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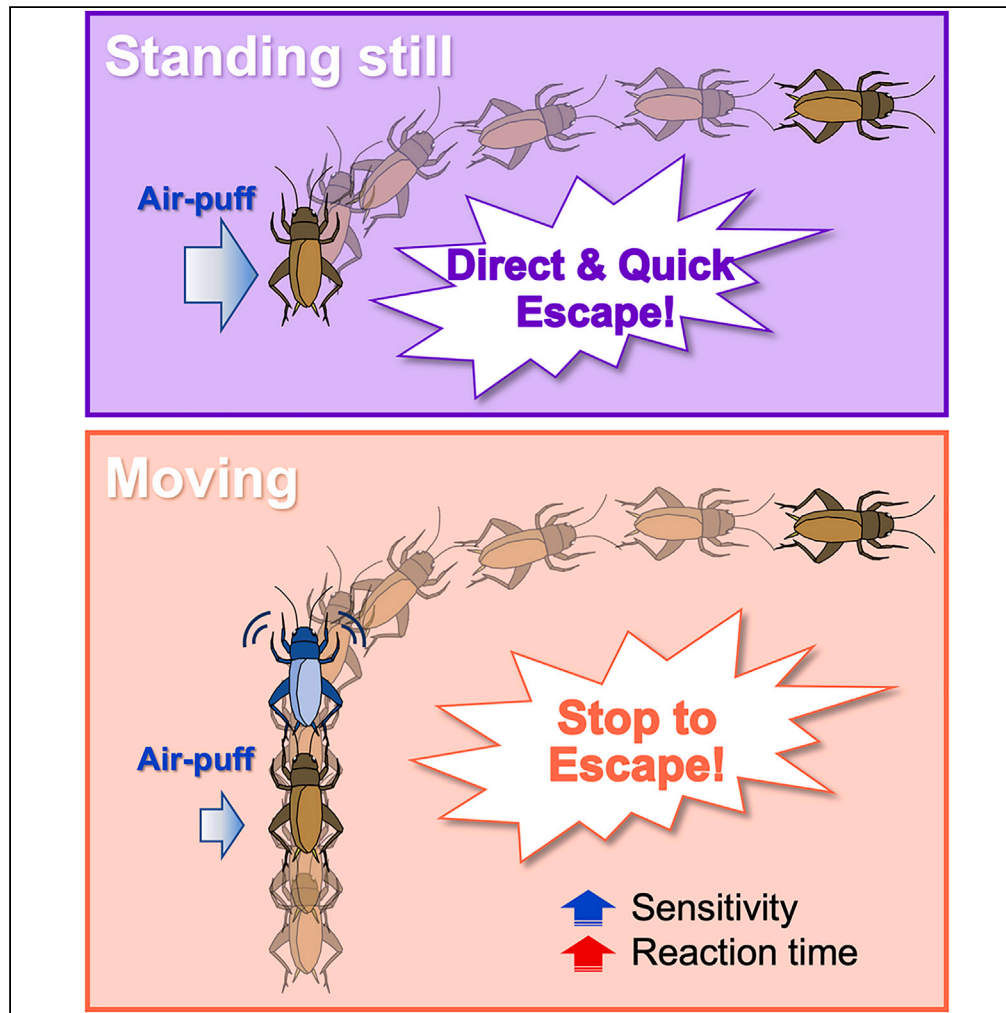
Motor state changes escape behavior of crickets

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Highlights
Motor states when stimulated affect cricket wind-elicited escape behavior

When stimulated during locomotion, crickets quickly stop prior to escape

Moving crickets are more sensitive to airflow stimuli than stationary ones

Response delay during moving may be compensated by the high sensitivity

Article

Motor state changes escape behavior of crickets

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SUMMARY

Animals change their behavior depending on external circumstances, internal factors, and their interactions. Locomotion state is a crucial internal factor that profoundly affects sensory perception and behavior. However, studying the behavioral impacts of locomotion state in free-moving animals has been challenging due to difficulty in reproducing quantitatively identical stimuli in freely moving animals. We utilized a closed-loop controlled servosphere treadmill system, enabling unrestricted confinement and orientation of small animals, and investigated wind-induced escape behavior in freely moving crickets. When stimulated during locomotion, the crickets quickly stopped before initiating escape behavior. Moving crickets exhibited a higher probability of escape response compared to stationary crickets. The threshold for pausing response in moving crickets was also much lower than the escape response threshold. Moving crickets had delayed reaction times for escape and greater variance in movement direction compared to stationary crickets. The locomotion-related response delay may be compensated by an elevated sensitivity to airflow.

INTRODUCTION

Animals' motor states, i.e., whether they are stationary or mobile, have great and complex impacts on their neural and behavioral responses. Sensory perception is sometimes gated, enhanced, or suppressed during locomotion.[1–7] These motor state–dependent changes in sensory perception could be interpreted as the effects of arousal and attention.[8,9] Furthermore, it has been reported that both behavioral and neural responses are affected by the motor state in an inverted U-shaped relationship, rather than a monotonous increase or inhibition.[10,11] Thus, this complexity of the effects of the motor state on both neural and behavioral responses makes it difficult to elucidate the relationships between them. In particular, it is more difficult for learned tasks because the motor state may have impacts on multiple levels of sensory perceptions, cognitive process, and learning ability. To determine the effects of the motor state on the sensory process alone, therefore, we investigated the innate escape behavior elicited during locomotion in insects with simple nervous systems.

The reaction time, escaping speed, accuracy in controlling the escape direction, and choice of escape actions are performance measures of escape behavior in response to threat stimuli or predator signals.[12–14] Because quantitative reproducibility of identical threat stimuli in freely moving animals is essential for determining locomotion effect on these behavioral parameters, we employed a closed-loop controlled servosphere treadmill system that allows small animals such as insects to be fixed in a specific position and head orientation without restriction.[15] The closed-loop controlled spherical treadmill system has also been used in pioneer studies on cricket's phonotaxis.[16,17] The advanced high-speed video-based closed-loop control and integration with a stimulator enable precise stimulation in orientation and flow speed according to the animal's motor state (Figure 1A). Using the proposed system, we investigated how crickets changed their escape behavior elicited by short airflow depending on their motor state, which was either stationary or mobile when stimulated.

Crickets, as well as other orthoptera, exhibit escape response to a short airflow stimulus, which is perceived as a threat signal suggesting that a predator is approaching[18–20], and move quickly in an opposite direction to the stimulus by running or jumping.[21] These wind-elicited escape behaviors are mediated by a pair of mechanosensory organs called cerci, and about 500–700 filiform hair sensilla are distributed on each.[22] Notably, crickets detect the flow velocity and direction of surrounding air currents using the cercal sensory system.[23] Previous studies on freely moving crickets in an experimental arena reported that crickets change the wind-elicited escape behavior in the selection rate of either jumping or running and in locomotion

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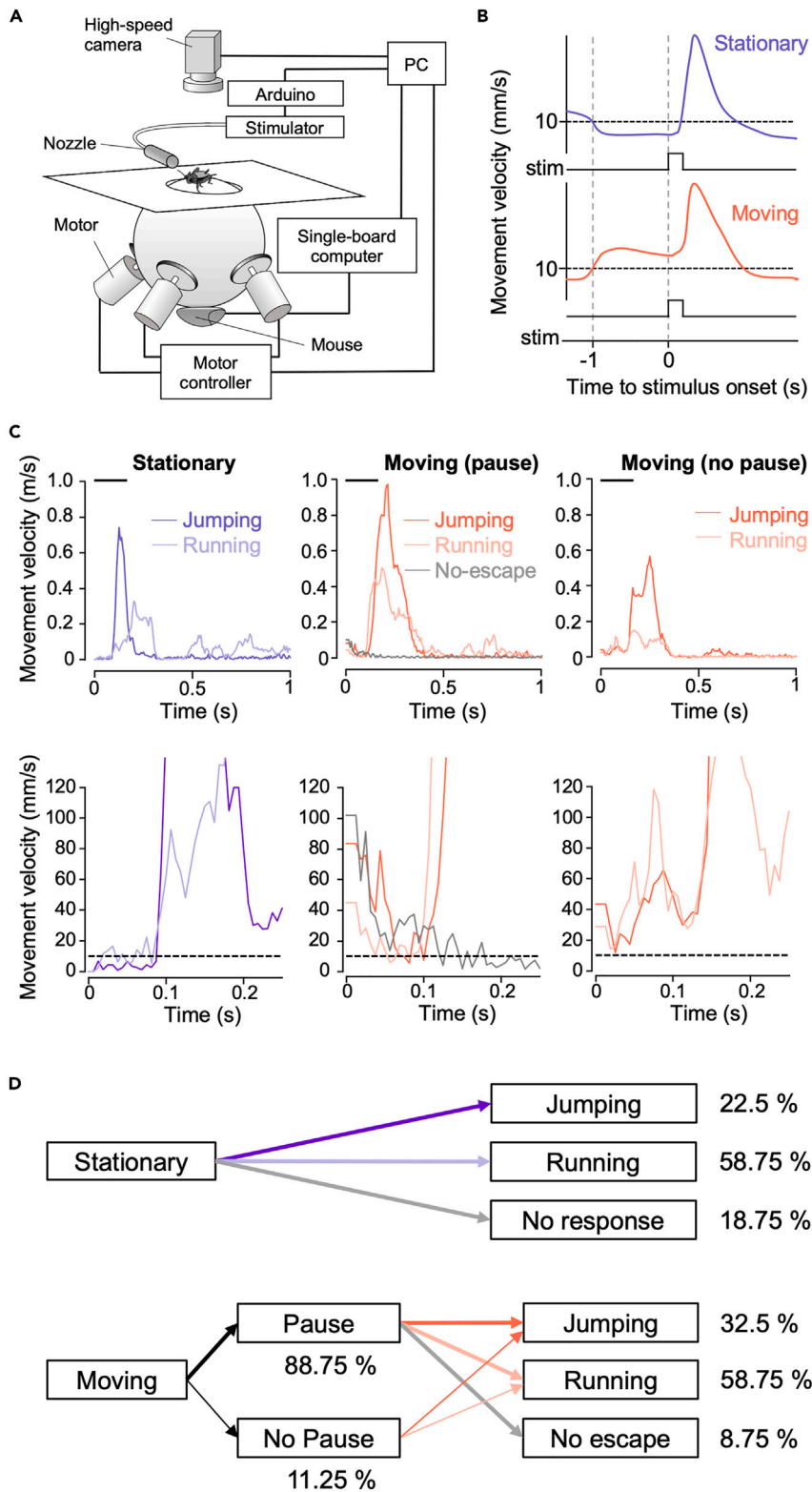https://doi.org/10.1016/j.isci.2023.107345

Figure 1. Escape behaviors while crickets are standing still and moving

(A) A servosphere treadmill system for the stimulation and monitoring of a non-tethered cricket.

(B) Stimulation criteria by different motor states based on movement velocity.

Figure 1. *Continued*

(C) Time course of translational velocity in typical responses to 1.00 m/s of stimulus under different motor states. The upper panels indicate whole-time courses of the initial escape response in stationary (left) and moving conditions (middle and right). The middle and right panels show the responses with (middle) and without (right) pausing before the escape movements, respectively. Black bars above the traces indicate the duration of the stimulus (200 ms). Lower panels indicate magnified data during the earlier period (see STAR Methods). Dashed lines in lower panels indicate a threshold to determine the start of the escape response and the pausing time. Darker colored traces represent jumping response, and lighter colored ones represent running response. Gray traces in the middle panels represent no movement following the pausing response.

(D) Patterns of the behavioral sequence in stationary and moving conditions. The upper chart indicates the response when the crickets were stimulated during stationary, and the lower chart indicates the response when they were during moving. The numbers on the side of boxes are the percentage of each behavior in response to 1.00-m/s air currents.

parameters such as movement velocity and distance, depending on the airflow velocity and duration.[24] In addition, studies of crickets tethered on air-lifted treadmills reported that cricket escape behavior is modified by additional sensory inputs of different modalities that indicate the surrounding contexts.[25–27] Another similar experiment demonstrated that the probability of the escape response to airflow increases with forward speed when crickets walking spontaneously are stimulated.[28] These findings suggest that cricket wind-elicited escape behavior, the motor parameter characteristics of which have been explicitly described, is a promising model to explore behavioral modulation by an animal's motor state, but the experiments should be ideally conducted in unconstrained conditions with quantitatively manipulated stimulation.

In this study, we examined the wind-elicited escape behaviors of freely moving and stationary crickets using a closed-loop controlled servosphere system that provided a trigger signal to the airflow stimulator. This system allowed a freely moving and untethered cricket to be kept in a specific location and orientation, allowing an airflow stimulus of quantitatively identical velocity and duration to be applied to the animal from a specific direction. Analyses of escape behaviors elicited by quantitatively controlled stimuli revealed that motor states affected both sensitivity to threat signals and motor parameters.

RESULTS

Airflow stimulus caused the moving cricket to pause, followed by an escape response

When we applied the air-current stimuli of 1.00-m/s velocity to the stationary crickets, they immediately exhibited escape responses, either "running" or "jumping" regardless of the stimulus angle, as previously reported[21] (Figure 1C left; Videos S1 and S2). In contrast, when the crickets received airflow while in motion, they quickly stopped moving and then escaped by running or jumping (Figure 1C center; Videos S3 and S4). In some cases, the crickets directly escaped without stopping in which the movement velocity did not decrease below 10 mm/s after the stimulation (Figure 1C, right). In addition, some crickets that paused in response to airflow remained stationary without any movement. Meanwhile, the crickets that did not pause always exhibited either "running" or "jumping" as escape movements (Figure 1D). Consequently, when stimulated by the airflow, the crickets' escape responses varied depending on their motor state.

The crickets that were stimulated while stationary escaped immediately, whereas those that were stimulated while moving paused or slowed before escaping. The servosphere treadmill system used in this study was designed to stop once the airflow stimulus started, indicating that the pausing response of the moving cricket may have been caused by the stopping of the treadmill ball but not by the air-current stimulus. To confirm this possibility, we examined the response to the stopping of the treadmill ball without airflow stimulation. The time taken for the crickets to stop after the treadmill ball was stopped was significantly shorter when airflow was provided (Figure S1). This indicated that the pausing response was significantly caused by the airflow stimuli although the effect of treadmill stoppage was not completely denied.

Moving crickets were more sensitive to the airflow in order to exhibit the escape response

To investigate the effects of the motor state on sensory sensitivity to the airflow stimulus in the escape response, four different velocities of airflow stimuli were applied to moving and stationary crickets, respectively. When the crickets were stimulated under both conditions, the response probability increased as the airflow speed increased (Figure 2A). However, the response probability of moving crickets exceeded that of stationary crickets, and the difference between them seemed to be more pronounced with a weaker stimulus. We estimated the response probability using GLMM with stationary or moving motor states, stimulus velocity, and their interaction as explanatory variables (see STAR Methods). In this model, all explanatory
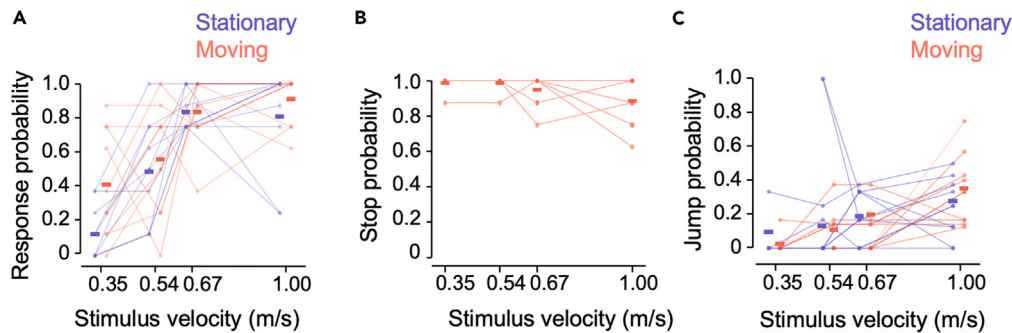
Figure 2. Probability of the behavioral responses in stationary and moving crickets

(A–C) Probabilities of the escape response including both running and jumping (A), of the pausing response (B), and the jumping response (C) for different stimulus velocities. Colored-filled circles connected with thin lines indicate the probability for each individual, and colored bars represent the mean of the individuals' probabilities for the stationary (blue) and moving conditions (red). N = 10 individuals.

variables had significant effects on the response probability (Data S1A). The significant effects of the motor state and its interaction with the stimulus velocity indicated that crickets stimulated while in motion exhibited escape responses to weaker airflow stimuli more frequently than those stimulated while stationary (Figure 2A). Comparing the response probability between the stationary and moving conditions for each stimulus angle and velocity, the moving crickets responded more to the lowest velocity (0.35 m/s) of airflow stimuli from the side (Figure S2A, Data S1B). In addition, the moving crickets exhibited the "pause response" to the weakest stimuli, whereas the stationary crickets rarely exhibited escape behaviors (Figure 2B), suggesting that the pause response threshold was much lower than the escape response threshold.

We examined the effects of the motor state on the proportion of the escape response involving jumping. No differences in the jump probability between the motor states were observed (Figure 2C, Data S1C). In both moving and stationary conditions, a stronger stimulus resulted in a greater frequency of jumping responses from crickets. Notably, no difference was observed when comparing the behavioral preferences for stationary and moving conditions for each stimulus angle and velocity (Figure S2B, Data S1D). Overall, moving crickets were more sensitive to air stimuli than stationary crickets, but their behavioral choice was unaffected by their motor state.

Moving crickets exhibited delayed escape time due to pausing response

During movement, crickets exhibited a remarkable sequence of escape behavior, which was accompanied by a "pause" before escape movements. To investigate the time sequence of escape behavior during movement, we defined reaction times. For the stationary conditions, the reaction time (RT) was defined as the delay from stimulus onset to the start of the initial response, as in previous studies.[21,24] For the movement condition, we defined three kinds of reaction times: T1, T2, and RTm (See STAR Methods, for details). T1 was the delay from stimulus onset to pausing, which was determined as the time when the cricket speed became lower than 10 mm/s. T2 was the interval between pausing and the start of the initial response. RTm was the sum of T1 and T2 (Figure 3A). Because T1 and T2 were almost comparable to or slightly shorter than RTs (Figure 3B), the time window for the definition of the pausing and escape responses in the moving condition was set to 250 ms after the stimulus onset, as in the stationary condition.[21,24]

We first compared the RTs and RTm, which were the delays between stimulus onset and escape initiation in stationary and moving conditions (Figure 3C). The effects of the motor state on these reaction times were assessed using GLMM. The explanatory variables of motor state, stimulus velocity, and their interaction were significant (Data S1E). RTm was longer than RTs, and both of these reaction times decreased as the stimulus velocity increased, in which this dependency was different between RTm and RTs. Furthermore, we separated RTm into T1, which was the time to stop, and T2, which was the time from stop to escape, and compared them with RTs based on stimulus velocity, respectively. GLMM used to estimate RTs and T2 indicated that stimulus velocity and motor state were significant variables, revealing the T2 was shorter than RTs (Figure 3D, Data S1E). This finding suggests that the escape movement that follows a transient pause in the moving condition is initiated more quickly than an escape response in the stationary
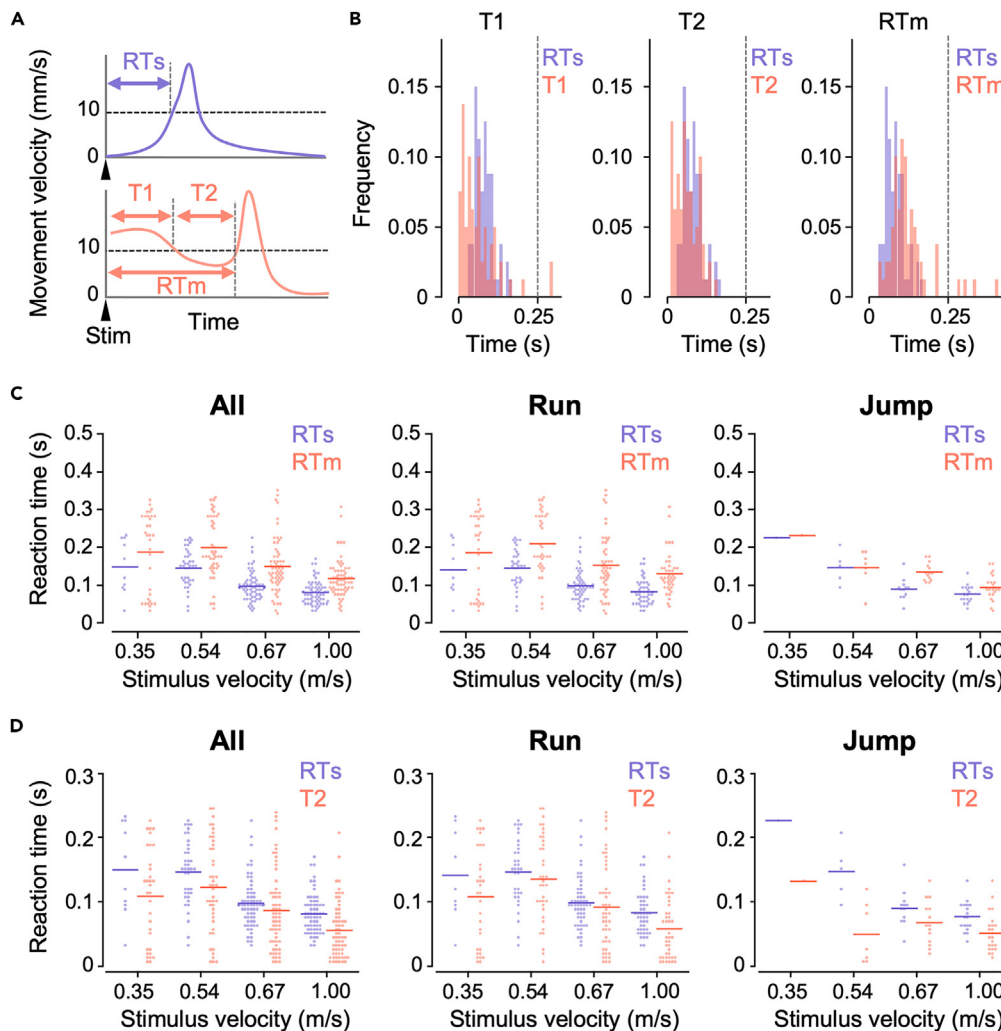
Figure 3. Reaction times of escape behavior in stationary and moving crickets

(A) Schematic diagram indicating the definitions of the reaction times based on the time course of translational velocity. RTs and RTm are delays from stimulus onset to the start of escape movements under stationary (blue) and moving (red) conditions, respectively. T1 is the delay from stimulus onset to pausing, and T2 is the delay from pausing to the start of the escape movement.

(B) Distribution of reaction times under stationary and moving conditions. The data were obtained from trials in which the crickets were stimulated by 1.00 m/s of airflow.

(C and D) Reaction times, RTs (blue in C, D), RTm (red in C), and T2 (red in D), for different stimulus velocities under stationary and moving conditions. Each column of the panels indicates the data for all (left), running (middle), and jumping (right) responses. Colored dots represent the data for each trial, and bars represent the mean of all trials for each type of response. N = 10 individuals.

condition. Next, we compared RTs and T1 (Figure S3A). Because T1 was the time elapsed between the stimulus onset and pause and differed from the delay from RTs and T2, we used GLMM with a different explanatory variable, reaction time type (*type*), to assess the effects of this variable and stimulus velocity. All explanatory variables, including the interactions among them, were significant (Data S1E), suggesting that T1 was significantly shorter than RTs regardless of the stimulus velocity. Meanwhile, greater stimulus velocity implied shorter RTs but not T1. In addition, different GLMM used to estimate T1 only in the moving cricket indicated no significance for the explanatory variables of "escape or not" (*response*) but for the moving speed when the crickets were stimulated (V_0) (Figures S3B and S3D, Data S1Fi). This result indicated that the delay to pausing was unrelated to the decision of whether to escape or not, and that the faster the cricket moved when stimulated, the longer it took them to stop. Notably, the other GLMM used to estimate
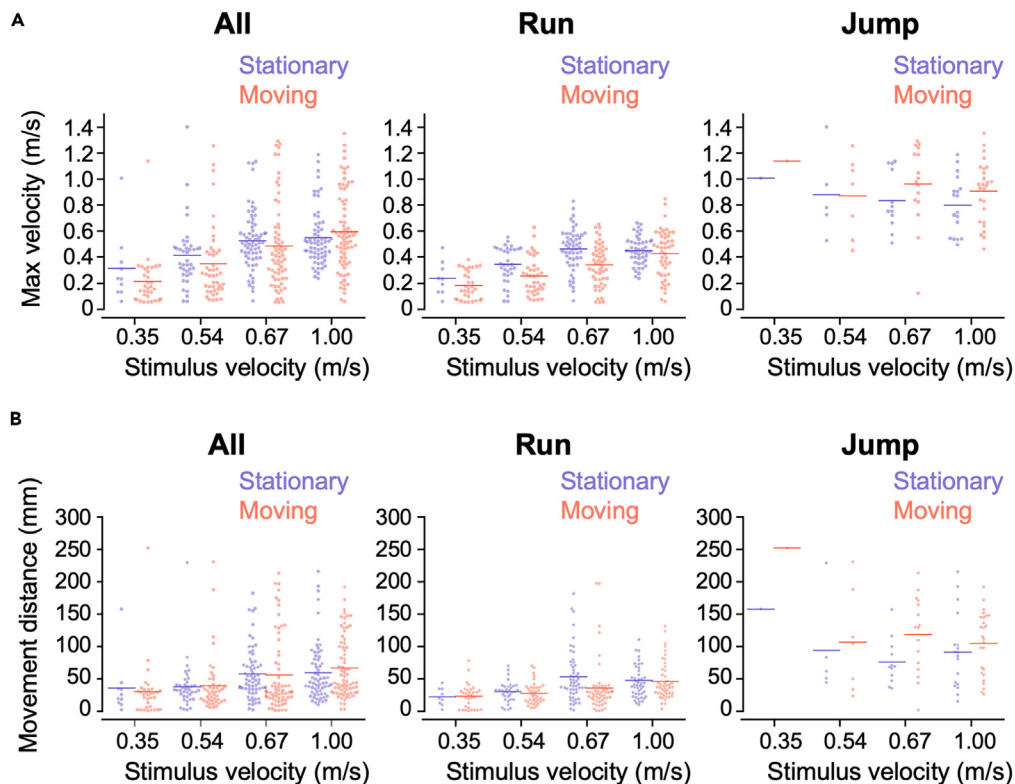
Figure 4. Maximum translational velocity and movement distance in stationary and moving crickets
(A and B) Maximum translational velocity (A) and movement distance (B) for different stimulus velocities. The left, middle, and right columns of panels show the data for all escapes, running, and jumping responses, respectively. Colored dots represent the data for each trial, and bars represent the mean of all trials for each type of response under stationary (blue) and moving conditions (red). N = 10 individuals.

T1, which included the explanatory variables for escape action type (*action*), either running or jumping, instead of (*response*) revealed the significance of the effects of action type and its interaction with stimulus velocity, in addition to V_0 (Figures S3C and S3E, Data S1Fii). The findings indicated that the pause time before jumping decreased as the stimulus velocity increased, whereas that before running remained relatively constant. The fact that T1 before jumping was shorter than before running and that jumping occurred in response to faster stimuli would explain the significance of the escape response types. Collectively, moving crickets require more time to decelerate and stop than stationary crickets, thereby delaying escape initiation.

Moving crickets exhibited similar speed and distance traveled as stationary crickets, but their escape direction was more varied

The effects of motor states on the various locomotion parameters of escape behavior were investigated. We first assessed these effects on the maximum translational velocity using GLMMs with the moving state, stimulus velocity, behavioral selection, and their interactions as explanatory variables (Data S1Gi). The significant effect of the interaction between the motor state and escape action type revealed that moving crickets had a lower maximum velocity in running escape than stationary crickets (Figure 4A). In addition, the significance of the effects by escape action types and their interaction with stimulus velocity indicated that the translational velocity of a jumping response always exceeded that of a running response, and that this velocity of a running escape response increased as stimulus velocity increased. These results corroborated previous results for stationary crickets.[24] In addition, the significant effect of the interaction of the motor state with stimulus velocity suggested that the maximum velocity increased to a greater extent for the moving crickets than for stationary crickets, depending on the stimulus velocity. Furthermore, GLMM used to estimate the movement distance revealed significant effects of escape action type, stimulus velocity, and their interaction (Data S1Gii), suggesting that the movement distance was longer in the jumping response

than in the running response, regardless of motor state, as previously reported.[24] In addition, the significance of the interaction effects suggested that the movement distance increased in running escape as stimulus velocity increased (Figure 4B). However, as revealed by no significance of motor state variables, there was little difference in the movement distance between the motor states.

Next, we examined the effects of the motor state on the escape behavior's directional control. Sato et al. (2019) revealed that stationary crickets escaped in the opposite direction to the stimulus source by running or jumping when stimulated by a short air puff.[21] The present results revealed that moving crickets could also move in the direction opposite the stimulus when stimulated (Figure 5A). Therefore, to quantitatively analyze accuracy in the directional control, the movement directions were plotted against the stimulus angle, which was defined as shown in Figure 5B.[21,24,29] The plots for both stationary and moving conditions were distributed around the line $y = x$ against all ranges of the stimulus angles (Figure 5C). This finding suggested that the crickets responded to stimuli from all directions by moving basically in the opposite direction to the stimuli. However, as the stimuli slowed, the plots of the running responses in the moving condition became more dispersed and further away from the $y = x$ line (Figure S4), indicating that the crickets may have regulated movement direction less accurately in the moving conditions than in the stationary condition. To quantitatively analyze the accuracy of the directional control, we calculated the absolute value of the difference between movement direction and stimulus angle as a directional accuracy index, after which the effects of motor state on this value were assessed using GLMM. The model revealed the significance of the explanatory variables, motor state, stimulus velocity, and escape action type (Data S1Giii), demonstrating that the movement direction was less accurately controlled against the stimulus angle in the moving condition than in the stationary condition (Figure 5D). In addition, regardless of the motor states, the displacement of the movement direction relative to the opposite direction of the stimulus was larger in the running response than in the jump response, and the movement direction was controlled more accurately in both types of escape action as the stimuli speed increased. Notably, escape behavior appeared to be more delayed and less accurately controlled under moving conditions than under stationary conditions. However, the moving crickets exhibited similar motor performance, such as their speed and distance during escape behavior compared to stationary crickets.

The pause response had little effect on subsequent escape behavior

When stimulated while in motion, in most trials, crickets paused transiently and then escaped. In some cases, however, they decreased their moving speed but escaped without pausing (Figure 1D). The difference in whether the crickets paused or not in response to airflow might affect subsequent escape movements. The escape behavior may also depend on the spontaneous walking activity that was indicated as the moving speed when the crickets were stimulated (V_0). GLMM used to estimate the stop probability, or the probability of the "pausing" response, also indicated that V_0 and stimulus velocity were insignificant (Data S1H), suggesting that the crickets stimulated during locomotion paused regardless of their movement speed or stimulus intensity. In addition, GLMMs used to estimate the response and jump probabilities indicated that both the "pause" or "no pause" and V_0 were insignificant (Data S1I and S1J), indicating that the presence or absence of pausing response and velocity when crickets were stimulated had no effect on the response probability and selection of subsequent escape behaviors.

GLMMs used to estimate the maximum translational velocity and movement distance revealed that the explanatory variable, "pause" or "no pause" (Data S1K), was insignificant, indicating that no difference was observed in the motor performance of the escape behavior, whether or not a pausing response was followed by it (Figure S5A). Although the explanatory variable of "pause/no-pause" was significant in the model for angular difference between movement direction and stimulus angle, the data for "no-pause" escape response was too small to discuss the meaning of this effect. These models revealed no significant effects of V_0 on the motor parameters and movement direction (Data S1K), suggesting that the velocity of the moving cricket when stimulated had no effect on the escape behavior (Figure S5B). Notably, neither the pausing response nor the speed at which the crickets moved in response to airflow stimulation had significant effects on their subsequent escape behavior. These results indicated that the pausing response was ineffective in facilitating escape movements.

DISCUSSION

Freezing response as a behavioral strategy

In this study, we examined how the cricket motor state influenced its escape behavior in response to airflow stimuli. The behaviors of insects in response to air currents have been well studied for over 40 years.[18–20]
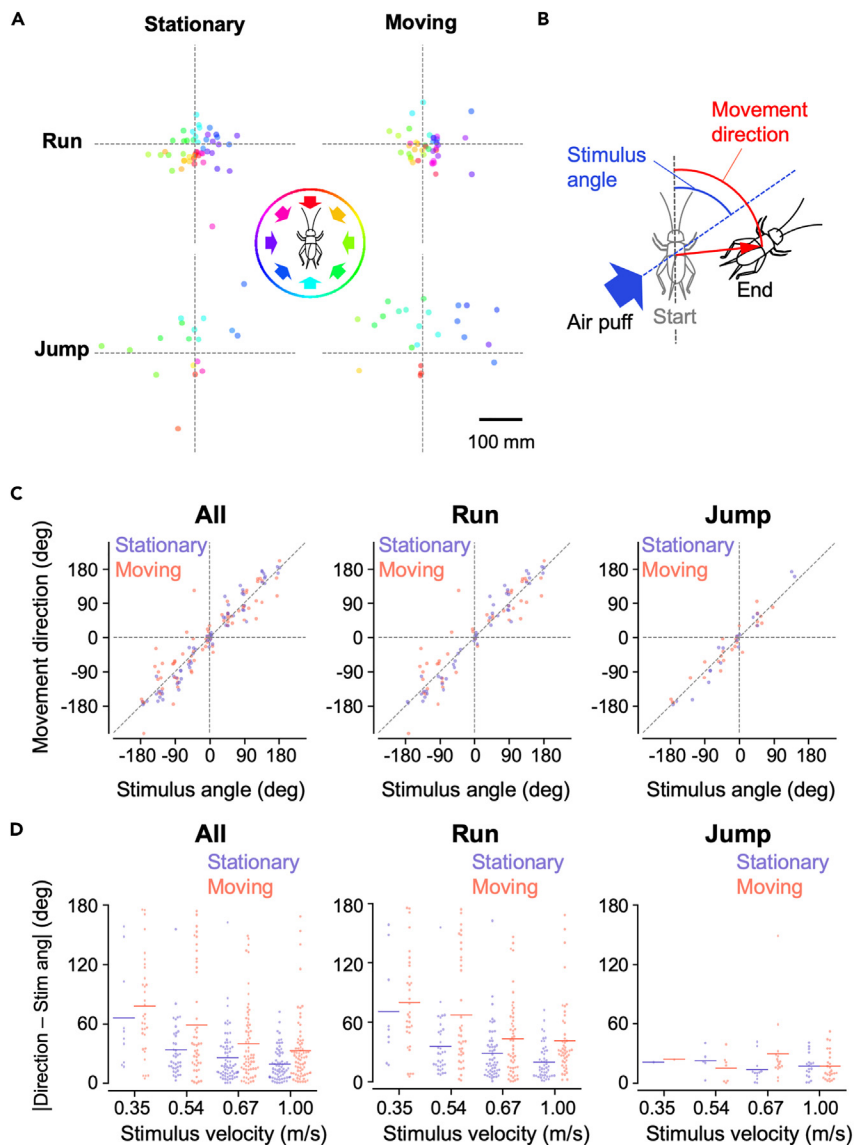
Figure 5. Control of escape direction in stationary and moving crickets

(A) Finish points of the initial escape response for all trials under different motor states. The right and left columns of panels are for the stationary and moving conditions, respectively. The upper and lower panels are for the running and jumping responses, respectively. Colored dots indicate the data for each trial, and the colors correspond to the stimulus direction shown in the center inset.

(B) Schematic diagram showing the definition of movement direction and stimulus angle. Blue indicates stimulus angle, and red indicates movement direction. Both of these angles are defined with a positive value clockwise to the cricket's anterior direction.

(C) Relationships between movement direction and stimulus angle. The diagonal-dotted lines indicate the line of $y = x$.

(D) The accuracy of directional control for different stimulus velocities. The absolute values of the angular difference between the movement direction and stimulus angle serve as the directional accuracy index. The left, middle, and right columns of panels in C and D show the data for all, running, and jumping responses, respectively. Colored dots represent the data for each trial, and bars represent the mean of all trials of each type of response under stationary (blue) and moving conditions (red). The velocity of the stimuli was 1.00 m/s. N = 10 individuals.

Airflow detected at the cerci can elicit multiple distinct responses, including evasion, flight, offensive reactions, scanning, and freezing, and response can depend on the behavioral state of the animal as well as the context of the environment.[30,31] Several studies on the wind-elicited escape behavior of stationary crickets have revealed that they move in the opposite direction of airflow stimulus in their initial responses of

running or jumping immediately after receiving it.[21,29] In addition, a previous study involving tethered crickets walking on a treadmill revealed that escape behavior probability increased as spontaneous walking velocity increased.[28] Using a closed-loop controlled servosphere treadmill system that provided quantitative stimulation to freely moving crickets, the current study revealed for the first time that when moving crickets were stimulated, they momentarily stopped and then exhibited either running or jumping as escape behavior. When the airflow velocity was slower than 0.54 m/s, most crickets stopped, but roughly half did not move after pausing. This response could be considered a "freezing response" observed in numerous animal species in response to a threat stimulus. For example, voles, a type of rodent, exhibit a threat response by combining freezing and fleeing in an unpredictable sequence to deceive predators.[32] Similarly, the sudden pausing of crickets may make it difficult for predators to predict their transient movement, thereby increasing their survival rate. Furthermore, the crickets remained stationary without showing escape behavior after a pause response to weak airflow stimuli. Most fruit flies show a sustained freezing response to visual looming stimulus.[33] The freezing response may be maintained in order to avoid detection by predators, rather than moving unnecessarily when the threat stimulus is not so imminent.

Considering the situation in which crickets did not move in response to weak airflow, previous studies on the escape behaviors of stationary crickets were unable to distinguish whether they dared to "freeze" or did not respond because their airflow velocity was below their detecting threshold.[29,34] Unfortunately, the high-speed camera used in this study had a magnification and spatial-temporal resolution insufficient to quantitatively determine the freezing response in the stationary crickets based on detailed movements of appendages and antennae. However, it remains possible that the stationary crickets that did not respond to airflow stimuli exhibited the freezing response. In any case, the results of the present experiments demonstrated that the crickets stimulated while in motion possibly exhibited a "freezing response" to the airflow stimulus. Interestingly, the pause response threshold was markedly lower than the escape response threshold. Previous electrophysiological studies revealed that the threshold for the firing response of wind-sensitive ascending projection neurons, identified as giant interneurons (GIs) within the terminal abdominal ganglion (TAG), to airflow is significantly lower than that for inducing escape behavior.[35,36] Overall, crickets can perceive airflow stimuli even when they are not displaying escape behavior. Notably, whether or not the cricket paused had little effect on the probability of subsequent escape responses and the selection ratio of either jumping or running. Consequently, the freezing response may be mediated by a neural mechanism distinct from that underlying subsequent escape behavior.

High sensitivity to threat signals may compensate for response delay during moving

The escape behaviors elicited when moving were similar in the locomotion characteristics to those elicited when the crickets were stationary. For example, as the airflow speed increased, the response probability and ratio of jumping response rose, running speed and distance increased, and reaction time decreased. Focusing on the escape response of the stationary condition, these characteristics were consistent with the previous report.[24] Unlike the previous report, however, the escape direction was controlled more accurately as the airflow velocity increased, even when the crickets were stimulated under stationary conditions. This dependence of directional accuracy on stimulus intensity may be attributable to differences in experimental conditions between the servosphere system used in this study and conventional arena used in the previous study. In a previous study,[24] crickets were contained within a cup until just prior to stimulation, after which they were exposed to a bright environment and stimulated. However, in the current experiments conducted under dark conditions, the crickets relied solely on the cercal mechanosensory inputs to regulate the escape direction,[27] suggesting that directional accuracy may have been relatively low for the slow airflow and increased with the stimulus velocity.

In the escape response to the same stimulus velocity, the response probability was higher when the cricket was in motion than when it was stationary, corroborating a previous study.[28] This finding indicated that crickets were more sensitive to airflow stimuli when in motion. This result is consistent with some studies that animals' sensory perception is enhanced while in motion.[10,11] For example, voles exhibit escape behaviors more frequently in an active state, where they can be easily detected by predators rather than in an inactive state.[37] Similarly, crickets that are easily detected by predators may increase their sensitivity to threat signals during movement. In addition, weaker stimuli from the side of the crickets were associated with a higher probability of an escape response during movement. This may be a defense mechanism to counteract the fact that moving crickets can be easily spotted from the side by a predator. Why animals'

sensory perception is affected by locomotion is explained by various theories, including the effects of the corollary discharge as efferent copy signals, predictive processing during locomotion, and template matching. The cercal sensory processing in crickets may also be modified by descending corollary discharges to cancel the self-generated stimuli. For example, the ventral GIs' spontaneous firing activity was elevated in moving crickets.[38] These self-movement–related modulations of the cercal sensory system may contribute to the enhanced airflow sensitivity experienced while moving.

In this study, moving crickets had a longer reaction time and a greater variance of escape direction than stationary crickets, although the locomotion had little apparent impact on movement speed and traveled distance in the escape behavior. These results indicated that moving crickets had a disadvantage compared to stationary crickets in terms of the time delay to start the escape behavior. Crickets may compensate for the longer delay in their escape start by increasing their sensitivity to airflow stimuli during movement. In contrast, the greater variance in movement direction is not generally a disadvantage for escape behavior. Cockroaches have been reported to flee in multiple directions in their escape behavior, which may help to increase the success rate of escape by surprising the predator's expectations.[13,39–41] Unlike cockroaches, crickets do not exhibit multiple peaks in the distribution of escape direction,[21,24] suggesting that they follow a simple escape strategy of moving away from the stimulus source. This tendency in the escape direction was also observed under the moving condition.

Neural mechanisms for quick pausing in response to airflow stimulus

The present results demonstrated that when the crickets were stimulated by airflow while in motion, they quickly paused and then ran or jumped to escape. The delay from stimulus onset to pausing under the moving condition, which was defined as T1 in this study, was 68.57 ± 37.26 ms, and much shorter than the delay from pausing to the start of following escape (T2) or the reaction time under the stationary condition (RTs). The first spikes of descending signals from the brain were recorded approximately 40 ms after the onset of an airflow stimulus, according to our previous research.[36] In contrast, the first ascending spikes evoked by airflow were recorded within about 15 ms after stimulus onset.[34] Considering the time required for activation (or inhibition) of motor circuits and for muscle contraction to stop, the pausing response may be caused by the direct suppression of the thoracic motor circuitry by ascending neurons, such as GIs, not by the descending commands from the brain that direct escape behavior.[24,29] As in locusts, the motor neurons and central pattern generator (CPG) circuits of crickets are thought to be located in the thoracic ganglia.[42] Cricket GIs extend their axon collaterals into the thoracic ganglia,[43,44] suggesting that the thoracic motor neurons or interneurons of CPGs could receive direct synaptic inputs from GIs. Compared to T1, the delay from pausing to the onset of escape behavior (T2) was 87.16 ± 65.31 ms and was similar to the reaction time under the stationary condition (RTs), 104.25 ± 48.08 ms. In addition, the escape behavior itself after the pause exhibited similar characteristics to that under stationary conditions, as revealed by the dependence of moving speed, travel distance, and directional control on airflow velocity. As a result, the brain's descending command signals are likely to trigger and regulate escape behavior during movement, just as they do during rest. In the insects' escape responses to threatening visual stimuli, a pair of descending projection neurons, which are identified as giant fibers in flies[45–49] or descending contralateral movement detector neurons (DCMD) in locusts,[50–53] activate thoracic motor neurons to trigger a takeoff or jump. However, as discussed previously, the pause as the freezing response may be mediated by neural mechanisms distinct from that underlying the subsequent escape movements. Notably, the freezing response when stationary, which is visually indistinguishable from "no response," may also be executed by a direct pathway from the TAG to the thoracic ganglia without brain involvement. Further electrophysiological studies on the functional connections between GIs and local circuits in the thoracic ganglia will reveal the neural substrates for pause and escape responses.

Limitations of the study

This study revealed that crickets during locomotion temporarily pause and then exhibit escape behavior when stimulated by airflow stimuli. This pausing response was likely a "freezing response." However, to determine whether crickets also exhibit freezing response when stationary, it is necessary to check more detailed movements of their antennae and appendages by using cameras with higher spatiotemporal resolution. In addition, future electrophysiological studies are required to elucidate the neural mechanisms underlying the differences in escape behavior between locomotion and stationary conditions.

STAR★METHODS

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

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.107345.

AUTHOR CONTRIBUTIONS

K.K., H.S., and H.O. designed research. K.K. performed experiments. Y.I. designed and developed the experimental apparatus. K.K. and H.S. analyzed the data. KK., H.S., and H.O. discussed the data interpretation and statistical methods and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental models: Organisms/strains		
Gryllus bimaculatus, Hokudai WT	Watanabe et al.[54]	N/A
Software and algorithms		
R programming software, ver. 3.5.0	CRAN	https://www.r-project.org
R package "glmmML" ver. 1.1.1	CRAN	https://www.r-project.org
R package "lme4" ver. 1.1-31	CRAN	https://www.r-project.org
Others		
High-speed camera, acA1920-155um	BASLER	https://www.baslerweb.com/en/products/cameras/area-scan-cameras/ace/aca1920-155um/
Pneumatic picopump, PV820	World Precision Instruments	https://www.wpi-europe.com/solutions/applications-knowledgebase/in-vivo-research/sys-pv820.aspx
Thermal anemometer, 405-V1	Testo	https://www.testo.com/ja-JP/testo-405-p/0560-4053#

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Hiroto Ogawa (hogawa@sci.hokudai.ac.jp).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The data that support the findings of this study are available from the lead contact, upon reasonable request.

- This paper does not report original code.

- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Animals

We used a wild-type strain of crickets (*Gryllus bimaculatus*, Hokudai WT[54]) bred in our laboratory in this study. Adult male crickets that were less than two weeks after imaginal molting were used for experiments. They were reared under 12:12-hour light/dark conditions at a constant temperature of 27°C. All experiments were conducted during the dark phase at room temperature (26°C–28°C).

Ethics statement

The guidelines of the Institutional Animal Care and Use Committee of the National University Corporation, Hokkaido University, Japan, specify no requirements for insect treatment in experiments.

METHOD DETAILS

Treadmill system

A marker-less visual feedback servosphere treadmill system[15] was used to hold a freely moving cricket on top of a Styrofoam ball (ø = 200 mm) supported by three motor-driven omni wheels (Figure 1A). The treadmill system was installed within a sound-proofed wood box, and its top was covered with a white paper board of 500 × 500 mm except for the top 100-mm diameter of the ball. A region of 350 × 450 mm, including the ball top and whiteboard, was monitored using a high-speed digital video camera (acA1920-155um, BASLER, Ahrensburg, Germany) hanged above the treadmill (resolution, 1216 × 1200 pixels; shutter speed, 1 ms; sampling rate, 160 frames/s). To track a cricket, full size of captured image was used and a half size of them was recorded for the offline analysis of escape behavior. While an animal was on the treadmill ball, the animal's location and head orientation were estimated automatically by an image processing technique proposed in a previous report from each captured image.[55] The treadmill ball was regulated by rotating the omni wheels so that the center of the estimated animal's body always corresponded to the center at the top of the treadmill, and its major axis was kept to the specific orientation. This feedback regulation allowed the animal moving on the treadmill to be kept at a specific location and orientation against the stimulation nozzle. This system was feedback controlled at a rate of 160 times per second. The rotation of the treadmill ball by an optical mouse placed beneath the treadmill ball was detected as the cricket's spontaneous movement before air-current stimuli.

Stimulation

An air-current stimulus was provided to a cricket on the treadmill ball by a short puff of nitrogen (N_2) gas from a plastic nozzle (ø = 15 mm) connected to a pneumatic picopump (PV820, World Precision Instruments, Sarasota, FL, USA). The nozzle end was positioned at a distance of 100 mm from the center of the servosphere on the same horizontal plane as the animal. By adjusting the picopump delivery pressure, the air-current velocity was controlled at 0.35, 0.45, 0.67, or 1.00 m/s, which was measured at the center of the treadmill ball with a 405-V1 thermal anemometer (Testo, Yokohama, Japan). The duration of the air-current stimulus was 200 ms. By rotating the treadmill ball to adjust the cricket's body axis to a specific angle against the stimulus nozzle, the air currents were applied from 8 different directions spaced 45° apart around the cricket.

Experimental procedure

A cricket was placed on the treadmill top, and the servosphere's feedback control was immediately initiated for the cricket to be oriented at the same position and treadmill center. Based on the cricket movement velocity measured with an optical mouse, the cricket locomotion state was determined, either moving or stationary (Figure 1B). When the movement velocity exceeded 10 mm/s for more than 1 s, the state was defined as moving; otherwise, it was stationary. After each trial was started and once the locomotion state was determined, the air current was automatically provided, and the feedback control of the servosphere treadmill was stopped. The cricket movements in response to the air-current stimulus were recorded using a high-speed camera, which was also used for the treadmill's feedback control.

The specific velocity of air-current stimuli was applied from any one of eight different directions alternately for moving and stationary, resulting in sixteen trials for each stimulus velocity. The stimulus direction was randomized so that it was not applied sequentially from the same direction. Four different velocities of stimuli were used, and the stimulus velocity order was also randomized. Thus, sixty-four trials in total for eight directions, four velocities, and two states were conducted for each individual. The intertrial interval was at least 60 s. In addition, a 10-min interval was provided every 30 min to allow the crickets free access to food and water.

Image processing

To compensate for sample-by-sample errors in distance measurements due to the angle of view and camera positions, only the area of 350 × 450 mm square on the opposite side to the stimulus nozzle, which was indicated by four black spots, was cut out from the video image, and transformed into a 406 × 522 pixel image. After approximating the contour of the cricket body as an ellipse based on a binarized image, the location and body axis of the cricket were determined by the centroid and the long axis of the ellipse, respectively. The head orientation was set manually on the first image for each sample.

Criteria for wind-elicited escape responses

The stationary cricket's wind-elicited responses were analyzed as previously described[21,24,34,36] (Figure 1C). The translational velocity of the cricket's movement determined whether or not it responded. If the translational velocity exceeded 10 mm/s during the period from stimulus onset to 250 ms after stimulus onset and surpassed 50 mm/s at its maximum value, the cricket was considered to "escape" in response to the air current. If the cricket failed to move within 250 ms of the response definition period, the trial was considered "no response". The wind-elicited responses of the moving crickets were classified based on the movements during two periods after stimulus onset: earlier and later periods (Figure 1C). The period of 250 ms after the stimulus onset was defined as the "earlier period". If the spontaneously moving cricket's translational velocity fell below 10 mm/s during this period, the cricket was considered to "pause" in response to the air-current stimulus, and that time point was defined as the time of stopping. However, if the cricket continued to move faster than 10 mm/s during this period, the trial was considered "no pause". Furthermore, the period of 250 ms after the time of stopping for the "pause" trial or the stimulus onset for the "no pause" trial was defined as the "later period". During this later period, if the translational velocity exceeded 10 mm/s and also surpassed 50 mm/s at its maximum, the cricket was considered to "escape" in response to the air current. However, if the cricket did not move faster than 50 mm/s during the later period, the trial was considered a "no escape". All trials in the moving cricket were classified into either "pause" or "escape" (Figure 1D). All "escape" responses were further categorized into "jumping" or "running" according to leg movements during escape action, which were determined visually for all escaping trials by frame check of the video, as described previously.[21,24] If all six legs were off the ground simultaneously, that response was defined as "jumping", but if any one of six legs touched the ground during movement, that response was defined as "running".

Data analysis

The response probability was calculated from the number of all "escaping" trials, including running and jumping, per total trial for each stimulus velocity or each stimulus angle (Figures 2A and S2A). The stop probability was calculated from the number of pausing trials per total trial in which the crickets were stimulated while in motion (Figure 2B). The jump probability was calculated from the number of jumping responses per total escaping trial for each stimulus angle (Figures 2C and S2B).

As in previous studies,[21,24,29,34,36] the movement in the escaping trials was analyzed for the "initial response" that was defined as described below. In the escape trials for the stationary crickets or after "no pause" for the moving crickets, the start of the initial response was defined as the time when the translational velocity exceeded 10 mm/s for the first time just before reaching 50 mm/s. However, in the escape trials after "pause" for the moving crickets, it was defined as the time when the translational velocity exceeded 10 mm/s for the first time just before reaching 50 mm/s during the movement after pausing. The finish was defined as the time when the velocity fell below 10 mm/s for the first time after the response had started. Reaction time, movement distance, and maximum translational velocity were measured as metric parameters that characterized escape movement. The reaction time in the escape trials for stationary crickets or after "no pause" for moving crickets (RTs) was calculated as the time elapsed between the stimulus onset and start of the initial response (Figure 3A). The reaction time in the escape trials after "pause" for the moving crickets (RTm) was calculated as the sum of two periods, T1 and T2. T1 was the time elapsed between the stimulus onset and stopping time, and T2 was the interval between the stopping time and response start (Figure 3A). Furthermore, the movement distance was measured as a straight-line distance between where the cricket was stimulated and where it completed the initial response. Movement direction as the angular parameter was measured as the angle between the body axis at the stimulated location and the line connecting the stimulated location and the response finish location. The stimulus angle was defined as the angle between the downstream orientation of the air-current stimulus and the animal's body axis at the stimulated location (Figure 5B). For both the movement direction and stimulus angle, clockwise was defined as positive, and counterclockwise as negative relative to the cricket head direction that was 0°. In this analysis, because the crickets move in the direction opposite the stimulus source in their wind-elicited escape behavior, the movement direction would be close to the stimulus angle.[21,24,36] Thus, the absolute values of the difference between the moving direction and stimulus angle were used to assess the accuracy in directional control of the escape movements.

QUANTIFICATION AND STATISTICAL ANALYSIS

R programming software (ver. 3.5.0, R Development Core Team) was used for all statistical analyses. To statistically assess the effects of motor state and other parameters on escape behavior, we used a generalized linear mixed model (GLMM). The packages "glmmML" ver. 1.1.1 and "lme4" ver. 1.1-31 were used for the models with Poisson and gamma distribution, respectively. All of models include a variable r as the random effect that indicates the animal's ID. The Poisson GLMMs were selected as the equations that included all explanatory variables and their interactions as described below. On the other hand, the gamma GLMMs with a log link function were selected based on the AIC values. First, we made models that included all explanatory variables and their interactions, as described below. Then, we reduced only their interactions steps by steps and made models for all combinations. Among them, the model with the lowest AIC value of which estimation was converged was selected (the selected models can be found in Data S1).

GLMMs with Poisson distribution were used to assess the effect of motor state and stimulus velocity on response and jump probabilities (Figures 2A and 2C). We assumed the GLMMs with the following two parameters as the explanatory variables: the stimulus velocity (*velocity*) and motor state that was either moving or stationary when cricket was stimulated (*state*) as follows:

$$(Response\ probability) = \beta_0 + \beta_1(velocity) + \beta_2(state) + \beta_3(velocity : state) + r,$$

$$(Jump\ probability) = \beta_0 + \beta_1(velocity) + \beta_2(state) + \beta_3(velocity : state) + r.$$

To assess the effect of the motor state on various locomotor parameters, such as reaction time, maximum translational velocity, movement distance, and accuracy in controlling the moving direction (Figures 3C, 3D, 4A, 4B, and 5D), gamma GLMMs were used. We assumed the GLMMs with the stimulus velocity (*velocity*), motor state (*state*), and type of escape action that was either jumping or running (*action*) as the explanatory variables, as follows:

$$(Parameter) = \beta_0 + \beta_1(\log(velocity)) + \beta_2(state) + \beta_3(action) + \beta_4(\log(velocity) : state)$$

$$+ \beta_5(\log(velocity) : action) + \beta_6(state : action) + \beta_7(\log(velocity)$$

$$: state : action) + r.$$

T1 was measured for a "pausing" response that was followed by three types of behavior: running, jumping, and not moving. Meanwhile, the RTs for two types of escape actions, running and jumping, were measured. To assess the effect of T1 and RT reaction time types (Figure S3A), we assumed gamma a GLMM with the stimulus velocity (*velocity*) and reaction time type (*type*) as the explanatory variables, as follows:

$$(Reaction\ time) = \beta_0 + \beta_1(\log(velocity)) + \beta_2(type) + \beta_3(\log(velocity) : type) + r.$$

To assess the effect of moving cricket-specific parameters on their escape behavior, we assumed Poisson GLMMs for the response probability, which was either escape or not, and the jump probability, which indicated the behavioral choice of jumping or running, with the effects of the cricket's movement velocity when stimulated (V_0) and the movements that were either "pause" or "no pause" (*pause*) just before escaping, in addition to the stimulus velocity (*velocity*), as the explanatory variables, as follows:

$$(Response\ probability)$$

$$= \beta_0 + \beta_1(velocity) + \beta_2(V_0) + \beta_3(pause) + \beta_4(velocity : V_0),$$

$$+ \beta_5(velocity : pause) + \beta_6(V_0 : pause) + \beta_7(velocity : V_0 : pause) + r,$$

$$(Jump\ probability) = \beta_0 + \beta_1(velocity) + \beta_2(V_0) + \beta_3(pause) + \beta_4(velocity : V_0)$$

$$+ \beta_5(velocity : pause) + \beta_6(V_0 : pause) + \beta_7(velocity : V_0 : pause) + r.$$

In addition, for locomotor parameters other than reaction time (Figure S5), we assumed a gamma GLMM with the effects of escape action types—running or jumping (*action*)—in addition to (V_0), (*pause*), and (*velocity*) as the explanatory variables, as follows:

$$(Parameter) = \beta_0 + \beta_1(\log(velocity)) + \beta_2(\log(V_0)) + \beta_3(action) + \beta_4(pause) + \beta_5$$

$$(\log(velocity) : \log(V_0)) + \beta_6(\log(velocity) : action) + \beta_7(\log(V_0) : action)$$

$$+ \beta_8(\log(velocity) : pause) + \beta_9(\log(V_0) : pause) + \beta_{10}(action : pause)$$

$$+ \beta_{11}(\log(velocity) : \log(V_0) : action) + \beta_{12}(\log(velocity) : \log(V_0)$$

$$: action) + \beta_{13}(\log(velocity) : \log(V_0) : pause) + \beta_{14}(\log(velocity)$$

$$: action : pause) + \beta_{15}(\log(V_0) : action : pause) + \beta_{16}(\log(velocity)$$

$$: \log(V_0) : action : pause) + r.$$

In the GLMMs for the reaction time of the moving crickets, the variable (*pause*) was discarded because neither T1 nor T2 could be measured during the "no pause" trial.

$$(Reaction\ time) = \beta_0 + \beta_1(velocity) + \beta_2(V_0) + \beta_3(response) + \beta_4(velocity : V_0) + \beta_5$$

$$(velocity : response) + \beta_6(V_0 : response) + \beta_7(velocity : V_0 : response) + r.$$

$$(Reaction\ time) = \beta_0 + \beta_1(velocity) + \beta_2(V_0) + \beta_3(action) + \beta_4(velocity : V_0) + \beta_5$$

$$(velocity : action) + \beta_6(V_0 : action) + \beta_7(velocity : V_0 : action) + r.$$

Here, two types of explanatory variables for the behavior were used in the GLMMs for T1, one of which was either escape or not escape (*response*), and the other was either running or jumping (*action*) (Figures S3B and S3C).

The significance of the coefficients for each explanatory variable and their interactions (*β*) was used to assess their effects on the various parameters of escape behavior. To assess the physical effect of the stop of the servosphere rotation by the feedback shutdown on the moving cricket motion, we compared T1 between the trials where the treadmill ball was stopped with and without air-current stimulation using the Wilcoxon rank sum test (Figure S1). Fisher's exact test was used to assess the effects of motor state on response probability and behavioral selection for each stimulus angle (Figure S2).