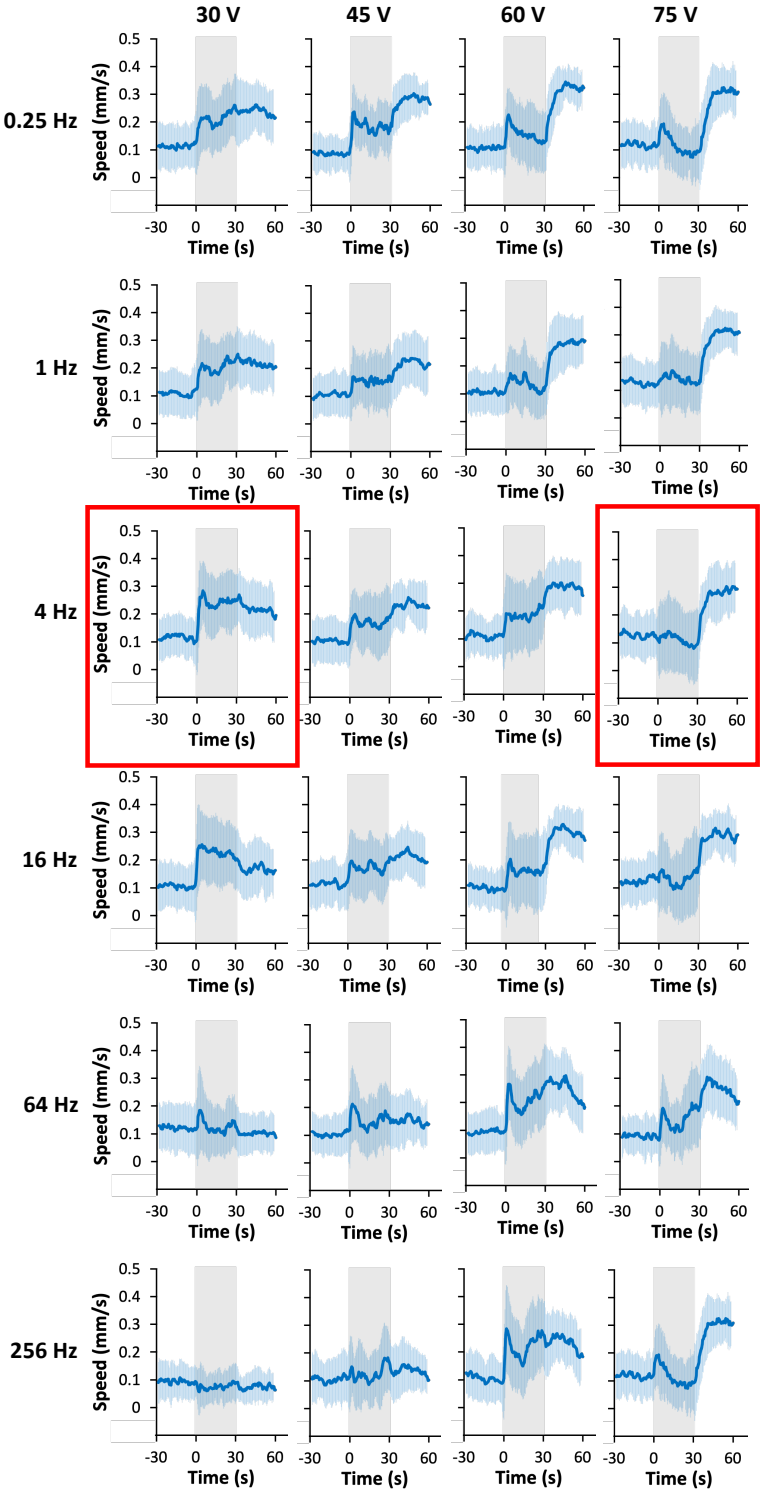

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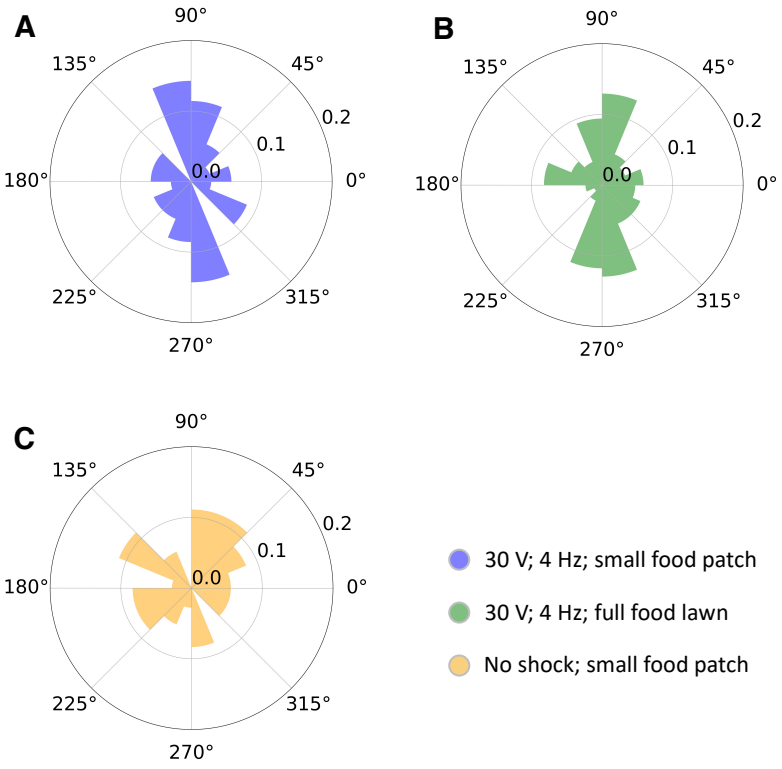

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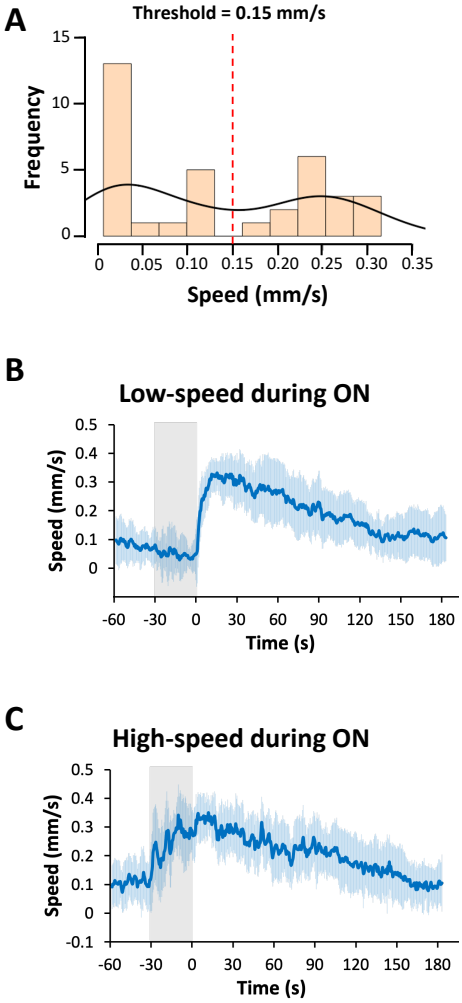

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