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Action selection in the escape behavior of crickets

(コオロギの生得的逃避行動における戦略選択)

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Action selection in the escape behavior of crickets

（コオロギの生得的逃避行動における戦略選択）

捕食者の接近のような生命を脅かす状況では、動物は捕食者の接近を示す刺激を検出し、捕食者から逃れるための逃避行動を行う。逃避行動は動物の生存を左右する生得的行動として、脊椎・無脊椎動物を含む多様な動物で普遍的に備わっている。また、動物は複数の異なる逃避行動を持つことが多い。最も典型的な行動は素早く刺激源から遠ざかる移動行動だが、あえて捕食者に気付かれないように動かない場合もある。このように逃避行動を状況に応じて適切に選択することは、逃避を成功するために有効である。しかし、各逃避行動がもたらす利益や捕食者の接近を示す刺激特性と行動選択の関係については不明な点が多い。そこで本研究では、動物がどのように異なる逃避行動を選択するか、さらにその行動選択に関与する神経機構を明らかにすることを目的とし、フタホシコオロギ (*Gryllus bimaculatus*) を用いて行動実験及び電気生理学実験を行った。コオロギは腹部末端に尾葉という機械感覚器官を持ち、捕食者の動きによって生じる気流を検出し、これに対し逃避行動として running または jumping を示す。コオロギの気流感覚系については既に多くの神経行動学的知見が蓄積されており、気流刺激情報の処理から行動選択に至るシステムに迫るために有用な実験材料である。

第一章では、running と jumping の二種の逃避行動がそれぞれどのような行動的利点を持つかを検証した。コオロギの running はどの方向から与えられた気流刺激に対しても正確に反対側に逃避するよう移動方向を制御できる運動であり、一方で jumping はバッタの jumping と同様に前方にのみ高速で移動する運動であると思われる。したがって、running には「常に刺激の反対側へ逃げられる」という利点があり、jumping には「より速く遠くへ逃げられる」という利点があると予想された。そこで、高速度カメラでそれぞれの行動を計測し、移動速度や移動方向などの運動パラメータを定量的に解析・比較した。その結果、jumping では running よりも速く長距離を移動できるうえ、running と同様に正確に移動方向を制御できることがわかった。そこで、running の別の行動学的利点として、常にいずれかの肢が接地しているため、続けて捕食者に攻撃されたときでも柔軟に対応できる利点があると予想した。短い間隔で二回の気流刺激を連続して与えたところ、一度目の刺激に対して jumping した場合よりも running した場合の方が、行動中に与えられた二度目の刺激に対して高頻度で応答することができた。したがって、running は jumping に比べて「行動の柔軟性」という行動的利点をもつことが示唆された。

第二章では、running と jumping がどのような刺激特性に基づいて選択されるかを調べた。他の動物では逃避行動の選択が危機刺激の「速度」に基づくことが示唆されているが、その他の刺激特性が行動選択に及ぼす影響は不明である。そこで、コオロギに「角度」、「流速」、「持続時間」の異なる気流刺激を与え、行動選択の割合や反応時の運動パラメータへの影響を調べた。逃避行動の選択は気流刺激の「流速」だけでなく「持続時間」に伴って変化し、刺激の流速が高いほど、持続時間が長いほど jumping が選ばれやすかった。また、running では反応時の移動速度や距離が刺激パラメータに伴って変化したのに対し、jumping では変化しなかったことから、running がどのような刺激かに基づいて運動を変えられる行動である一方、jumping はより定型的な行動であることがわかった。

第三章では、第二章で明らかにした、気流刺激に応じて逃避行動を選択し、運動を制御するための神経機構に着目した。コオロギでは、気流刺激の強度や角度の情報は最終腹部神経節内で抽出され、そこから巨大介在ニューロン (GIs) によって脳や胸部神経節に運ばれる。これらの高次神経中枢で行動選択や運動制御に影響する気流刺激の情報処理が行われていると考えられるが、脳や胸部神経節がどのような役割を持つかは不明である。そこで、腹側縦連合神経束を異なる位置で切断して脳や胸部神経節への上行性信号（感覚情報入力）や脳からの下行性信号（運動司令出力）を遮断し、逃避行動における各高次中枢の機能を調べた。まず、左右の縦連合を脳-胸部神経節間で切断すると逃避行動が消失したため、気流逃避行動を行うには脳と胸部の神経連絡が必須であることが分かった。次に、第4-最終腹部神経節間または脳-胸部神経節間で縦連合を片側だけ切断した。前者では脳・胸部神経節共に片側の上行性信号が失われ、後者では胸部神経節脳へは両側性の上行性信号が維持されているが、脳に対しては上行性信号と胸部への下行性信号がそれぞれ片側遮断されている。その結果、胸部神経節への両側性の上行性信号入力があれば下行性信号が片側だけでも running することができたが、jumping には脳への両側性の上行性信号入力が必要であった。また、移動方向や移動速度、距離の制御には脳からの下行性信号が必要であった。さらに、running と jumping に先行して脳から送られる下行性信号を細胞外記録によって調べたところ、jumping の際には running より行動開始直前に急激な下行性信号の活動増加が生じることが確認された。これらの結果から、脳で決定された選択結果に基づいて異なる運動司令が胸部神経節に送られていると考えられる。

本学位論文では、コオロギの二種の逃避行動についてその特徴を明らかにした上で、これらの逃避戦略が脳内で複数の気流刺激パラメータの感覚情報処理を経て選択されることを示した。危機刺激は捕食者がどのように接近するかを示す手掛かりであり、刺激の様々な情報に基づいて行動を選択したり運動内容を変化させたりすることは、逃避の成功に重要であると考えられる。本研究を足がかりとして、さらに危機刺激を受けたときの状況による逃避行動選択の違いや逃避行動を決定する脳内神経回路を調査することによって、動物が適切な行動を選択する神経メカニズムの全容を解明することが期待できる。

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General Introduction

Action selection in the intrinsic behaviors

It is necessary for animals including humans to make a decision for actions in every situation in daily life. Animals choose the adaptive behavior to the various situations depending on external stimuli (Briggman et al., 2005; De Franceschi et al., 2016; Gold and Shadlen, 2007; Preuss and Faber, 2003; Turner et al., 2016) and internal states (Betley et al., 2015; Dickinson and Balleine, 1994). Numerous studies in behavioral economics have demonstrated that this behavioral choice is based on the trade-off between the paid effort and the advantages of that behavior or the amount of expected benefits as results (Doya, 2008; Fehr and Rangel, 2011; Ydenberg and Dill, 1986). Thus far, most studies have focused on the relationship between the choice in a learned task and the expected value of rewards such as food intake resulting from their responses (Cromwell and Schulz, 2003; Rangel et al., 2008; Schulz, 2006).

In contrast, when animals choose a response in the intrinsic behaviors, the advantage of that response is related to the contribution to survival and reproduction in natural environment, which cannot be measured as the amount of artificial rewards in the learned task in a laboratory. Instead, the behavioral advantages are often linked directly to the performance of the response itself that increase the success rate of avoiding predator or of mating (Lind and Cresswell, 2005; Walker et al., 2005; Byers et al., 2010; Soma and Garamszegi, 2011). Therefore, quantitative assessment for the performance of the responses is necessary to understand how animals choose responses adaptively in intrinsic behaviors.

Escape behavior to survive attack of predator

Various species of animals, including vertebrates and invertebrates universally exhibit escape behavior to survive predator's attacks (Domenici et al., 2011a, b; LeDoux and Daw, 2018). This means that the escape behavior is one of the most evolutionarily important and conserved behaviors, along with reproductive behavior. In the typical escape response, animals quickly move to distant from the predator, which greatly elevates their survival rate. The study in fish about the interaction between the behaviors of prey and predator has reported that the preys not exhibiting escape response are mostly caught by the predator (Stewart et al., 2013). Compared to other defensive behaviors such as freezing, escape response is predominantly chosen in the more emergent situation (Baba and Shimozawa, 1997; De Franceschi et al., 2016; Fadok et al., 2017; Evans et al., 2018; Turner et al., 2016).

While the complex neural mechanism including multiple brain regions are involved in the escape behavior in mammals (Evans et al., 2018; Gross and Canteras, 2012; Shang et al., 2015; Wang et al., 2015), the relatively simple neural circuits mediate the escape behavior in fish and invertebrates (Card, 2012; Eaton et al., 2001). Such simple neural systems for the escaping have been well studied, reporting that a few neurons trigger the escape responses to the specific sensory stimulus indicating predator's approach. Mauthner cells, which are one of the most studied neurons eliciting the escape response, are a pair of descending projection neurons in the brainstem of fish. The activation of either of them triggers a fast bending of fish body, termed "C-turn" (Eaton et al., 2001; Gazzola et al., 2012; Korn and Faber, 2005), in response to the several modalities of stimuli (Casagrand et al., 1999; Dunn et al., 2016; Kohashi and Oda, 2008). In crayfish and flies, the key neurons called giant fiber directly induce the escape response (Allen et al., 2006; Edwards et al., 1999). The simplicity of the neural systems is useful

to address neural mechanism mediating escape behavior.

Selection between multiple escape responses

Various species of animals employ several types of responses in escape behavior (Briggman et al., 2005; Liu and Hale, 2017; Takagi et al., 2017). The multiple escape responses are accompanied by distinct movement, resulting in difference in the behavioral performance. For instance, flies exhibit two types of escape take-off, short- and long modes (Card and Dickinson, 2008). They jump without wing elevation in the short-mode, whereas they take-off after elevating the wings in the long-mode. Compared to the long-mode, the short-mode enables flies to leave the ground immediately responding to the stimulus, but with less controllability of their body after taking-off. However, how animals choose the distinct escape responses remains unclear.

In the selection between the multiple escape responses, the locomotor performance contributing to the successful avoidance of the predator's attack is regarded as the advantage of the response. Since each escape response has distinct advantages and disadvantages in the performance, the trade-offs between the advantages/disadvantages would be considered as a fundamental factor of the action selection. Therefore, I have to assess quantitatively the performance for each response in order to understand biological meaning of the selection in the escape responses. In addition, to utilize the multiple escape responses effectively, animals should choose appropriate one to the situation, in which the benefit of the chosen response increases the survival rate of animals. Thus, it is an important biological issue to clarify under what situation each response is chosen.

Wind-elicited escape behavior in cricket

In this thesis, I used the wind-elicited escape behavior of the field cricket, *Gryllus bimaculatus* as experimental material to address the issue about action selection in the escape behaviors. Crickets exhibit either running or jumping in response to a short air-current detected as predator's approach (Dupuy et al., 2011; Sato et al., 2017). These responses are different in movement from each other; the crickets run on the ground or jump by strongly kicking the ground. Interestingly, the cockroaches and locusts, the orthopteras evolutionally close to crickets, just exhibit either of running or jumping as escape response (Tauber and Camhi, 1995). Unlike them, crickets can utilize both running and jumping as the escape responses.

The locomotion of the running has been analyzed by using a spherical treadmill-system (Fukutomi et al., 2015; Grass and Hörner, 1992; Oe and Ogawa, 2013); the crickets immediately start to run in response to the air-current stimulus and move toward the direction opposite to the stimulus source. In this condition, however, the crickets rarely jump because they are suspended with a metal wire on the treadmill ball, as reported in locusts (Santer et al., 2008). In contrast, free-moving experiments allows the crickets to exhibit both running and jumping in response to the air-current stimulus (Dupuy et al., 2011; Sato et al., 2017). Here, I adopted the free-moving experiment to monitor both escape responses and to assess their behavioral benefits by analyzing the locomotor performances quantitatively.

The cercal sensory system, which mediates the wind-elicited escape behavior of crickets, is well studied (Casas and Dangles, 2010; Dupuy et al., 2011). An air current is detected as air-particle displacement by filiform hairs on the cerci (Miller et al., 2011; Shimozawa and Kanou, 1984), and the receptor neurons of these hairs project their axons into the terminal abdominal ganglion (TAG) in a directionally orderly manner (Jacobs

and Theunissen, 1996, 2000; Ogawa et al., 2006). In TAG, several wind-sensitive interneurons including giant interneurons (GIs) receive excitatory synaptic inputs from the sensory afferents of the receptor neurons. The GIs encode various characteristics of surrounding air-currents such as velocity, direction and frequency in their firing activity (Miller et al., 1991; Ogawa et al., 2008), and project their long axons via ventral nerve cord (VNC) to higher centers including the thoracic ganglia and the brain (Hirota et al., 1993). These facts suggest that GIs convey the information of the air currents to the thoracic and cephalic ganglia. If crickets choose either running or jumping based on the stimulus properties, this model can provide an answer to the question what neural mechanism underlies choice of appropriate escape responses to the predator's approach.

Goal of this thesis

The goal of my study is to understand how the animals choose a certain escape response that have distinct behavioral benefits. First, I compared the escaping performance of running and jumping and assessed the advantages of each response (Chapter 1). Then, I examined if the threatening level of the stimulus has an influence on whether crickets run or jump to escape by manipulating the parameters of air currents (Chapter 2). Lastly, I looked into how the higher centers of the nervous system mediated choice and regulation of running/jumping escape responses, by performing partial ablation of VNC and by extracellular recording of descending neural activities from brain (Chapter 3).

Chapter 1

Trade-off in locomotor performance of escape responses

1.1 Introduction

When animals escape from predators, performance of the response directly affects the survival of them. The advantage to exhibit the response can be regarded as the characteristics of its performance which make escape more successful. To successfully evade predator attacks, the speed of the movement is one of the most important features (von Reyn et al., 2014; Walker et al., 2005). However, as mentioned in General Introduction, several animal species exhibit multiple types of escape responses; some responses are slower than others (Bhattacharyya et al., 2017; Card and Dickinson, 2008; Edwards et al., 1999; Hale, 2002; Herberholz and Marquart, 2012; Kohashi and Oda, 2008; Liu and Hale, 2017; von Reyn et al., 2014). This suggests that slower escape responses may have other advantages to compensate for speed.

For animals with multiple escape responses, choosing appropriate response based on the trade-off between the behavioral advantages would be important for successful escape. For instance, fishes display the following two different escape responses: C-turn triggered by firing of giant nerve cells identified as Mauthner cells and another without Mauthner cell activity. The C-turn allows fishes to escape at high speed (Eaton et al., 2001; Gazzola et al., 2012), but the stereotypic locomotion of the C-turn is considered as disadvantage because it makes the prey's movement easily predictable by the predator (Catania, 2009). Meanwhile, the slower response not mediated by Mauthner

-cells is more variable in escape direction than that of the C-turn (Bhattacharyya et al., 2017; Kohashi and Oda, 2008), making it harder to predict by predators. Thus, the selection of escape responses appears to be based on the trade-off between speed and unpredictability in fish. However, little is known on the trade-off between behavioral advantages/disadvantages underlying the selection in escape responses. To address this question, it is required to analyze locomotor performances of escape responses quantitatively and comprehensively.

In crickets, short air-current stimulus indicating predator's approach elicit running or jumping (Sato et al., 2017). Jumping of the crickets are similar to those in locusts in which animals escape exclusively in a forward direction with high speed (Bennet-Clark, 1975; Gabbiani et al., 1999; Heitler and Burrows, 1977; Santer et al., 2005). These features of jumping seem to be advantageous for a successful escape. However, crickets exhibit running more frequently than jumping for escape (Sato et al., 2017), suggesting that running may have advantages other than speed compared to jumping. Previous experiments using a treadmill system revealed that crickets run away from the stimulus source in a direction opposite to that of the airflow (Oe and Ogawa, 2013). Their direction of movement in the wind-elicited escape response is precisely controlled by, and dependent on, the stimulus angle. The accurate control of movement direction reported in various species (Card, 2012; Domenici et al., 2011a, b) is critical for escape behavior to get as far away from the predator as possible. Thus, a trade-off between speed and directional control possibly exists in the cricket escape responses: jumping would allow crickets to escape quicker but to control their movement direction less accurately than running.

Here, I hypothesized that running and jumping would have different advantages

in terms of escaping performance, e.g. speed and directional control. To test this hypothesis, I quantitatively assessed several aspects of motor performance of running and jumping and examined distinct benefits of these escape actions. Furthermore, I focused on behavioral flexibility as another important benefit for successful escape. Even if animals successfully avoid the first attack, predators may continue to chase and attack them (Combes et al., 2012). If so, it is important for the prey animals to flexibly adapt their behavior in response to additional attacks. The crickets running away may be able to change their movement in response to additional attacks but jumping ones cannot. To mimic the predator's chase, I applied two successive air currents and examined the response to the second stimulus that was applied during/after the running or jumping elicited by the first stimulus.

1.2 Materials and Methods

Animals

I used two strains of crickets (*Gryllus bimaculatus*): one is obtained from a local breeder and the other was the wild-type strain (Hokudai WT; Watanabe et al., 2018). The former strain was used for the single stimulus experiment and the later was done for the double stimulus experiment. Both strains were inbred in my laboratory, respectively. Throughout the experiments, 20 adult male crickets, less than 14 days after adult molting, were used. 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 26-28°C.

Behavioral experiment

To analyze the movement trajectory and locomotor parameters of two distinct wind-elicited escape behaviors, running and jumping (Fig 1-1A), these responses were monitored using a high-speed digital camera (CH130EX, Shodensha, Osaka, Japan) installed above the experimental arena ($\phi = 260$ mm) throughout experiments (Fig. 1-1B). After anesthetized by cooling for 10 min, crickets were marked with two white spots on the dorsal surface of the head and thorax, of which size were large enough to detect the moving of crickets in images (Fig. 1-1C). The cricket was placed in the center of the arena within an inverted beaker ($\phi = 50$ mm) covered with aluminum foil. After the beaker was carefully lifted up, an air-current stimulus was immediately applied to the cricket standing still and the wind-elicited response was recorded by the camera. Based on the video data (shutter speed, 1 ms; sampling rate, 120 frames/s; total recording duration, 1660 ms), the two markers on the animal were automatically traced, and locomotor parameters that will be mentioned in a later section were measured with motion analyzing software (Move-

tr/2D, Library, Tokyo, Japan). To monitor the whole trajectory during movement, we adopted 285.7×285.7 mm frame size in 1024×1024 pixels resolution which covered whole arena (Fig. 1-1B).

Stimulation

The air-current stimulus was a short puff of nitrogen gas from a plastic nozzle ($\phi = 15$ mm) connected to a pneumatic picopump (PV820, World Precision Instruments, Sarasota, FL, USA). One air-current nozzle was installed on the inside wall of the arena to be positioned on the same horizontal plane as the animal (Fig. 1-1B). Since the crickets were oriented randomly within a beaker, the stimulus angles against the cricket were varied across the trials. The stimulus (618 mm/s) used in our previous study exclusively elicited the running, but rarely caused jumping (Sato et al., 2017). Then, in order to elicit not only running but also jumping for each cricket, a faster stimulus (834 mm/s) was used in this study. The travel time of the air to the center was 14.6 ± 0.1 ms, which was measured as a delay in stimulus-evoked ascending spikes of projection neurons that were extracellularly recorded ($n = 2$ animals, 10 trials) at the center of the arena.

Two experiments were performed with different stimulus conditions: single- and double-stimulation. In single stimulus experiment, only one air-current of 200-ms duration was applied in a trial. Twenty trials were repeatedly performed for each individual at inter-trial intervals of 60–90 sec. In double stimulus experiment, three sessions with distinct stimulus conditions were performed for each individual (Fig. 1-5C). To apply two successive stimuli during the initial response, air currents of 100-ms duration were used throughout all sessions. For the control (“Single”), only one stimulus was applied in a trial. In “Double 100” session, two successive stimuli were applied at

100-ms intervals in a trial to examine the responsiveness to the stimulus during escape. In “Double 400” session, two successive stimuli were applied at 400-ms intervals in a trial to examine the responsiveness to the stimulus just after escape. Twenty trials were performed for each session, so in total 60 trials were performed for each individual in the double stimulus experiments. Three sessions were conducted on each individual in the order of Single, Double 400 and Double 100. Inter-trial intervals in each session were 60–90 sec. Between different sessions, crickets rested within a plastic container (138 mm × 220 mm × 135 mm) for about 10 min and freely accessed food and water. For each experiment, 10 animals were tested.

Behavioral analysis

The wind-elicited responses were analyzed in the similar way as our previous study (Sato et al., 2017). Responding or not was defined based on translational velocity. If the translational velocity exceeded 10 mm/s during the period from the stimulus onset to 50 ms after the stimulus termination and the maximum translational velocity was greater than 50 mm/s, the cricket was considered to respond to the air current. If the cricket did not begin to move within the response definition period, which was 250 ms for single stimulus experiment or 150 ms for double stimulus experiment, that trial was considered as “no response”. In the double stimulus experiment of “Double 100”, because the second stimulus was applied to the cricket during movement elicited by the first stimulus, the cricket was considered to respond if the translational velocity increased during the 150-ms period after the second stimulus onset.

Wind-elicited responses were categorized into “running” or “jumping” according to movement of legs during the locomotion, which was confirmed visually for

all responded trials by frame check of video images. If all six legs were off the ground simultaneously, that response was defined as “jumping”. If any one of six legs touched the ground during movement, that response was defined as “running” (Fig. 1-1D). I rarely observed complex behaviors combined with running and jumping, such as jumping after running and *vice versa*. The response probabilities in the single stimulus experiment and the first response probability in the double stimulus experiment were calculated for each individual from the number of responses categorized as running or jumping over 20 trials (Figs. 1-1E and 1-5D). Second response probability was calculated as the proportion of trials with response to the second stimulus relative to all trials with response to the first stimulus categorized as running or jumping (Figs. 1-7B and C, 1-9B and C). In the double stimulus experiment, crickets did not respond to the second stimulus responded if they did not respond to the first stimulus.

Cricket’s movement during initial response was analyzed as in previous studies (Fukutomi et al., 2015; Oe and Ogawa, 2013; Sato et al., 2017). The response start was defined as the time when the translational velocity exceeded 10 mm/s after stimulus onset; the finish was defined as the time when the velocity was less than 10 mm/s. Movement distance, maximum translational velocity, and reaction time were measured as metric locomotor parameters (Fig. 1-2C–E). The movement distance was measured as the entire trajectory of crickets. The reaction time was calculated by subtracting the mean travel time of the air current (mentioned above) from the response delay to operation of the picopump. Angular parameters including movement direction and turn angle were calculated based on the cricket’s body-axis, which is a vector connecting the thoracic and head markers. The movement direction was measured as the angle between the body axis at the start point of the response and a line connecting the thoracic markers at start and

finish points (Fig. 1-3A). If the cricket moved to the opposite direction of the stimulus source, the movement direction would be equivalent to the stimulus angle. The turn angle was measured as the angle between the body axes at the start and finish points (Fig. 1-4A). If the cricket oriented to the opposite side of the stimulus source at the finish points, the turn angle would be equivalent to the stimulus angle.

In the double stimulus experiment, the total movement distance of the cricket was measured as entire trajectory length from the onset of the first stimulus to the end of the video data (Fig. 1-8C and D). In “Double 400”, the second response began after the first response had finished, which enabled measurement of stimulus angle and movement direction of the second response (Fig. 1-9E).

Statistical analysis

R programming software (ver. 3.3.2, R Development Core Team) was used for all statistical analyses. Response probabilities were compared using Wilcoxon signed-rank test. To compare metric locomotor parameters between running and jumping, Paired t-test was used. For angular parameters including moving direction and turn angle, the absolute values (0° - 180°) were compared between running and jumping in three different ranges of the stimulus angle, which corresponded to behind (-60° to 60°), lateral (60° to 120° and -60° to -120°), and front (120° to 180° and -120° to -180°) using Student t-test.

Since a strong linear correlation between the stimulus angle and movement direction was observed as in previous studies (Oe and Ogawa, 2013; Sato et al., 2017), movement direction was considered as non-circular data and linear regression analysis was used as follows:

$$(Movement\ direction) = a(Stimulus\ angle) + b,$$

where a is the estimated coefficient and b is the intercept. In contrast, to analyze the relationship between stimulus angle and turn angle that was considered as circular data, circular-circular regression analysis was used as follows:

$$(Turn\ angle) = \tan^{-1} \left(\frac{A+B\cos(Stimulus\ angle)+C\sin(Stimulus\ angle)}{a+b\cos(Stimulus\ angle)+c\sin(Stimulus\ angle)} \right),$$

where A is the intercept, B is the estimated coefficient of cosine and C is the estimated coefficient of sine in numerator, a is the intercept, b is the estimated coefficient of cosine and c is the estimated coefficient of sine in denominator in the model. Permutation test was adopted to assess the difference in variability between running and jumping in the distribution of the movement direction or turn angle against stimulus angle. Initially, the mean value of residual sum of square (RSS/trials) was measured for each plot from the regression line or curve in running (RSS_R) and jumping (RSS_J), and the ratio of RSS_J to RSS_R was calculated. If the RSS_J/RSS_R was larger than 1, it meant that the movement directions in jumping were more varied than those in running. Then, the category of the data, “running” and “jumping”, was shuffled 1000 times, and the distribution of RSS_J/RSS_R for all shuffled data was plotted. If RSS_J/RSS_R for the original (non-shuffled) data was outside the range of the mean $\pm$ 2 SD of the ratio for shuffled data, the difference in variability of the angular parameters between running and jumping was considered significant.

In the double stimulus experiment, to compare “Single” with “Double 100” or “Double 400” in total movement distance of individuals, paired t-test with Bonferroni correction for multiple comparisons was used. To determine whether the difference between running and jumping in total movement distance was altered by two stimulations, the ratio of the difference between the distance of running (D_R) and jumping (D_J) to their

total sum was compared using Wilcoxon signed-rank test with Bonferroni correction for multiple comparisons. If the p value corrected by Bonferroni method became over 1, that was represented as “ $p = 1.000$ ” in the main text.

1.3 Results

Crickets escaped farther and faster in jumping than in running.

To test the hypothesis that the action selection of either running or jumping would be based on the trade-off between the speed and the accuracy of the directional control, I compared several locomotor parameters between running and jumping. Each cricket chose running or jumping from time to time (Wilcoxon signed-rank test, $V = 41$, $p = 0.190$; Fig. 1-1E, Table. 1-1). Distribution of the arrival points and translational velocities of escape responses suggested that crickets escaped farther away from the stimulus source with higher speed in jumping than in running (Fig. 1-2A and B). Indeed, I found significant difference in the metric locomotor parameters including movement distance, maximum translational velocity and reaction time; crickets moved longer distance (Paired t-test, $t_{(9)} = -6.556$, $p < 0.001$; Fig 1-2C) with higher velocity (Paired t-test, $t_{(9)} = -10.70$, $p < 0.001$; Fig. 1-2D) in jumping than in running and they initiated jumping more quickly than running (Paired t-test, $t_{(9)} = -6.556$, $p = 0.038$; Fig. 1-2E). These results demonstrated that the jumping had advantage in terms of speed compared to running.

The moving direction was accurately controlled in both running and jumping.

Next, to investigate how accurately crickets controlled escape direction during running and jumping, I compared movement direction against the stimulus angle between these two responses (Fig. 1-3). Unexpectedly, the crickets controlled the movement direction in jumping as accurately as in running. The regression line for the plot of movement direction against stimulus angle in running (estimated slope = 0.839, $t = 23.36$, $p < 0.001$; blue in Fig. 1-3B) was similar to those of previous results (Oe and Ogawa, 2013; Sato et al., 2017), meaning that crickets moved precisely in the opposite direction to that of the

stimulus source in running. The linear relationship between the movement direction and stimulus angle was also observed in jumping (estimated slope = 0.851, $t = 18.35$, $p < 0.001$; red in Fig. 1-3B). I divided all trials into three categories, “behind”, “lateral” and “front”, based on the stimulus angle and compared the magnitude of the movement direction (Fig. 1-3C). There was no significance in the angular magnitude of the movement direction between running and jumping for all categories (Student t-test, $t_{(82)} = 0.605$, $p = 0.547$; “behind”, $t_{(56)} = 1.368$, $p = 0.177$; “lateral”, $t_{(37)} = -0.192$, $p = 0.848$; “front”). Thus, the crickets controlled not only running but also jumping to escape in the opposite direction to the stimulation source.

To statistically test the variability of the movement direction against stimulus angle, the mean of the residual sum of square (RSS/trials) for all plots from the regression line was calculated for each response (1152 for running and 1029 for jumping; Fig. 1-3D). To assess the difference in the RSS/trials between running and jumping, I performed the permutation test for the ratio of the RSS/trials of the responses (RSS_J/RSS_R , see Materials and Methods). Figure 1-3E shows the distribution of RSS_J/RSS_R in all shuffled datasets. The RSS_J/RSS_R value of the original data (0.893; black triangle in Fig. 1-3E) was close to 1 and within the range of the mean ± 2 SD of the shuffled data. Thus, the variabilities of the movement direction were not significantly different in running and jumping.

The turn angle was more varied in jumping than in running

I also investigated the turn angle because it was also regulated depending on the stimulus angle, similar to the movement direction (Oe and Ogawa, 2013; Sato et al., 2017). In contrast to the movement direction, the plots for jumping (red in Fig. 1-4B) were distributed more widely than those for running (blue in Fig. 1-4B) and the regression

curves of running and jumping did not overlap. Unlike the responses to stimuli from the lateral direction and behind, the magnitude of the turn angle for the stimulus from the front was significantly larger in jumping than in running (Student t-test, $t_{(82)} = -1.934$, $p = 0.057$; “behind”, $t_{(56)} = -1.481$, $p = 0.144$; “lateral”, $t_{(37)} = -4.054$, $p < 0.001$; “front”; Fig. 1-4C). This suggested that stimuli from the front caused larger turning in jumping than in running.

Significant difference was observed in the variability in turn angle between running and jumping. The mean RSS/trials for jumping was approximately twice as large as that for running (996 for running and 2165 for jumping; Fig. 1-4D). The permutation test for the ratio of the RSS/trials in the turn angle showed that the RSS_J/RSS_R of the original data was much larger than the mean ± 2 SD of the shuffled data (Fig. 1-4E), indicating that the turn angle in jumping was more varied than in running. These results demonstrated that the turning movement in jumping was not as tightly controlled with regard to the stimulus angle compared to that in running.

Advantages of running in double-puffs stimulation with short interval

Comparing locomotor performance of running and jumping showed that crickets could move faster, farther, and more quickly in the opposite direction to the stimulus in jumping, in which only the advantage of the jumping could be observed. Then, I assumed flexibility as an advantage of running compared to jumping when crickets received continuous stimuli. Using three types of stimulation, I compared running and jumping in terms of behavioral flexibility (Fig. 1-5). Here, the air puff with shorter duration (100 ms) than that in the single stimulus experiment were used and it also elicited running and jumping equally as the first response (Wilcoxon signed-rank test, $V = 29.5$, $p = 0.441$; “Single”, V

= 17.5, $p = 0.594$; “Double 100”, $V = 10$, $p = 0.313$; “Double 400”, in Fig. 1-5D). The difference in locomotor performance between running and jumping in “Single” was consistent with those in the single stimulus experiment with 200-ms duration, although no significance was observed in reaction time between running and jumping (Paired t -test, $t_{(9)} = -6.155$, $p < 0.001$; Fig. 1-6A, $t_{(9)} = -16.91$, $p < 0.001$; Fig. 1-6B, $t_{(9)} = -0.783$, $p = 0.454$; Fig. 1-6C, $t_{(9)} = -4.033$, $p = 0.003$; Fig. 1-6D, linear regression analysis, estimated slope = 0.990, $t = 29.38$, $p < 0.001$; “Running”, estimated slope = 0.987, $t = 25.71$, $p < 0.001$; “Jumping”, in Fig. 1-6E). Since the crickets moved in precisely the opposite direction to the air nozzle in both running and jumping (Fig. 1-3B), crickets were still located on the straight course of airflow even after responding to the first stimulus and then they were also stimulated by the second stimulus (Fig. 1-5E and F).

First, I examined the responsiveness to the second stimulus applied at a 100-ms interval (“Double 100”). In this stimulation, the second air-puff was applied before the end of the initial responses; the duration of the initial responses was >250 ms in running and >300 ms in jumping after the first stimulus onset, respectively (Figs. 1-5A and B, 1-6C and D). The crickets responded to the second stimulus more frequently during running than jumping and were likely to choose running in response to the second stimulus (Fig. 1-10A). Fig. 1-7A shows the typical time course of the translational velocity if the crickets responded to the second stimulus during running (left) or jumping (right). Although the probabilities of running and jumping to the first stimulus were almost equal (Fig. 1-5D, center), those for the second stimulus were affected by the choice of the first response. The crickets that ran in response to the first stimulus responded to the second stimulus with higher probability than those that jumped (Wilcoxon signed-rank test, $V = 54$, $p = 0.004$; Fig. 1-7B). Regardless of the first response choice, the probability of jumping as

the second response was lower than that of running (Wilcoxon signed-rank test, $V = 45$, $p = 0.004$; “Running”, $V = 36$, $p = 0.008$; “Jumping”, in Fig. 1-7C).

As the different effects of the first choice on whole escape responses in “Double 100” experiments, the total movement distance increased only when the crickets chose running as the first response. In both running and jumping trials in response to the first stimulus, the distribution of the finish points in “Double 100” was similar to that in “Single” (Fig. 1-8A), but the total movement distance of the crickets running as the first response in “Double 100” was significantly longer than that in “Single” (Paired t-test with Bonferroni *post hoc* comparisons, $t_{(9)} = -3.147$, $p = 0.021$; “Running”, in Fig. 1-8C). In the case of jumping as the first response, there was no significant difference in the total movement distance between “Single” and “Double 100” (Paired t-test with Bonferroni *post hoc* comparisons, $t_{(9)} = -1.703$, $p = 0.369$; “Jumping”, in Fig. 1-8C). In addition, when crickets responded to the second stimulus, the movement direction measured at the finish points was controlled relatively to the angle of the first stimulus in both running and jumping (linear regression analysis, estimated slope = 1.012, $t = 24.25$, $p < 0.001$; “Running”, estimated slope = 0.963, $t = 14.91$, $p < 0.001$; “Jumping”, in Fig. 1-7D), implying little advantage of running in directional control.

Advantages of running in double-puffs stimulation with long interval

The high responsiveness to successive stimuli was shown as the advantage of running. I considered this advantage was possibly specific to “during locomotion” and then investigated the response to the second stimulus applied with a 400-ms interval (“Double 400”). In this experiment, the second air-puff was given just after the initial response finished (Figs. 1-5A and B, 1-6C and D). In this case, the first response did not

significantly affect the responsiveness to the second stimulus (Fig. 1-10B), indicating that the advantage of responsiveness to successive stimuli in running was specific to during locomotion. When the crickets responded to the second stimulus, a distinctive response was elicited both after running and jumping (Fig. 1-9A). The responsiveness to the second stimulus was not significantly different between running and jumping trials for the first response (Wilcoxon signed-rank test, $V = 43$, $p = 0.131$; Fig. 1-9B). In addition, the crickets also chose jumping as the second response after both running and jumping (Wilcoxon signed-rank test, $V = 14$, $p = 0.193$; “Running”, $V = 22$, $p = 0.625$; “Jumping”, in 1-9C), unlike the result in “Double 100”. Furthermore, even the distance from the air-current course at the second stimulated location did not affect the selection of the second responses (Fig. 1-5F).

In “Double 400” experiment, the total movement distance increased regardless of which response crickets chose to the first stimulus. The distribution of the finish points indicated that crickets moved farther from the start position in “Double 400” than those in “Single” for both running and jumping trials (Fig. 1-8B). Indeed, the total movement distance in “Double 400” was significantly longer than that in “Single” for both of the first response choice (Paired t-test with Bonferroni *post hoc* comparisons, $t_{(9)} = -8.107$, $p < 0.001$; “Running”, $t_{(9)} = -4.348$, $p = 0.002$; “Jumping”, in Fig. 1-8C). Interestingly, the difference in the total distance of running (D_R) and jumping (D_J) decreased in “Double 400” compared to that in “Single”, while those in “Double 100” and in “Single” were not significantly different (Wilcoxon signed-rank test with Bonferroni *post hoc* comparisons, $V = 34$, $p = 1.000$; “Double 100”, $V = 54$, $p = 0.012$; “Double 400”, in Fig. 1-8D). This meant that the advantage in the distance of jumping compared to running was reduced by the response to additional stimulus in “Double 400”.

Finally, I investigated directional control when crickets responded to the second stimulus in “Double 400”. I analyzed the relationship between the movement direction in the first or second response and the angles of the first or second stimulus, respectively. In terms of the first response, the movement direction was precisely controlled against the first stimulus angle in both running and jumping (linear regression analysis, estimated slope = 0.983, $t = 31.46$, $p < 0.001$; “Running”, estimated slope = 1.011, $t = 23.47$, $p < 0.001$; “Jumping”, in Fig. 1-9D). However, the choice of the first response had impact on the directional control in the second response. When the crickets chose running as the first response, the similar linear relationship between the movement direction and stimulus angle was observed in second running and jumping (estimated slope = 0.968, $t = 8.716$, $p < 0.001$; “Running”, estimated slope = 0.957, $t = 8.645$, $p < 0.001$; “Jumping”, in Fig. 1-9E, left). In contrast, if the cricket jumped in response to the first stimulus, the linear relationship was different between second running and jumping (estimated slope = 1.231, $t = 10.81$, $p < 0.001$; “Running”, estimated slope = 0.735, $t = 5.913$, $p < 0.001$; “Jumping”, in Fig. 1-9E, right). Thus, choosing jumping as the first response affected directional control during the subsequent. In addition, the effects of the position of the crickets when they received the second stimulus on the second response were checked, but significant effects were not observed on the locomotor parameters (Fig. 1-11, Table 1-2).

Collectively, if the second stimulus was applied after termination of the first response, the crickets could respond to it regardless of the choice in the first response, but there was no longer disadvantage in movement distance of running. Furthermore, the choice to jump in response to the first stimulus was inferior to that to run in terms of directional control in the second response.

1.4 Discussion

The ultimate goal of escape behavior is to successfully evade predators' attack, and the motor performance of escape has critical impact on the survival rate. Here, I quantitatively assessed the locomotor performance of two distinct escape responses of crickets, to clarify the behavioral advantages of each response that would be involved in the action selection. Firstly, I hypothesized that running had advantages in the control of movement direction in exchange for the moving speed. However, the trade-off between the speed and accuracy of directionality was rejected because crickets controlled their movement direction in jumping as precisely as in running. Next, behavioral flexibility was considered as another advantage of running. Double stimulus experiment revealed that running had the higher responsiveness and more precise control of movement direction in the case of continuous stimulation than jumping and that the disadvantage of shorter distance in running compared to jumping was compensated by the following response. Crickets that chose running in response to the first attack could respond flexibly to further attacks, which may be an advantage of running as an escape behavior. These results proposed that the trade-off in the performance of the escape responses underlying the selection of them would involve the flexibility for taking subsequent responses.

Crickets jumped with high speed and precise control of movement direction

In animals' locomotive behavior, moving speed often conflicts with the precise control of movement direction (Bhattacharyya et al., 2017). In the crickets' wind-elicited escape responses, however, they were able to control the movement direction of jumping that allows crickets faster away (Fig. 1-2 and 1-3). Surprisingly, the crickets jumped even sideways and backwards, which may be achieved by physically different systems from

that of locusts (Santer et al., 2005; Simmons et al., 2010). In the rapid escape responses of flies and cockroaches, movement direction is precisely controlled (Card, 2012; Domenici et al., 2008), with which my results were consistent. Variability in movement direction is considered as another advantage because it would provide unpredictability to the attacking predator (Bhattacharyya et al., 2017; Combes et al., 2012). However, there was no difference in directional variability between running and jumping (Fig. 1-3D and E). Significant difference was observed in the variability of the turn angle (Fig. 1-4), which would not be related to the success rate of escape directly. Taken together, comparison in the movement direction failed to explain the advantages of running that would trade-off for the advantages of speed and movement distance in jumping.

Running crickets flexibly changed their movement responding to additional stimulus

I assessed the behavioral flexibility by investigating responsiveness to subsequent predator attacks during the escape response to the first attack. Although a previous research has revealed that a pre-stimulus affects behavioral performance in response to a subsequent alert stimulus (Tureson et al., 2009), I focused on behavioral flexibility following the first response. The crickets running in response to the first stimulus more frequently responded to the second stimulus than jumping ones (Fig. 1-7B), indicating that the behavioral flexibility would be an advantage of running. Since a running cricket had any one of its legs on the ground, it could change the movement if receiving the second stimulus. In contrast, it would be difficult for the cricket to respond to the stimulus during jumping because they were away from the ground. Once the escape response was terminated, the behavioral choice for the first response did not affect the responsiveness to the second stimulus (Fig. 1-9B and C), which also supported the conclusion that the

responsiveness especially during the movement is the advantage of running. The present results demonstrated that animals' action selection affects subsequent responses.

Energy cost for each response would not affect the action selection

In this study, I focused on the response probability and observable locomotor parameters, but the other factors that are difficult to measure such as energy cost may affect action selection. In crickets, the energy cost to run is smaller than that to jump (Bennet-Clark, 1975; Tauber and Camhi, 1995), thus running may have more cost advantage compared to jumping. For jumping to take off the ground, the heavier crickets would need larger energy, so would more frequently choose running rather than jumping. However, neither the probability of running nor that of jumping depended on the animals' weights (Fig. 1-12A and B, Table. 1-3). In addition, the crickets that are repeatedly stimulated would lose more energy, so may also choose running rather than jumping as in later trials. However, the selection of running or jumping was not changed throughout the experiment (Fig. 1-12C and D, Table. 1-3). These results suggest that energy cost may not be critical for action selection of either running or jumping. For action selection in emergency situations of life or death (Kavaliers and Choleris, 2001; Lima and Dill, 1990; Ydenberg and Dill, 1986), lower energy cost would be an insignificant benefit and the performance of the responses including speed, directional control, and flexibility are considered to be more important factors.

1.5 **Figures**

(Figure 1-1)

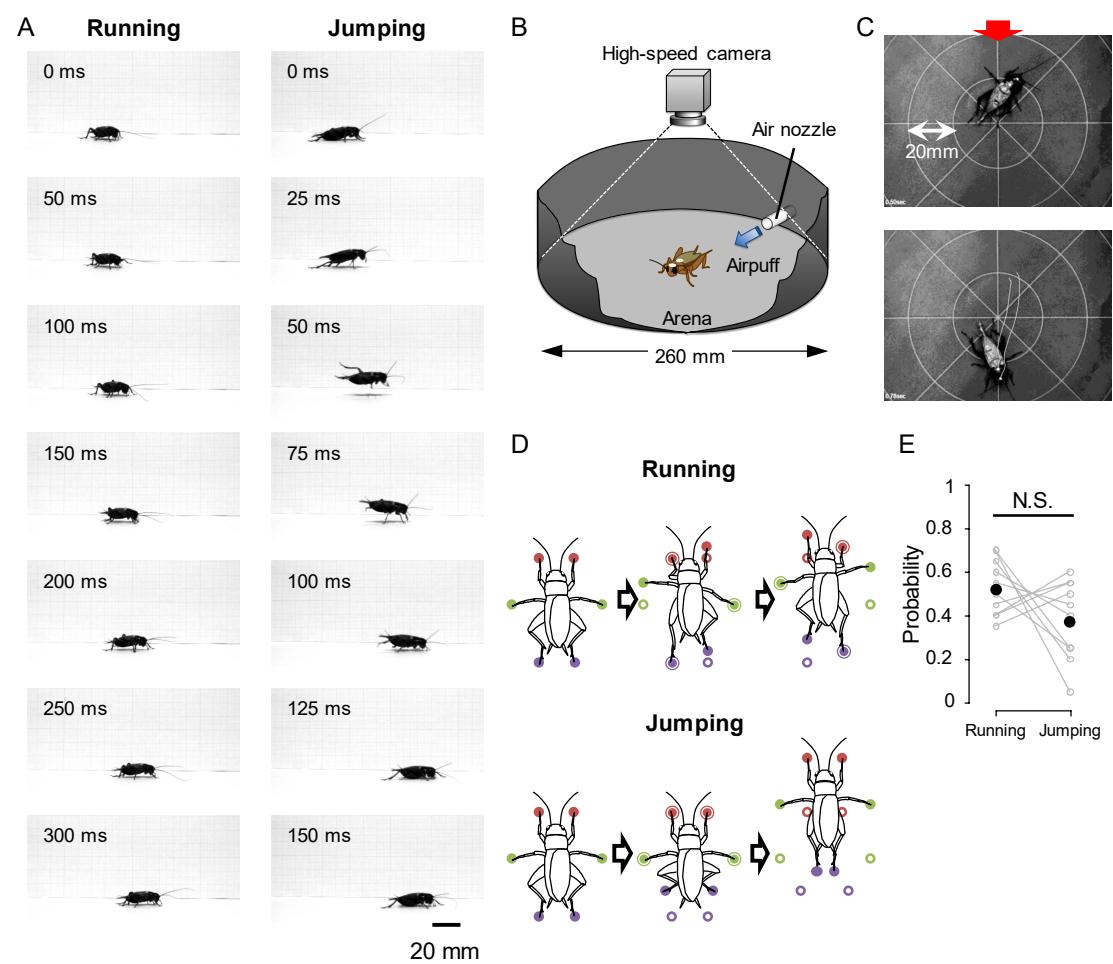


Figure 1-1. The wind-elicited escape responses of the cricket. (A) Typical running (left) and jumping (right) elicited by air-current stimulus, which were recorded from the side by a high-speed digital camera. An air current of 200-ms duration was applied to the cricket from left side on the images. The numbers on the left of each image indicate the elapsed time after response onset. **(B)** Experimental apparatus for monitoring cricket's movement. A cricket with two white markers on its dorsal surface was placed in the center of the circular arena. An air-current stimulus was applied horizontally to the cricket from one nozzle attached on the wall (blue arrow). The cricket's movement was monitored with a high-speed digital camera at 120 fps from above. **(C)** The cricket's movements recorded by the high-speed digital camera. Top image shows the cricket at the start point and bottom shows that at the finish point. Red arrow indicates the stimulus direction of air current. Two markers were traced using a motion analyzing software. **(D)** Schematic diagrams of leg movements during running (left) and jumping (right). During running, the left and right legs moved alternately, and at least any three of legs contacted on the ground. For jumping, the both hind legs were once flexed before kicking the ground, and all six legs simultaneously left the ground during the jumping. Filled circles indicate the current position of each tarsus and open circles indicate the previous position. So, double circles with inner filled and outer open circles indicates that tarsus position was not changed, meaning the leg did not move at that step. **(E)** Probabilities of running and jumping in response to the air-current stimulus. Gray open circles represent the response probabilities for each individual, and black filled circles represent the mean of the probabilities for all individuals. N.S., not significant, Wilcoxon signed-rank test ($\alpha = 0.05$). $n = 10$ animals.

(Figure 1-2)

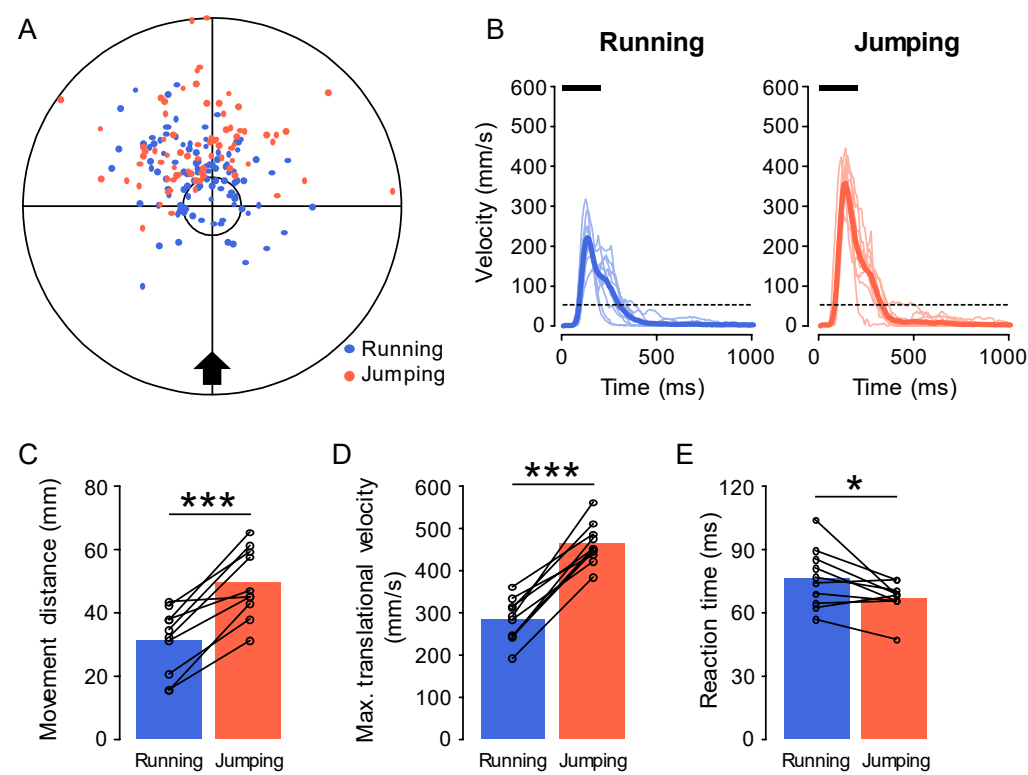


Figure 1-2. Difference in metric locomotor parameters between running and jumping. **(A)** Spatial distributions of finish points of running (blue) and jumping (red) for all trials. Black arrow indicates the direction of the air current. Small circle in the center indicates the range of starting position of the crickets enclosed within a beaker, and larger circle on the outside indicates the wall of the experimental arena. **(B)** Time course of translational velocity in running (left) and jumping (right). Thin colored lines represent the time course of mean velocity for each individual, and dark colored lines represent the average of the mean time courses for all individuals. Black bars indicate the stimulus duration. Dotted lines indicate the thresholds for criteria of the response. **(C-E)** Averages of mean movement distance (C), mean maximum translational velocity (D), and mean reaction time (E) for all individuals. Black open circles connected with lines indicate the mean value for each individual. $*p < 0.05$, $***p < 0.001$, paired t-test ($\alpha = 0.05$). $n = 10$ animals.

(Figure 1-3)

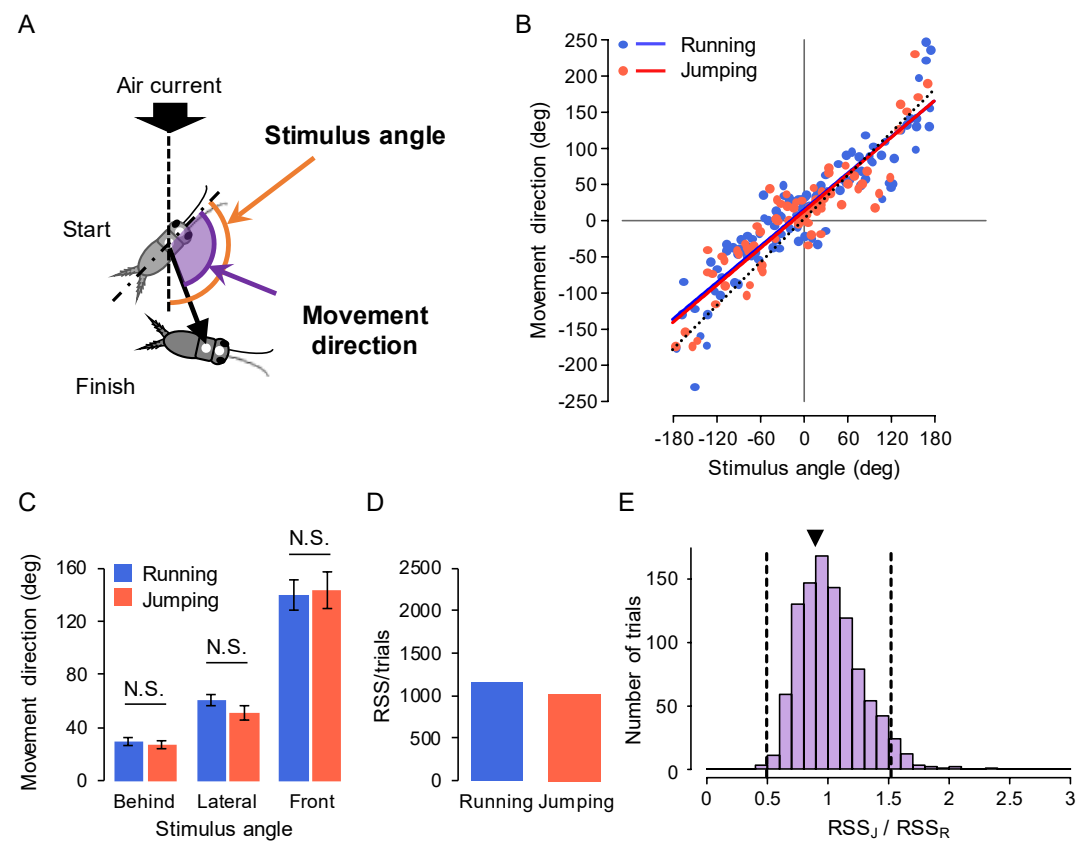


Figure 1-3. Movement direction for running and jumping. (A) Diagram showing the definition of movement direction (purple) and stimulus angle (orange). **(B)** Relationships between movement direction and stimulus angle in running (blue) and jumping (red). Colored lines represent linear regression lines for the data of running (blue) or jumping (red). The black dotted line indicates line of $y = x$. **(C)** Average absolute angular values of the movement direction in running and jumping. The data were divided into three ranges of the stimulus angle that corresponded to behind, lateral, and front (see Materials and Methods). Error bars indicate $\pm$ S.E.M. N.S. not significant, Student t-test ($\alpha = 0.05$). **(D)** RSS/trials values of regression lines for the movement direction. **(E)** Distribution of RSS_J/RSS_R values for the shuffled data. The area between two dashed lines indicate range of the mean $\pm$ 2S.D. of the shuffled data, and black triangle indicates the RSS_J/RSS_R of the real (non-shuffled) data.

(Figure 1-4)

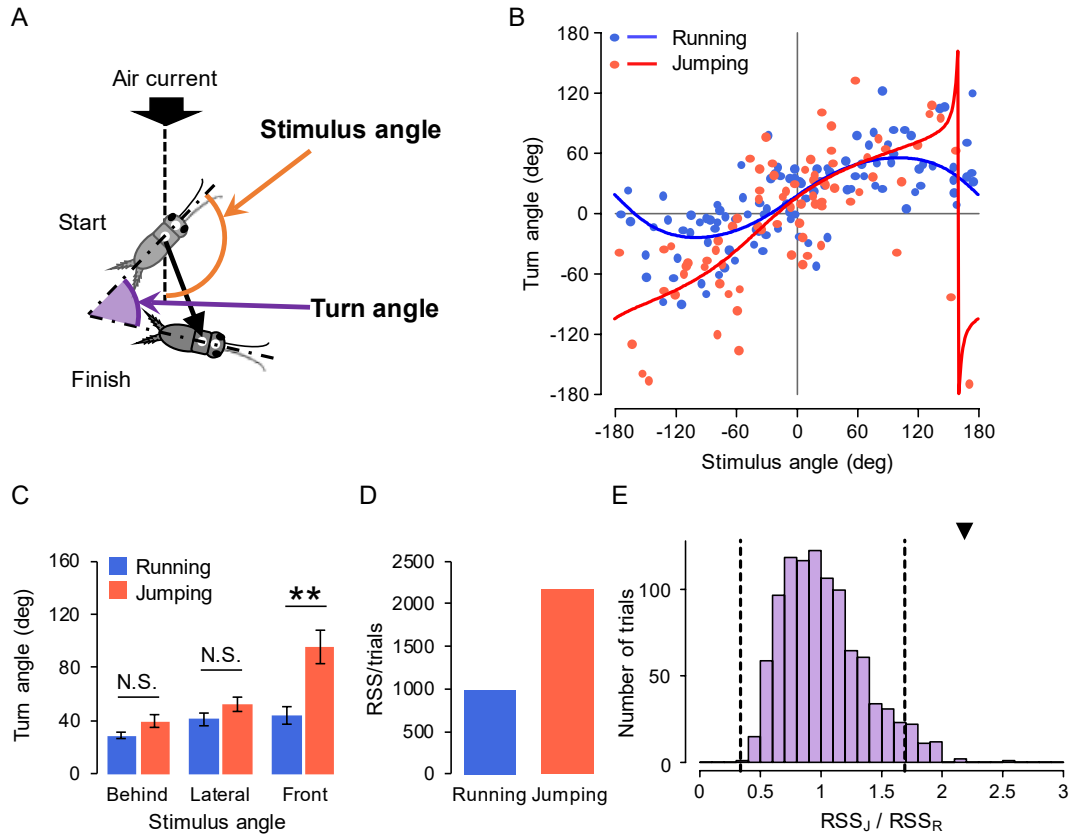


Figure 1-4. Turn angle for the movement in running and jumping. **(A)** Diagram showing the definition of the turn angle (purple) and stimulus angle (orange). **(B)** Relationships between the turn angle and stimulus angle in running (blue) and jumping (red). Curves represent circular-circular regression curves. **(C)** Average absolute angular values of the turn angle in running and jumping. The data were divided into three ranges of the stimulus angle corresponding to behind, lateral, and front. Error bars indicate $\pm$ S.E.M. ** $p < 0.01$, N.S. not significant, Student t-test ($\alpha = 0.05$). **(D)** RSS/trials values of regression curves for the turn angle. **(E)** Distribution of RSS_J / RSS_R values for the shuffled data. The area between two dashed lines indicate range of the mean $\pm$ 2S.D. of the shuffled data, and black triangle indicates the RSS_J / RSS_R of the real data.

(Figure 1-5)

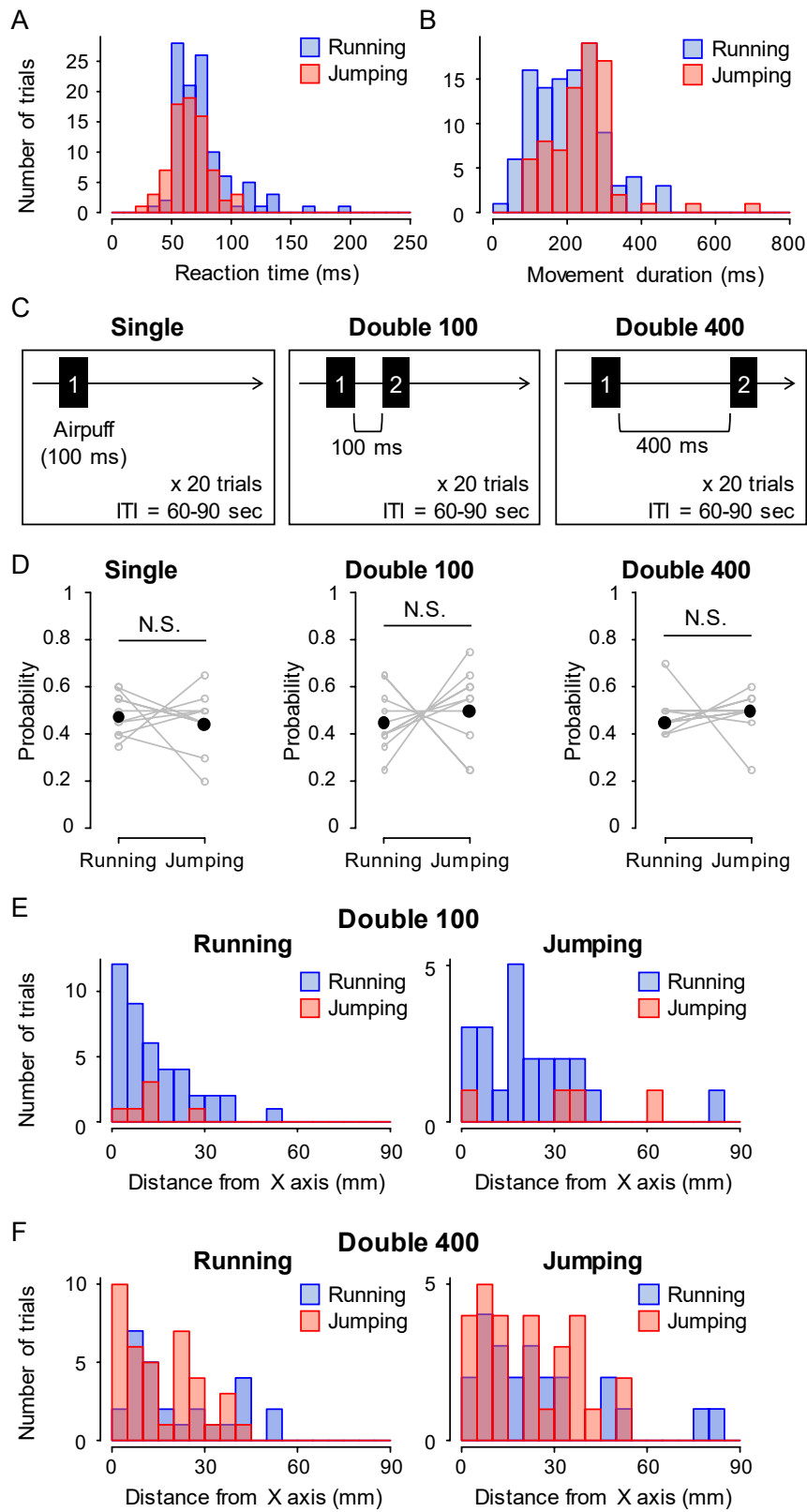


Figure 1-5. Response to the successive double stimulation. (A,B) Distribution of the reaction time (A) and movement duration (B) in the single stimulation experiment. Histograms indicates the number of trials in which the parameter values were measured for running (blue) and jumping (red). (C) Diagrams indicating the temporal scheme in “Single” (left), “Double 100” (center), and “Double 400” (right) stimulation experiments. (D) Probabilities of running and jumping as the first response in “Single” (left), “Double 100” (center), and “Double 400” (right) stimulation experiments. Gray open circles connected with lines represent the response probabilities for each individual, and black filled circles represent the mean of the probability in all individuals. N.S., not significant, Wilcoxon signed-rank test ($\alpha = 0.05$). $n = 10$ animals. (E,F) Distribution of the location where the animals stimulated by the second stimulus. Histograms indicate the number of secondly-responding trials against the distance from X axis, which corresponds to airflow course, to the position stimulated by the second puff in “Double 100” (E) and “Double 400” (F). The data are divided by the first response into running (left) and jumping (right).

(Figure 1-6)

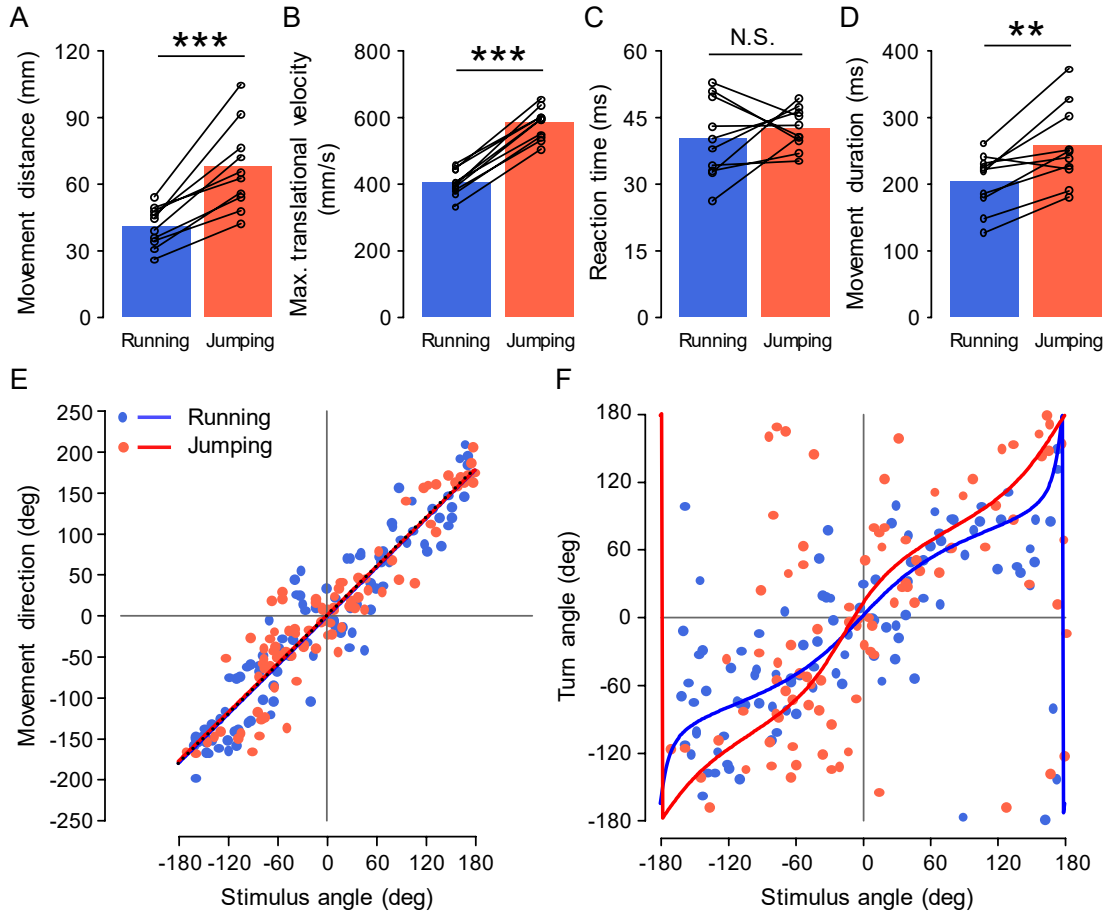


Figure 1-6. Locomotor performances of the responses in “Single” session of double stimulus experiment. (A-D) Averages of mean movement distance (A), mean maximum translational velocity (B), mean reaction time (C), and mean movement duration (D) for all individuals. Black open circles connected with lines indicate the mean value for each individual. ** $p < 0.01$, *** $p < 0.001$, N.S., not significant, paired t-test ($\alpha = 0.05$). $n = 10$ animals. **(E)** Relationships between the movement direction and stimulus angle in running (blue) and jumping (red). Lines represent linear regression lines for the data of running (blue) or jumping (red). Black dotted line indicates line of $y = x$. **(F)** Relationships between the turn angle and stimulus angle in running (blue) and jumping (red). Curves represent circular-circular regression curves.

(Figure 1-7)

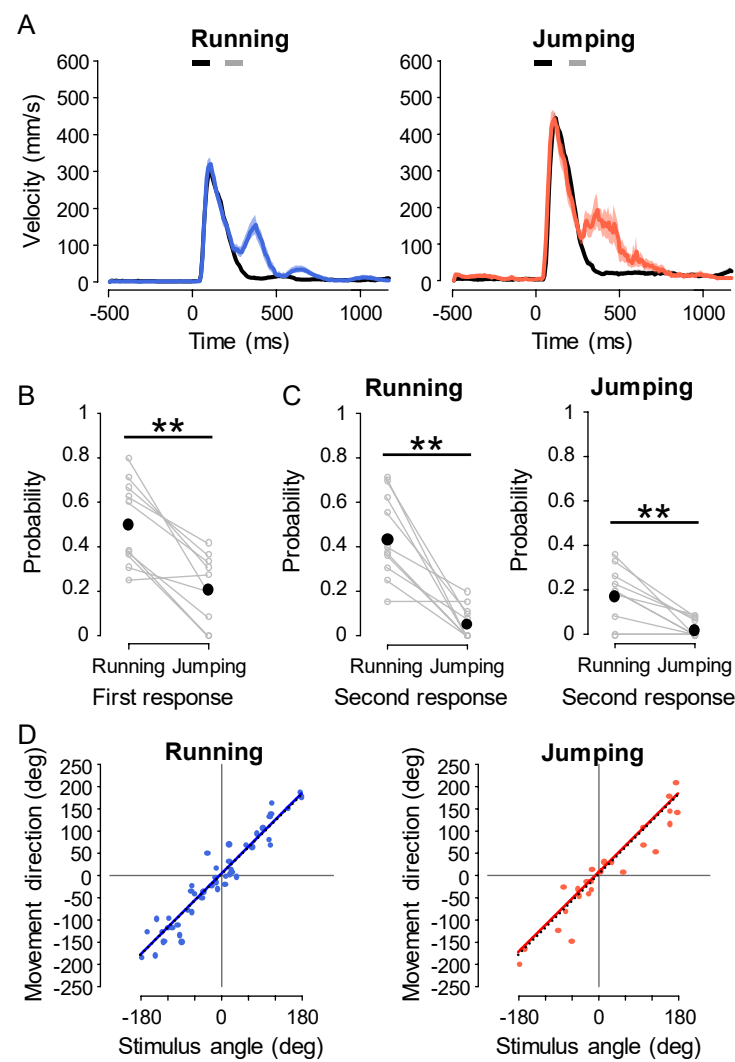


Figure 1-7. Response to successive double stimulation of short intervals. (A) Time course of translational velocity in the escape responses in the “Single” and “Double 100” experiments. Black lines indicate the average of the mean time course for all trials in “Single”. Colored lines indicate those for trials in which crickets responded to both the first and second stimuli in “Double 100”. The trials are divided by the first response into running (left) or jumping (right). Shaded areas represent range of mean $\pm$ S.E.M. Black and gray bars indicate the first and second stimuli, respectively. (B,C) Probabilities of the second response. Probability of second response including running and jumping during each of the first response (B), and those of running and jumping as the second response following the first response of running (left) or first jumping (right) (C). Gray open circles connected with lines represent the response probabilities for each individual, and black filled circles represent the mean of the probability in all individuals. $*p < 0.05$, $**p < 0.01$, Wilcoxon signed-rank test ($\alpha = 0.05$). $n = 10$ animals. (D) Relationships between the moving direction of whole escape responses and the first stimulus angle in the trials in which crickets responded to both stimuli in “Double 100”. The trials are divided by the first response into running (left) and jumping (right). Colored lines represent linear regression lines for the data of running (blue) or jumping (red), and black dotted lines indicate lines of $y = x$.

(Figure 1-8)

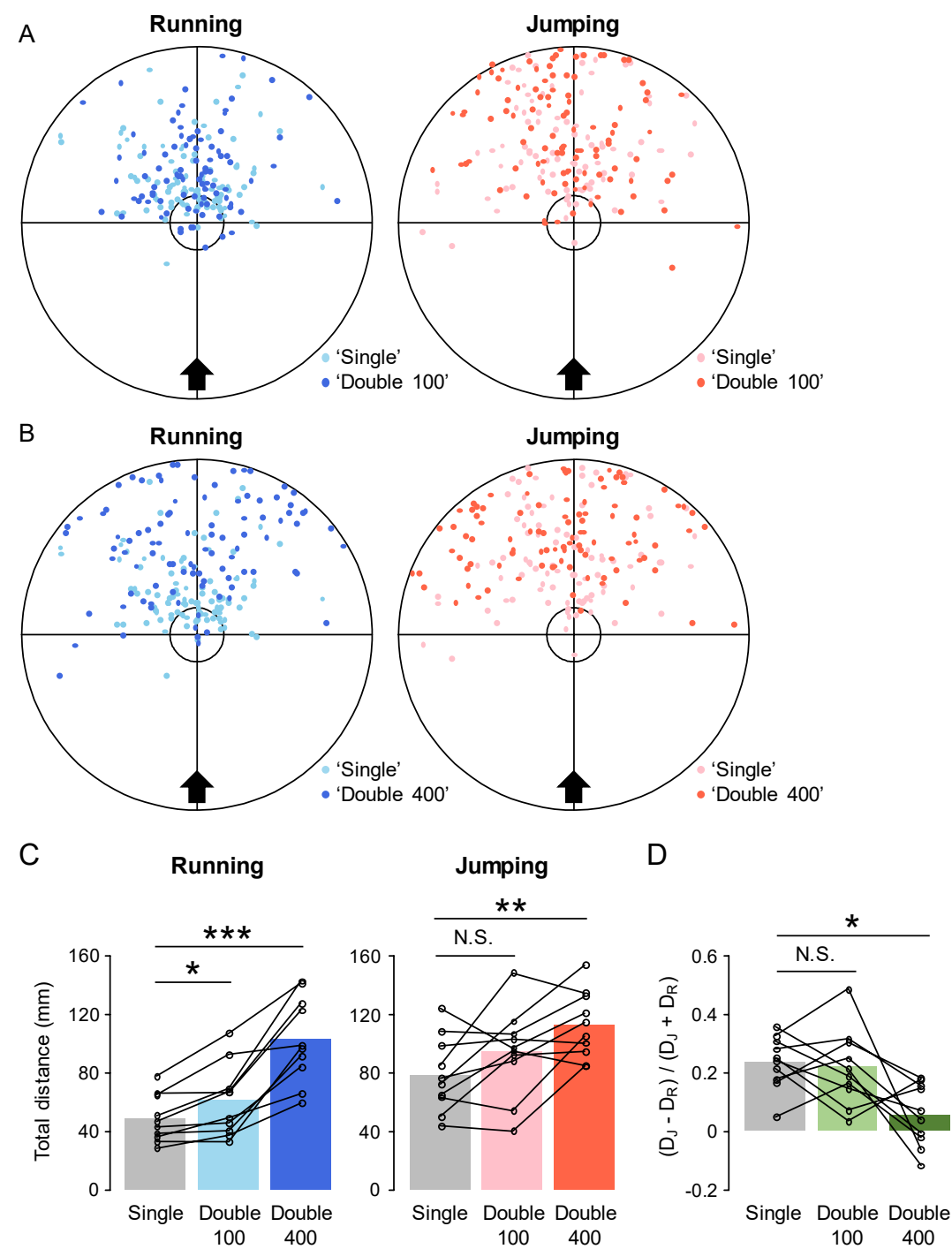


Figure 1-8. Movement distance in escape responses to single and double stimulation.

(A,B) Spatial distributions of finish points of whole escape locomotion in the trials. The data are divided by response to the first stimulus into running (left) and jumping (right). Thin colored circles represent the trials in “Single”, and dark colored circles represent the trials in “Double 100” (A) or “Double 400” (B). Black arrows indicate the direction of the air current stimuli. The center small circle indicates the starting location of the crickets enclosed within a beaker, and the large circle on the outside indicates the wall of the experimental arena. **(C)** The total movement distances of whole escape locomotion in trials where running (left) or jumping (right) was elicited by the first stimulus. Bars indicate the average of the mean value for all individuals in “Single” (gray), “Double 100” (light colored), and “Double 400” (dark colored) stimulation experiments. Open circles connected with lines represent the mean values for each individual. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, N.S. not significant, paired t-test with Bonferroni correction for multiple comparisons ($\alpha = 0.05$). **(D)** The normalized differences in total movement distance between running and jumping responses to the first stimulus. Bars indicate the average of the mean value for all individuals in “Single” (gray), “Double 100” (light green), and “Double 400” (dark green) stimulation experiments. $*p < 0.05$, N.S. not significant, Wilcoxon signed-rank test with Bonferroni correction for multiple comparisons ($\alpha = 0.05$). $n = 10$ animals.

(Figure 1-9)

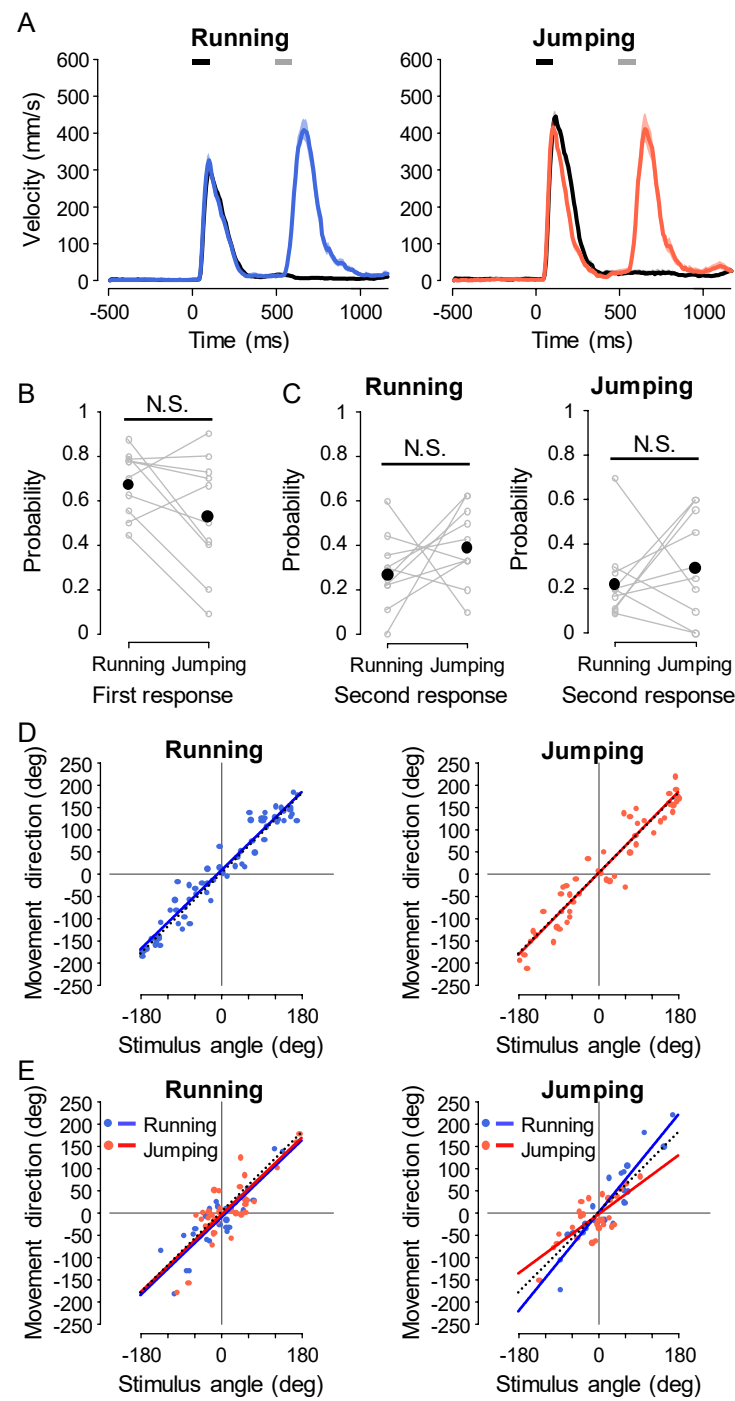


Figure 1-9. Response to successive double stimulation of long intervals. (A) Time course of translational velocity in escape responses in the “Single” and “Double 400” experiments. Black lines indicate the average of the mean time course for all trials of running (left) and jumping (right) in “Single”. Colored lines indicate those for trials in which crickets responded to both the first and second stimuli in “Double 400”. The trials are divided by the first response into running (left) or jumping (right). Shaded areas represent range of $\pm$ S.E.M. Black and gray bars indicate the first and second stimuli, respectively. (B,C) Probabilities of the second response. Probability of second response including running and jumping after each of the first response (B), and those of running and jumping after the first running (left) or first jumping (right). Gray open circles connected with lines represent the response probabilities for each individual, and black filled circles represent the mean of the probability in all individuals. N.S., not significant, Wilcoxon signed-rank test ($\alpha = 0.05$). $n = 10$ animals. (D,E) Relationships between the moving direction and stimulus angle of the first response (D) and the second response (E) in the trials in which crickets responded to both the two stimuli in “Double 400”. The trials are divided by the first response into running (left) and jumping (right). Colored lines represent linear regression lines for the data of running (blue) or jumping (red), and black dotted lines indicate lines of $y = x$.

(Figure 1-10)

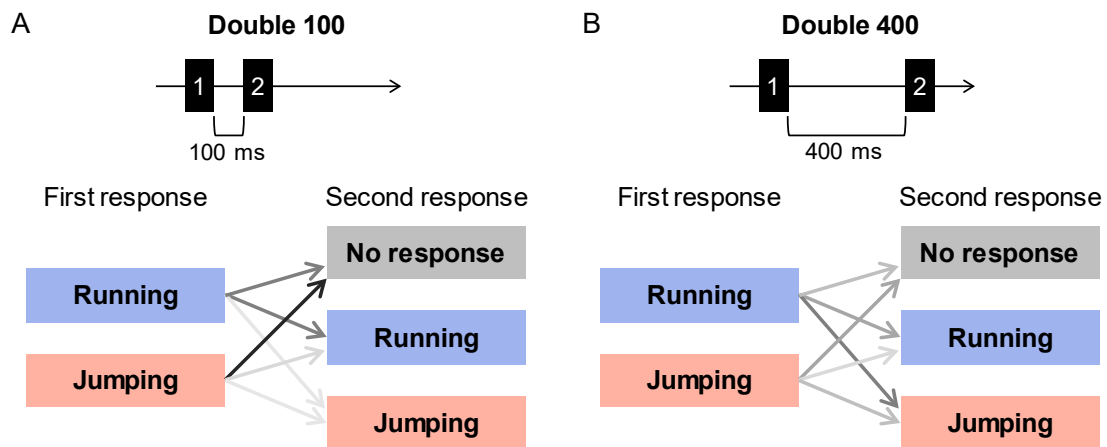


Figure 1-10. Summary of the sequence of the first and second response in the double stimulus experiments. Diagram showing the stimulation (top) and sequences of the first and second responses (bottom) in “Double 100” (**A**) and “Double 400” (**B**). The thickness of the arrows from the first response to the second response indicates the probability of the sequence of responses.

(Figure 1-11)

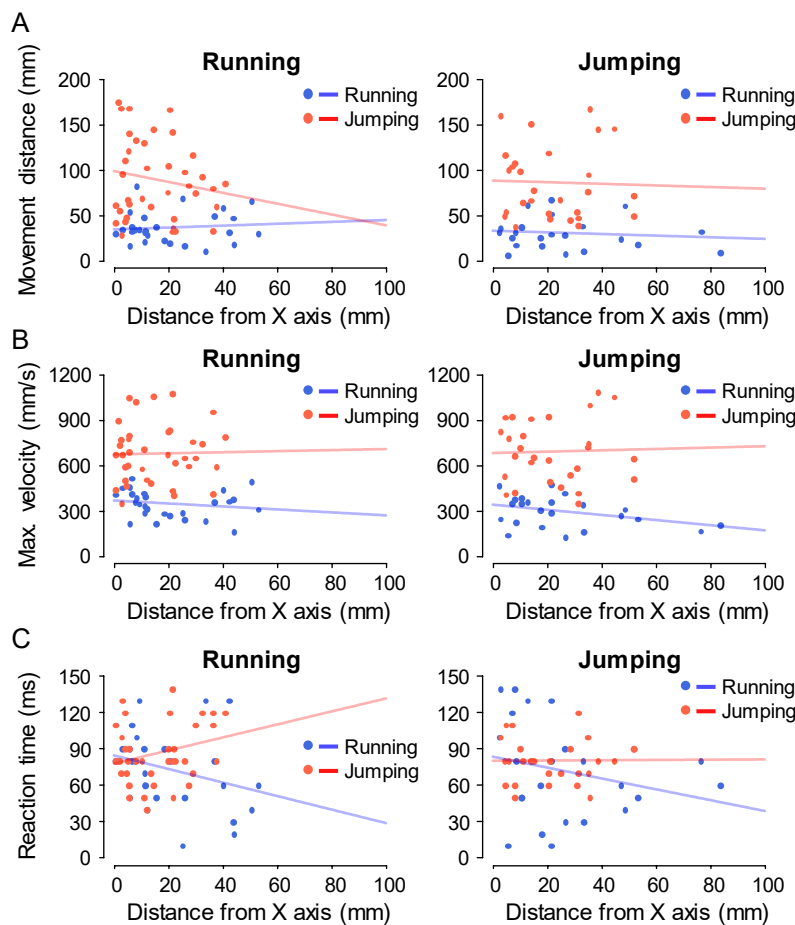


Figure 1-11. No significance in the effect of the position stimulated by the second puff in double stimulus experiments. (A-C) Relationship between the distance from X axis to the secondly-stimulated position and the metric locomotor parameters in the second response, including the movement distance (A), maximum translational velocity (B), and reaction time (C) in “Double 400”. The data are divided by the first response into running (left) and jumping (right). Lines represent linear regression lines for the data of running (blue) or jumping (red). No significant relationship was indicated by linear regression analysis ($\alpha = 0.05$).

(Figure 1-12)

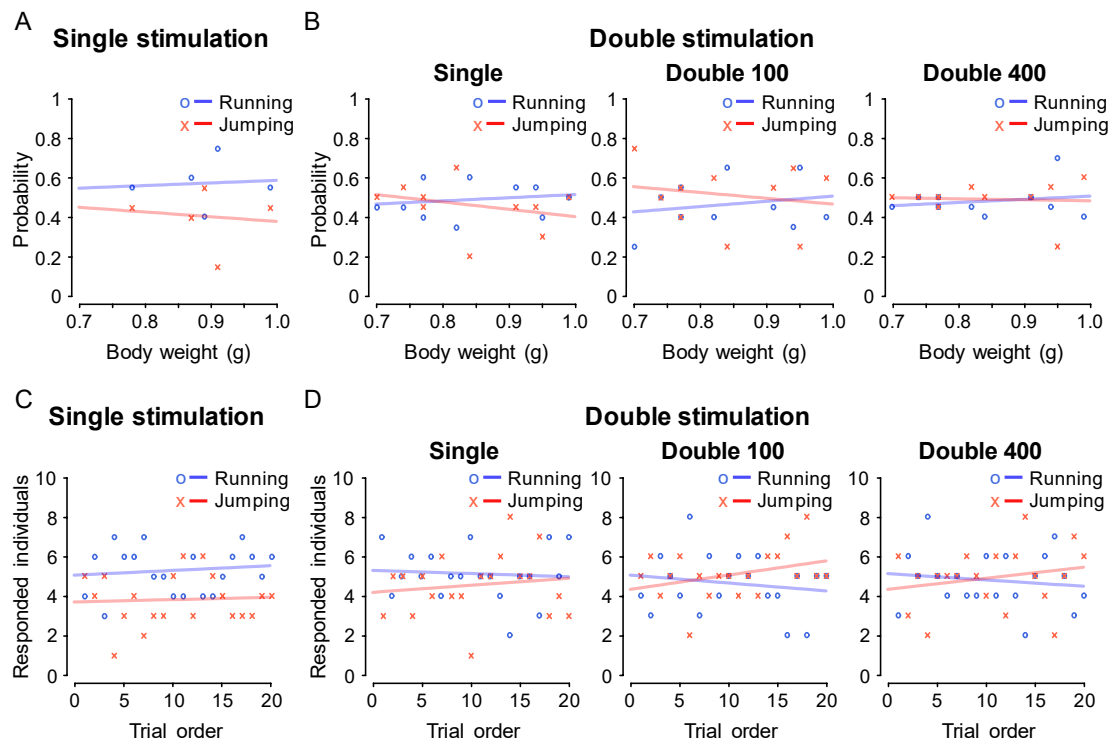


Figure 1-12. Behavioral choice did not depend on weight of the animals or trial order.

(A,B) Relationship between the weight of each individual and the probabilities of running (blue “o”) and jumping (red “x”) as the initial response in single- (A) and double stimulus experiments (B). Lines represent linear regression lines for the data of running (blue) or jumping (red). No significant relationship was indicated by linear regression analysis ($\alpha = 0.05$). $n = 5$ and 10 animals for single- and double stimulus experiments, respectively.

(C,D) Relationship between the trial order in each individual and the number of individuals responding running (blue “o”) or jumping (red “x”) as the initial response in single- (C) and double stimulus experiments (D). Lines represent linear regression lines for the data of running (blue) or jumping (red). No significant relationship was indicated by linear regression analysis ($\alpha = 0.05$). $n = 10$ animals for each stimulation experiments.

1.6 Tables

Table 1-1. Summary of the number of trials in each response.

Running	Jumping	No response
106	76	18

Table 1-2. Summary of the linear regression result for the effect of the crickets' position on the locomotor parameters in the second response in “Double 400”.

Parameter	First Response	Second response	Significance of coefficient		
			Estimated slope	t value	p value
Movement distance (Fig. 1-11A)	Running	Running	0.095	0.442	0.662
	(left panel)	Jumping	-0.604	-1.027	0.311
	Jumping	Running	-0.101	-0.611	0.548
	(right panel)	Jumping	-0.090	-0.171	0.866
Max. velocity (Fig. 1-11B)	Running	Running	-0.981	-0.888	0.383
	(left panel)	Jumping	0.336	0.126	0.900
	Jumping	Running	-1.667	-1.825	0.082
	(right panel)	Jumping	0.481	0.175	0.863
Reaction time (Fig. 1-11C)	Running	Running	-0.557	-1.475	0.153
	(left panel)	Jumping	0.537	1.642	0.109
	Jumping	Running	-0.450	-1.168	0.256
	(right panel)	Jumping	0.0129	0.055	0.956

Table 1-3. Summary of the linear regression result for the effect of weight of animals on the response probabilities.

Explanatory variable	Experiment	Response	Significance of coefficient		
			Estimated slope	t value	p value
Weight (Fig. 1-12 A and B)	Single stimulation	Running	0.140	0.147	0.893
		Jumping	-0.240	-0.211	0.846
	“Single” in Double stimulation	Running	0.145	0.469	0.652
		Jumping	-0.377	-0.885	0.402
	“Double 100” in Double stimulation	Running	0.277	0.620	0.552
		Jumping	-0.312	-0.543	0.602
	“Double 400” in Double stimulation	Running	0.158	0.529	0.611
		Jumping	-0.053	-0.159	0.878
Trial order (Fig. 1-12 C and D)	Single stimulation	Running	0.026	0.551	0.558
		Jumping	0.011	0.206	0.839
	“Single” in Double stimulation	Running	-0.017	-0.312	0.759
		Jumping	0.034	0.545	0.593
	“Double 100” in Double stimulation	Running	-0.041	-0.705	0.490
		Jumping	0.072	1.539	0.141
	“Double 400” in Double stimulation	Running	-0.032	-0.557	0.585
		Jumping	0.056	0.920	0.370

Chapter 2

Velocity and duration of stimulus affected selection of escape responses

2.1 Introduction

Animals choose behavioral response appropriate to the situation. For instance, mice exhibit flight or freezing depending on movement of the predator (De Franceschi et al., 2016); they choose flight in response to the predator approaching to them but exhibit freezing in response to the predator that is just cruising. When the predator is unaware of prey, the freezing is more efficient for the prey animal to avoid being targeted by the predator. However, once the predator is aware of the prey and began to approach it, the flight is appropriate response than freezing to escape from the predator attack (Eilam, 2005). The prey animals receive attacks from various species of predators (Bulbert et al., 2015; Evans et al., 2019) and the approaching manner of the predator is varied trial by trial (Dangles et al., 2006; Walker et al., 2005). Thus, it is critical for the prey animals to know how the predator is approaching them and to choose an appropriate response from several escape responses with distinct behavioral advantages.

Animals detect “how the predator is approaching” as various parameters of sensory stimuli that are directly related to the escape behavior (Domenici, 2010). The preys use the information about from which direction the predator approaches to control their escaping direction (Bhattacharyya et al., 2017; Card, 2012; Domenici et al., 2011a, b; Dunn et al., 2016), which was also observed in the cricket wind-elicited escape behavior (Fig. 1-3). The visual size of stimulus indicating the distance to the approaching

predator is reported to determine the escape initiation in several species (Bhattacharyya et al., 2017; Dunn et al., 2016; Fotowat and Gabbiani, 2007; Preuss et al., 2006). Recently, the approaching speed of the predator has been reported to have impact not only on the distance of escaping (Stewart et al., 2013) but also on the action selection between distinct escape responses (Bhattacharyya et al., 2017; von Reyn et al., 2014, 2017). In flies, the more rapidly a visual stimulus looms, the more likely the flies choose escape flight with shorter reaction time instead of being unstable (Card and Dickinson, 2008; von Reyn et al., 2014, 2017). However, it is unknown whether and how the other parameters such as the direction or duration of the stimulus are involved in the action selection.

Although the velocity of the stimulus directly indicates the approaching speed of the predator, the other two stimulus parameters are also important for the escape behavior. The stimulus with higher velocity indicates a larger predator and quicker attack. The stimulus of longer duration suggests greater movement of the approaching predator (Casas and Dangles, 2010). Therefore, fast and long stimulus means more emergent situations for the prey animals. In addition, the stimulus angle indicating from which direction the predator approaches may affect the selection of escape responses. Considering the result in Chapter 1, the faster and longer stimulus that indicate the emergent situation may cause jumping more frequently because the crickets can move more quickly in jumping. However, the stimulus angle would have small effect on the choice of either running or jumping because the crickets could move to any direction in both running and jumping.

To clarify which parameters of the stimulus affect the action selection between distinct escape responses, it is necessary to manipulate the particular stimulus parameters quantitatively. In the wind-elicited escape behavior of crickets, the animals not only

control their movement direction based on the stimulus angle, but also increase the running distance in response to the longer stimulus (Oe and Ogawa, 2013). Therefore, the crickets may be able to take such stimulus parameters into account when they choose either running or jumping. In this chapter, I manipulated the three stimulus parameters, which were the angle, velocity and duration, and examined their effects on the response probabilities. Furthermore, to reveal how the stimulus parameters had impacts on the performance in the escape responses, I checked the relationships between the locomotor parameters such as the movement speed and directionality and the stimulus parameters.

2.2 Materials and Methods

Apparatus of the behavioral experiment, stimulators and recording procedures were the same as in Chapter 1.

Animals

I used the wild-type strains of crickets (*Gryllus bimaculatus*, Hokudai WT; Watanabe et al., 2018) which were inbred in my laboratory. Throughout the experiments, 30 adult male crickets, less than 14 days after adult molting, were used. 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 26-28°C.

Stimulation and experimental procedure

In Chapter 2, data of three kinds of experiments was analyzed to test the effect of the three stimulus parameters on the escape responses. Throughout the experiments in Chapter 2, the stimulus angles against the cricket were varied across the trials because the crickets were oriented randomly within a beaker, as in Chapter 1. In order to test the effect of wind stimulus angles on escape response (“angle test”), the data obtained from single stimulus experiments in Chapter 1 was used, in which the duration and velocity of the stimulus were constant (200-ms duration and 834-mm/s velocity). In order to test the effect of wind stimulus velocities on escape response (“velocity test”), four types of stimuli with different velocities (340, 618, 734 and 834 mm/s) and fixed duration (200 ms) were used. The travel times were 21.2 ± 1.0 , 15.5 ± 0.1 , 14.6 ± 0.1 and 14.6 ± 0.1 ms for 340, 618, 734 and 834 mm/s of air currents, respectively. These values were measured as a delay in stimulus-evoked ascending spikes of projection neurons that were extracellularly

recorded ($n = 2$ animals, 10 trials at each stimulus velocity) at the center of the arena. In order to test the effect of wind stimulus durations on escape response (“duration test”), the stimuli with fixed velocity (834 mm/s) and different durations (20, 40, 60, 80 and 100 ms) were used. The velocity and duration of the air current were regulated as injection pressure and open/close of the valve of the picopump.

In both of the “velocity test” and “duration test”, the stimuli with different velocity or duration were applied to the same individuals at increasing ($n = 5$ animals) or decreasing order ($n = 5$ animals). Twenty trials were performed in one session for each type of stimulus, so in total 80 or 100 trials were performed for each individual in “velocity test” or “duration test”, respectively. Inter-trial intervals in each session were 60–90 sec, and during the inter-session interval for about 10 min the crickets rested within a plastic container (138 mm× 220 mm × 135 mm) and freely accessed food and water, as in Chapter 1.

Behavioral analysis

Definition and categorization of escape response and measurement of all locomotor parameters were the same as in Chapter 1. The metric locomotor parameters including the movement distance, maximum translational velocity and reaction time were measured in each escape response. To investigate the angular control in the responses, the movement direction and turn angle were calculated.

In addition to the response probability, the selection ratio of running or jumping was calculated as the proportion between both responses for all responded trials including running and jumping. In “angle test”, all trials were pooled and categorized into four groups, trials with 0° to 45° , 45° to 90° , 90° to 135° and 135° to 180° of absolute

stimulus angle, and the selection ratio was assessed in each category (Fig. 2-1B). In “velocity test” and “duration test”, the selection ratio was calculated for each individual regardless of the stimulus angle and then obtained mean value of the ratios for all individuals (Fig. 2-1D and F).

In Chapter 1, the plot of movement direction against stimulus angle was close to $y = x$ in both running and jumping, meaning that crickets moved toward opposite direction to the stimulus (Figs. 1-3, 1-6E). Therefore, the accuracy in the directional control was assessed by the absolute value of the difference between the movement direction and stimulus angle (Figs. 2-7B, 2-8B).

Statistical analysis

R programming software (ver. 3.4.4, R Development Core Team) was used for all statistical analyses. Relationship between angular parameters and stimulus angle was checked by performing the same regression analysis as in Chapter 1.

To evaluate the effect of the stimulus parameter on the all response probability (responded or not, for whole trials) and selection ratio (running or jumping, for all responded trials), multiple logistic regression analysis was used in each of “angle test”, “velocity test” and “duration test”. In addition to the manipulated stimulus parameter, the trial order had to be considered as an explanatory valuable, as follows;

$$(\text{All response probability}) = \frac{1}{1 + \exp(-(a(\text{Stimulus parameter}) + b(\text{Trial order}) + c))},$$

$$(\text{Selection ratio}) = \frac{1}{1 + \exp(-(a(\text{Stimulus parameter}) + b(\text{Trial order}) + c))},$$

where a and b are the estimated coefficient and c is the intercept. Then, the significance of the coefficient for each explanatory valuable was checked to assess the effect on the

all response probability or selection ratio (Table. 2-1).

To analyze the relationships between the metric locomotor parameters such as movement distance, maximum velocity, or reaction time and the stimulus angle, which was indicated as absolute value from 0° (front) to 90° (lateral) and to 180° (behind), I used linear regression analysis as follows:

$$(\text{Metric locomotor parameter}) = a(\text{Stimulus angle}) + b,$$

where a is the estimated coefficient and b is the intercept.

Since the response probability was strongly affected by stimulus velocity and duration, the number of obtained data for the responses largely differed throughout the sessions (Tables. 2-2, 2-3). Especially, slow (340 mm/s) or short (20 ms) air current rarely or never elicit jumping so that I could not calculate the mean value of the locomotor parameters in all of the tested individuals at the session for such stimulus. Considering the small and varied sample size, a linear mixed-effects model (LMM, the package ‘lme4’ ver. 1.1-23 in R) was adopted to evaluate the effect of the stimulus velocity or duration on the locomotor parameters, in which the animal IDs were considered as random effects. I supposed the LMMs with explanatory variable of stimulus parameter as follows:

$$(\text{Locomotor parameter}) = \beta_0 + \beta_1(\text{Stimulus parameter}) + r,$$

where r was the random effect. The significance of the effect of stimulus parameters was tested by comparing the LMMs with explanatory variable of stimulus velocity or duration and without one by the likelihood ratio test.

2.3 Results

Faster and longer stimulus elicited jumping more frequently.

First, I examined what parameters of the stimulus had effects on the action selection of either running or jumping. I tested the effects of the angle, velocity and duration of the stimulus respectively, and found that the crickets chose jumping more frequently in response to the faster and longer stimulus (Fig. 2-1). In the “angle test” (Fig. 2-1A and B), the data of single stimulation experiments in Chapter 1 showed that the running was elicited in about 50% probability regardless of the stimulus angles, whereas the jumping was more likely elicited by the stimulus from behind (around 0° in Fig. 2-1A). However, the effect of the stimulus angle on the selection ratio of running or jumping was not significant (Fig. 2-1B, Table 2-1). In contrast, the velocity and duration of the stimulus affected whether the crickets respond to the stimulus and which response the crickets chose for escaping. In the experiments of “velocity test” and “duration test” (Fig 2-1C–F), either the velocity or the duration of the stimulus was manipulated. Here, both of the response probability to the stimulus and the selection ratio of jumping for the escape response significantly elevated as the velocity or duration of the stimulus increased (Fig 2-1C–F, Table. 2-1). These results indicated that the crickets’ choice of the escape responses was affected by the two stimulus parameters, velocity and duration. Although I repeated multiple trials to calculate response probability in each individual, the trial order affected neither on the response probabilities nor the selection ratios in all of the three experiment (Fig 2-1, Table 2-1).

Stimulus angle affected the locomotor performance in running.

Next, I investigated how the tested stimulus parameters affected the locomotor

performance in running and jumping. Here, I checked the relationships between metric locomotor parameters and the stimulus angle in “angle test” experiment (Fig. 2-2), since the relationships of the moving direction or turn angle and the stimulus angle have already been analyzed in Chapter 1 (Figs. 1-3, 1-4). The crickets could change their locomotion speed according to the stimulus angle in running rather than in jumping. The stimulus angle significantly had impact on all of the three metric locomotor parameters in running but only on the maximum velocity in jumping (linear regression analysis, estimated slope = -0.104, $t = -3.346$, $p = 0.001$; “Running”, estimated slope = -0.073, $t = -1.213$, $p = 0.229$; “Jumping”, in Fig. 2-2A, estimated slope = -0.360, $t = -2.160$, $p = 0.033$; “Running”, estimated slope = 0.652, $t = 2.105$, $p = 0.039$; “Jumping”, in Fig. 2-2B, estimated slope = 0.140, $t = 3.076$, $p = 0.003$; “Running”, estimated slope = 0.021, $t = 0.545$, $p = 0.587$; “Jumping”, in Fig. 2-2C). The closer to behind the stimulus was applied from, the more quickly crickets responded and the faster and the farther they moved in running (Fig. 2-2A–C, left). By contrast, crickets jumped at higher speed for the frontal stimulus, but the moving distance and reaction time of jumping were not significantly affected (Fig. 2-2A–C, right).

Locomotor parameters changed depending on stimulus velocity and duration in running.

In “velocity test” and “duration test” experiment, I examined the effects of velocity or duration of the stimulus on the metric locomotor parameters in running and jumping (Figs. 2-3, 2-4, 2-5 and 2-6). Both of the velocity and duration of the stimulus affected the metric locomotor parameters in running rather than in jumping. In “velocity test”, the crickets increased the distance and speed in response to the faster stimulus in running, but not in jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 9.287$, $p = 0.002$; “Running”, $X^2_{(1)} = 0.254$,

$p = 0.614$; “Jumping”, in Fig. 2-4A, $X^2_{(1)} = 22.10$, $p < 0.001$; “Running”, $X^2_{(1)} = 0.035$, $p = 0.851$; “Jumping”, in Fig. 2-4B). In terms of the reaction time, the crickets initiated both running and jumping more quickly in response to the faster stimulus (likelihood ratio test for LMMs, $X^2_{(1)} = 254.7$, $p < 0.001$; “Running”, $X^2_{(1)} = 149.6$, $p < 0.001$; “Jumping”, in Fig. 2-4C). In “duration test” experiment, the longer stimulus, the faster and farther the cricket ran, but they did not change jumping locomotion with the stimulus duration (Fig. 2-6). The significant effect of the stimulus duration was observed in all of the locomotor parameters in running, whereas in none of the parameters in jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 17.24$, $p < 0.001$; “Running”, $X^2_{(1)} = 2.284$, $p = 0.131$; “Jumping”, in Fig. 2-6A, $X^2_{(1)} = 74.93$, $p < 0.001$; “Running”, $X^2_{(1)} = 0.785$, $p = 0.376$; “Jumping”, in Fig. 2-6B, $X^2_{(1)} = 36.20$, $p < 0.001$; “Running”, $X^2_{(1)} = 3.431$, $p = 0.064$; “Jumping”, in Fig. 2-6C). In running, the longer stimulus elicited the earlier start of the response, which seemed to be the confusing result. That was probably because that the shortest stimulus (20 ms) elicited the running responses with a long delay of over 100 ms after the stimulus terminated (Fig. 2-5C). Fig. 2-3 and 2-5 shows the all distributions of the metric locomotor parameters for the data in “velocity test” and “duration test” experiments, respectively.

Movement direction was controlled regardless of stimulus velocity and duration.

It was shown in Chapter 1 that the crickets precisely controlled their movement direction against the stimulus angle in both running and jumping (Fig. 1-3). Then, I examined whether the velocity and duration of the stimulus affected that directional control in the escape responses (Figs. 2-7, 2-8). The crickets could control their movement direction precisely against the stimulus angle independent of the velocity and duration of the

stimulus. Indeed, the plots of the movement direction against stimulus angle were distributed at close to $y = x$ line in both running and jumping throughout any stimuli of different velocities or durations (Figs. 2-7A, 2-8A, Table 2-4, 2-5). Here, how precisely the crickets moved to opposite direction to the stimulus was assessed by the absolute value of difference between the moving direction and stimulus angle. Regardless of the stimulus velocity, the absolute differences between the movement direction and stimulus angle were around 30° in both running and jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 0.3176$, $p = 0.573$; “Running”, $X^2_{(1)} = 0.530$, $p = 0.467$; “Jumping”, in Fig. 2-7B), indicating no effect of the air-current velocity on the directional control. Also in “duration test” experiment, the duration of stimulus had little impacts on the directional control in the escape responses (Fig. 2-8). The absolute difference between the direction and stimulus angle was constant for the stimuli of 40- to 100-ms durations in both running and jumping (Fig. 2-8B). The significant effect of the stimulus duration on the absolute difference value was observed in running, not in jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 7.681$, $p = 0.006$; “Running”, $X^2_{(1)} = 0.787$, $p = 0.375$; “Jumping”, in Fig. 2-8B), which may be due to that the movement directions in running elicited by the shortest (20-ms) stimulus were less correlated with the stimulus angle. No jumping was elicited by that shortest stimuli (Table. 2-2), resulting in no significance for the absolute difference value on the stimulus duration in jumping.

I also checked the impacts of the velocity and duration of the stimulus on the relationship between the turn angle and stimulus angle (Figs. 2-9, 2-10). I observed apparent difference in the distributions of the plot among the responses neither in “velocity test” nor in “duration test”. Thus, angular parameters of escape responses including the movement direction and turn angle were little affected by the velocity and

duration of the stimulus, except for extremely weak or short stimulus close to threshold to induce jump.

For a conclusion about the locomotor parameters, the crickets could alter their locomotion performance such as speed, distance, and reaction time in response to the changes in the angle, velocity and duration of the stimulus in running, but not in jumping. In contrast, the escape direction was controlled precisely independent of the velocity and duration of the stimulus in both running and jumping.

2.4 Discussion

It is crucial for animals to sense the predator's approach and to exhibit appropriate escape response. In this Chapter, I manipulated several parameters of the air-current stimulus including the angle, velocity and duration, and examined their effects on the elicited escape responses by analyzing the selection of either running or jumping and locomotion parameters of both responses. The crickets chose jumping rather than running in response to the faster and longer stimulus. Furthermore, the locomotion speed was elevated as the velocity or duration of the stimulus increased in running, whereas the locomotor parameters of jumping were almost constant independent of the stimulus velocity and duration. In contrast, the movement direction was precisely controlled regardless of the changes in stimulus velocity or duration. In conclusion, the crickets detected the multiple aspects of the air currents indicating the predator's approach and decided their escape responses based on that stimulus information.

Neural basis underlying the action selection between running and jumping.

Previously, the choice of escape response has been reported to depend on the stimulus velocity (Bhattacharyya et al., 2017; von Reyn et al., 2014, 2017). However, in the wind-elicited escape behavior in the crickets, not only the velocity but also the duration of the stimulus affected the action selection between running and jumping. This finding of the action selection based on multiple stimulus parameters strongly suggest that the neural system underlying this action selection involve integration of the multiple channels of distinct stimulus information. Thus, investigation of the neural system for selection in the cricket's escape responses would promote understanding the mechanism to choose appropriate behavior according to varied situation in nature field. The neural basis of the

cercal sensory system to process airflow information, which mediates the wind-elicited escape behavior, has been well studied (Reviewed in Jacobs et al., 2008; Baba and Ogawa, 2017).

Stimulus characteristics that were likely to elicit jumping.

The faster and longer air currents elicited jumping more frequently. Since the velocity and duration of the air-current stimulus are considered to be correlated with the size and speed of the approaching predator (Casas and Dangles, 2010), crickets would choose jumping in the imminent and critical situation in which a large predator was attacking to them quickly. It should be noted, however, that the probability of jumping was saturated at about 50% even if the fastest and longest stimulus was applied, which is the most inducible for the jump (Fig. 2-1). One possibility is that the additional sensory inputs may be required to further elevate the likelihood of jumping selection. Animals use various modality of sensory inputs to sense the predators. For example, the air-current stimulus combined with auditory stimulus alters the contents of the escape responses (Fukutomi and Ogawa, 2017; Fukutomi et al., 2015). Even the other stimuli that does not directly elicit the escape responses inform context or condition of the animals and affect the escape responses (Domenici et al., 2008; Matsuura et al., 2002). Therefore, the crickets may jump more frequently than 50% under different contexts or situations. The results in this chapter would provide useful and fundamental data for future studies exploring how the animals choose a response under the different conditions which contains multiple sensory signals.

On the other hand, the stimulus angle did not affect the selection of running and jumping significantly. This result would be due to the directional controllability in

crickets' jumping shown in Chapter 1 (Fig. 1-3). In this chapter, the strong relationships between the movement direction and stimulus angle were also observed in “velocity test” (Fig. 2-7) and “duration test” (Fig. 2-8). The crickets would not have to choose jumping depending on the stimulus angle because they could jump to any direction.

Stimulus dependency of the metric locomotor parameters.

The crickets changed their locomotion according to the stimulus parameters in running, but not in jumping. Therefore, in addition to the behavioral advantages of each escape response revealed in Chapter 1, I suggest that the other advantage of running would be a controllability of movement such as velocity and distance according to the stimuli. In contrast, the invariance of the speed and distance in jumping against the stimulus parameters illustrated that jumping was a more stereotypic response than running.

Interestingly, the reaction time became shorter as the stimulus was faster in both running and jumping responses (Fig. 2-4C). Previous studies demonstrate that the escape responses induced by visual looming stimuli initiates at the certain angular size of the stimulus, suggesting that the distance to the predators rather than their approaching velocity would be crucial cue for the prey to start escaping (Bhattacharyya et al., 2017; Dunn et al., 2016; Fotowat and Gabbiani, 2007). Contrary to such previous reports, my result revealed the strong relationship between the stimulus velocity and the time point to start the escape responses. It means that the crickets may decide to start escaping based on the stimulus velocity, which may reflect approaching speed of a predator and may indicate the imminent level for animals. The faster a predator approaches, the less time it takes to reach its prey. The reaction time is an important factor that directly affects the success rate of the escape behavior (von Reyn et al., 2014; Walker et al., 2005). Especially

in situations where animals can't rely on visual information, like in the dark, to decide the escape initiation based on the stimulus velocity would be effective for the survival of animals.

Movement direction was unaffected by the stimulus velocity and duration.

Unlike the metric locomotor parameters, the movement direction was accurately controlled independent of the velocity or duration of the stimulus. Previous study using treadmill has also reported that the direction of the running is controlled accurately to the stimuli of 50, 100 and 200 ms duration, suggesting no dependency of the movement direction on the stimulus duration (Oe and Ogawa, 2013).

Only in the running responding to the shortest stimulus, the accuracy of directional control decreased (Fig. 2-8B, left). It is possible that the 20-ms stimulus was too short for the cricket to perceive its direction. That was also supported by the result that reaction time for running response to this shortest stimulus was longer than that to the stimuli with longer duration (Fig. 2-6C, left). Probably, a certain duration of stimulus is required for the cricket to perceive the exact stimulus angle and to control the escape direction.

2.5 Figures

(Figure 2-1)

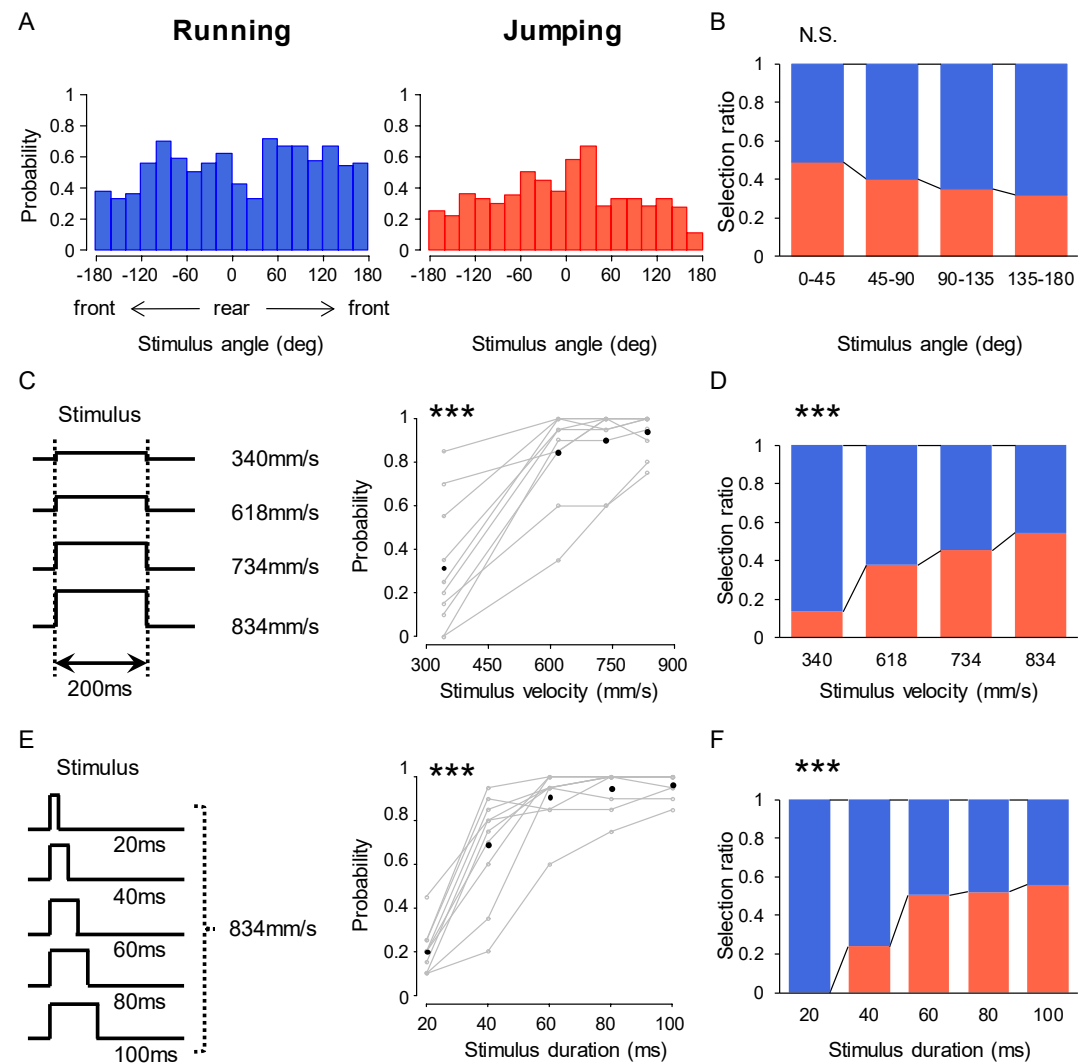


Figure 2-1. Effects of the stimulus parameters on the action selection of running and jumping. **(A)** Distribution of the response probability for running (left) and jumping (right) against the stimulus angle in “angle test”. Histograms represent the number of responding trials per the number of all trials for the range of every 20° of the stimulus angles. $n = 10$ animals. **(B)** The selection ratio of the running (blue) to jumping (red). All trials were categorized into four groups based on the absolute value of the stimulus angle. **(C)** Diagram indicating four types of stimuli with varied velocities and fixed duration (200 ms) used for “velocity test” (left). The all response probability to the stimuli with varied velocities (right). $n = 10$ animals. **(D)** The selection ratio of the running (blue) to jumping (red) in “velocity test”. **(E)** Diagram indicating five types of stimuli with varied durations and fixed velocity (834 mm/s) used for “duration test” (left). The all response probability to the stimuli with varied durations (right). $n = 10$ animals. **(F)** The selection ratio of the running (blue) to jumping (red) in “duration test”. In (C) and (E), gray open circles connected with gray lines represent the probability for each individual, and black filled circles represent the mean of the probability for each stimulus velocity or duration. *** $p < 0.001$, N.S. not significant, coefficients for each of the stimulus parameter in logistic regression analysis ($\alpha = 0.05$).

(Figure 2-2)

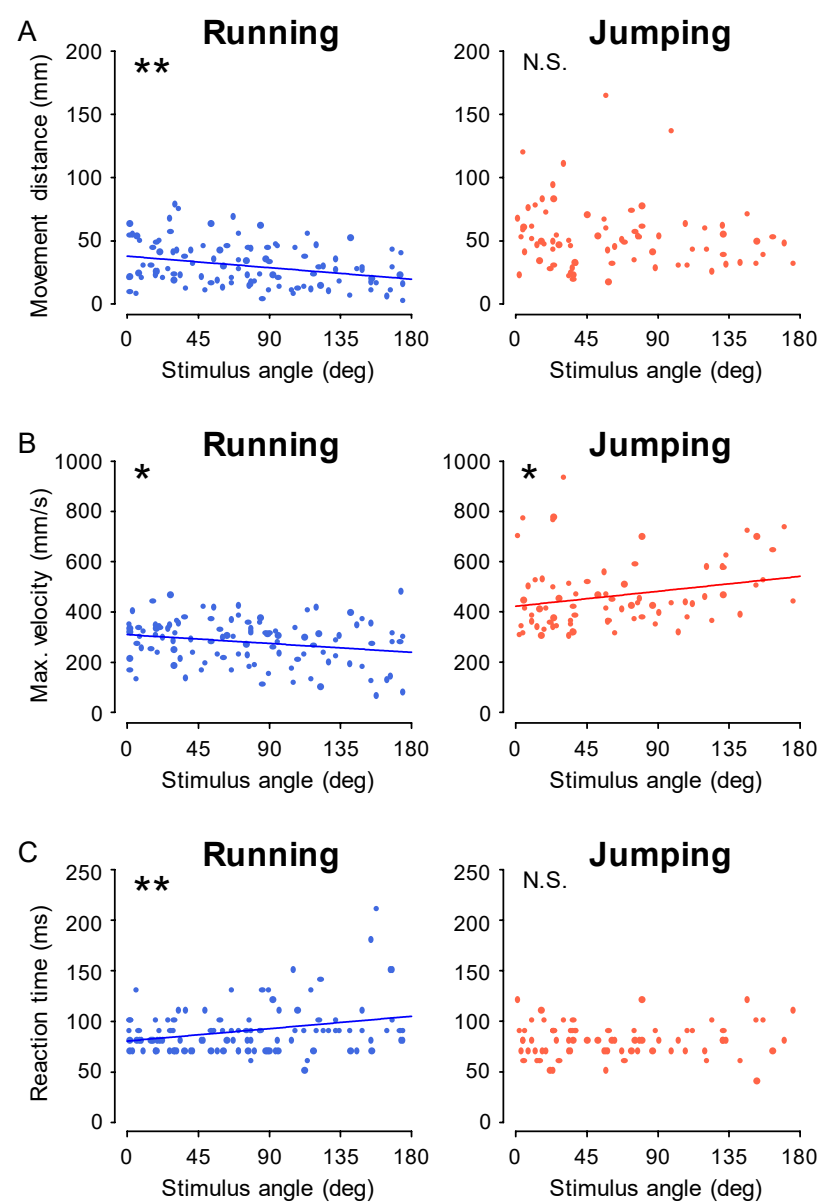


Figure 2-2. Effects of the stimulus angle on metric locomotor parameters in running and jumping. (A–C) Relationships between the stimulus angle and the movement distance (A), maximum translational velocity (B), or reaction time (C) in running (left) and jumping (right) in “angle test”. The air current with a velocity of 834 mm/s and a duration of 200-ms was used. Lines indicate the regression lines with significant slope. * $p < 0.05$, ** $p < 0.01$, N.S. not significant, linear regression analysis ($\alpha = 0.05$). $n = 10$ animals.

(Figure 2-3)

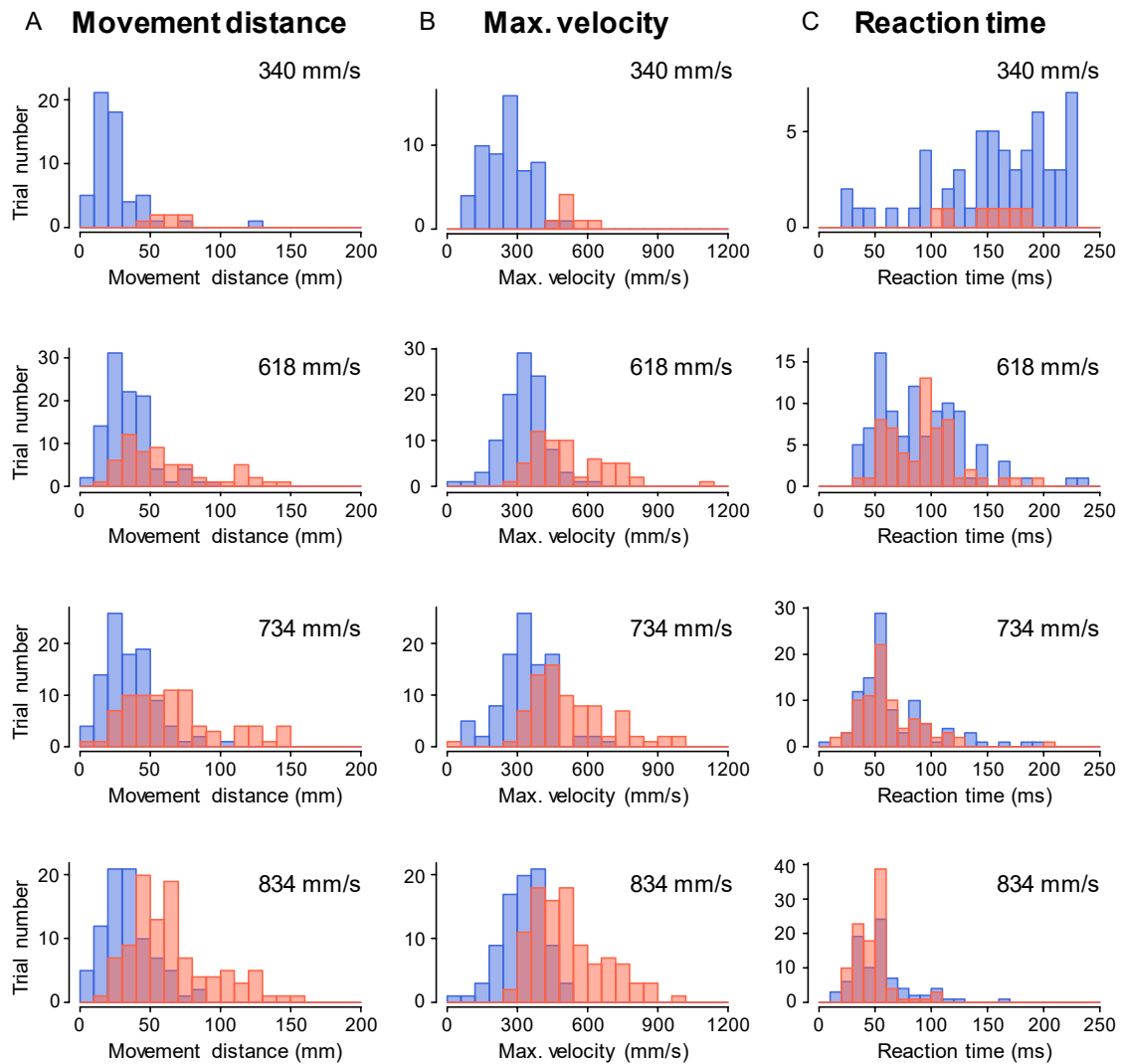


Figure 2-3. Distribution of metric locomotor parameters elicited by the stimulus with different velocities. (A-C) Histograms represent distribution of the movement distance (A), maximum translational velocity (B), or reaction time (C) in running (blue) and jumping (red) in response to the stimuli of 340, 618, 734, and 834 mm/s velocities. $n = 10$ animals.

(Figure 2-4)

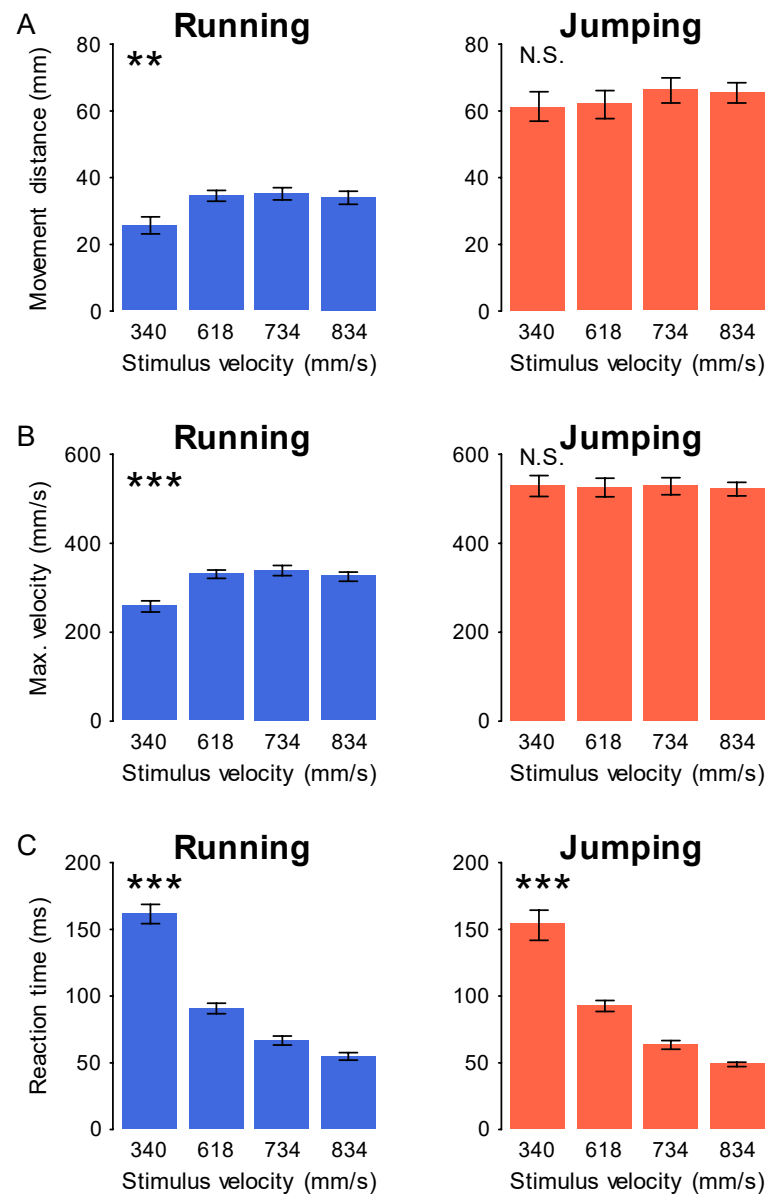


Figure 2-4. Effects of the stimulus velocity on metric locomotor parameters in running and jumping. (A–C) Mean values of the movement distance (A), maximum translational velocity (B), and reaction time (C) in running (left) and jumping (right). Error bars indicate $\pm$ S.E.M. $**p < 0.01$, $***p < 0.001$, N.S. not significant, likelihood ratio test for LMMs ($\alpha = 0.05$). $n = 10$ animals.

(Figure 2-5)

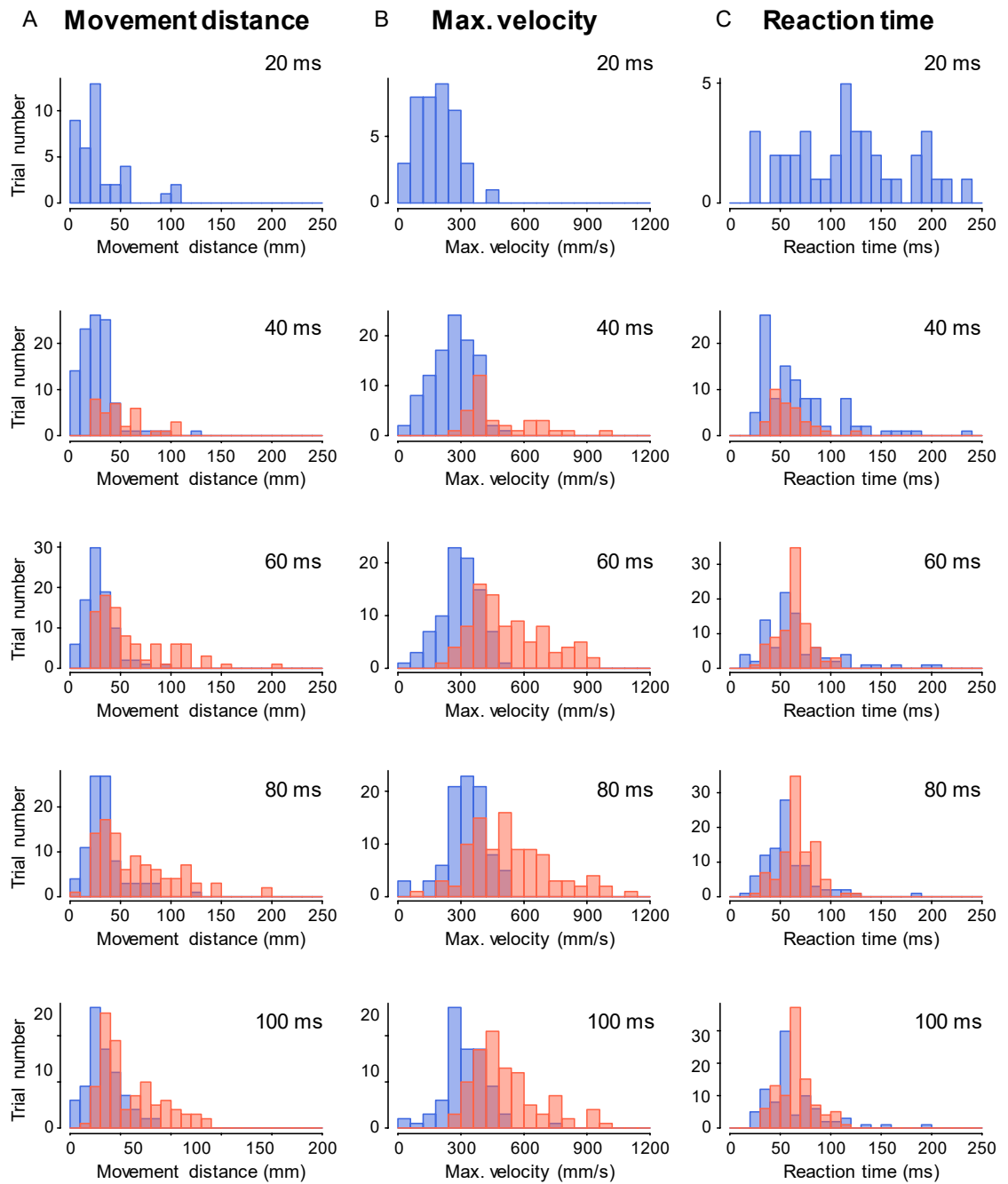


Figure 2-5. Distribution of metric locomotor parameters elicited by the stimulus with different durations. (A–C) Histograms represent distribution of the movement distance (A), maximum translational velocity (B), or reaction time (C) in running (blue) and jumping (red) in response to the stimuli of 20, 40, 60, 80, and 100 ms durations. No jumping was elicited by the stimulus of 20 ms duration. n = 10 animals.

(Figure 2-6)

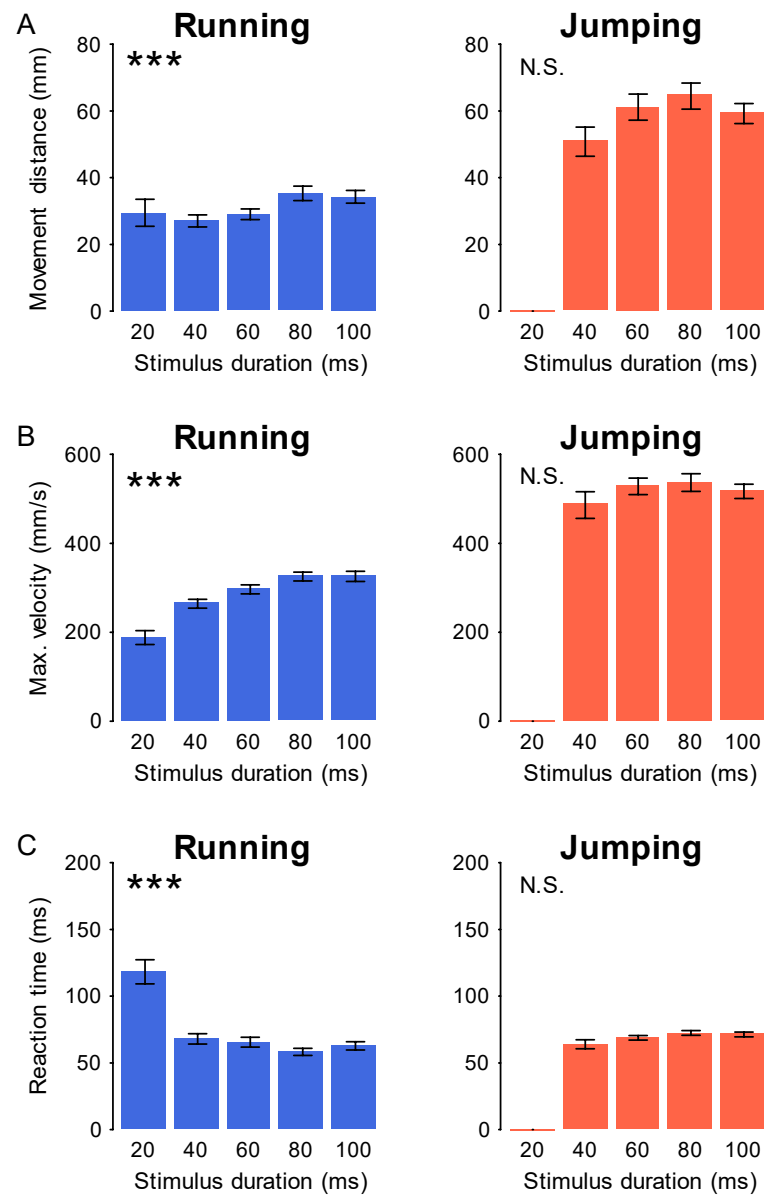


Figure 2-6. Effects of the stimulus duration on metric locomotor parameters in running and jumping. (A–C) Mean values of the movement distance (A), maximum translational velocity (B), and reaction time (C) in running (left) and jumping (right). No jumping was elicited by the stimulus of 20-ms duration. Error bars indicate $\pm$ S.E.M. *** $p < 0.001$, N.S. not significant, likelihood ratio test for LMMs ($\alpha = 0.05$). $n = 10$ animals.

(Figure 2-7)

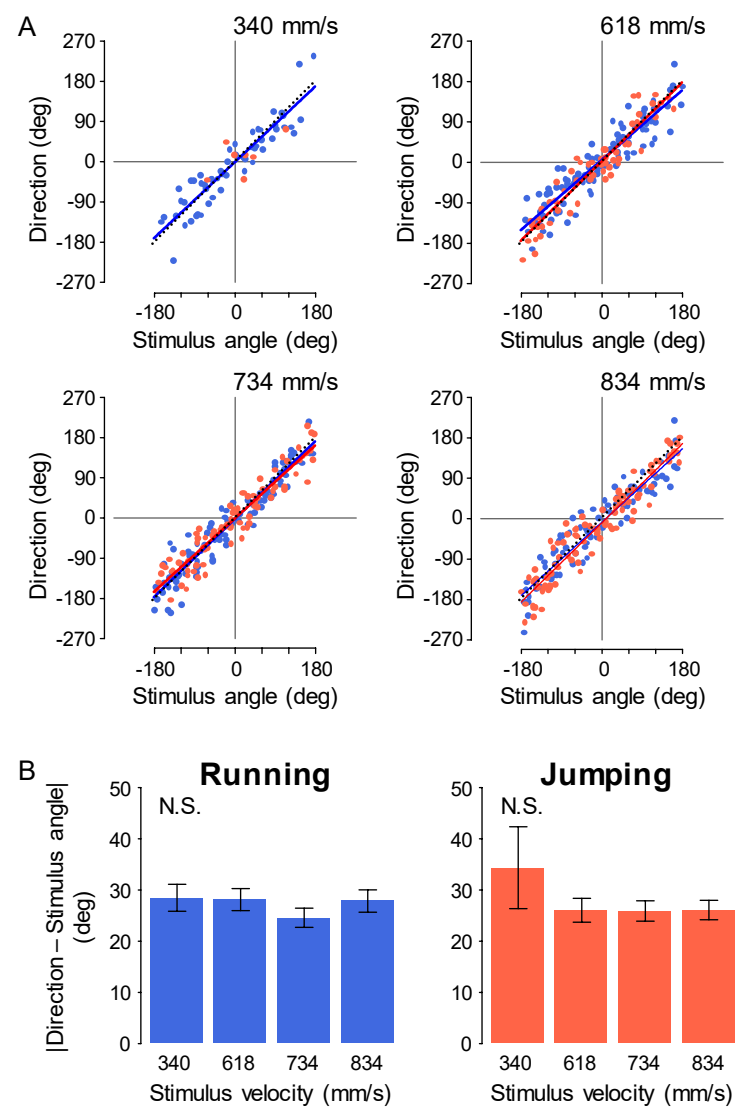


Figure 2-7. Effects of stimulus velocity on control of movement direction in running and jumping. (A) Relationships between the movement direction and stimulus angle in running (blue) and jumping (red) in response to the stimuli of 340, 618, 734, and 834 mm/s velocities. Colored lines represent linear regression lines for the data of running (blue) or jumping (red) with significant slope. Black dotted line indicates line of $y = x$. **(B)** Mean absolute values of the difference between the movement direction and stimulus angle in running (left) and jumping (right). Error bars indicate $\pm$ S.E.M. N.S. not significant, likelihood ratio test for LMMs ($\alpha = 0.05$). $n = 10$ animals.

(Figure 2-8)

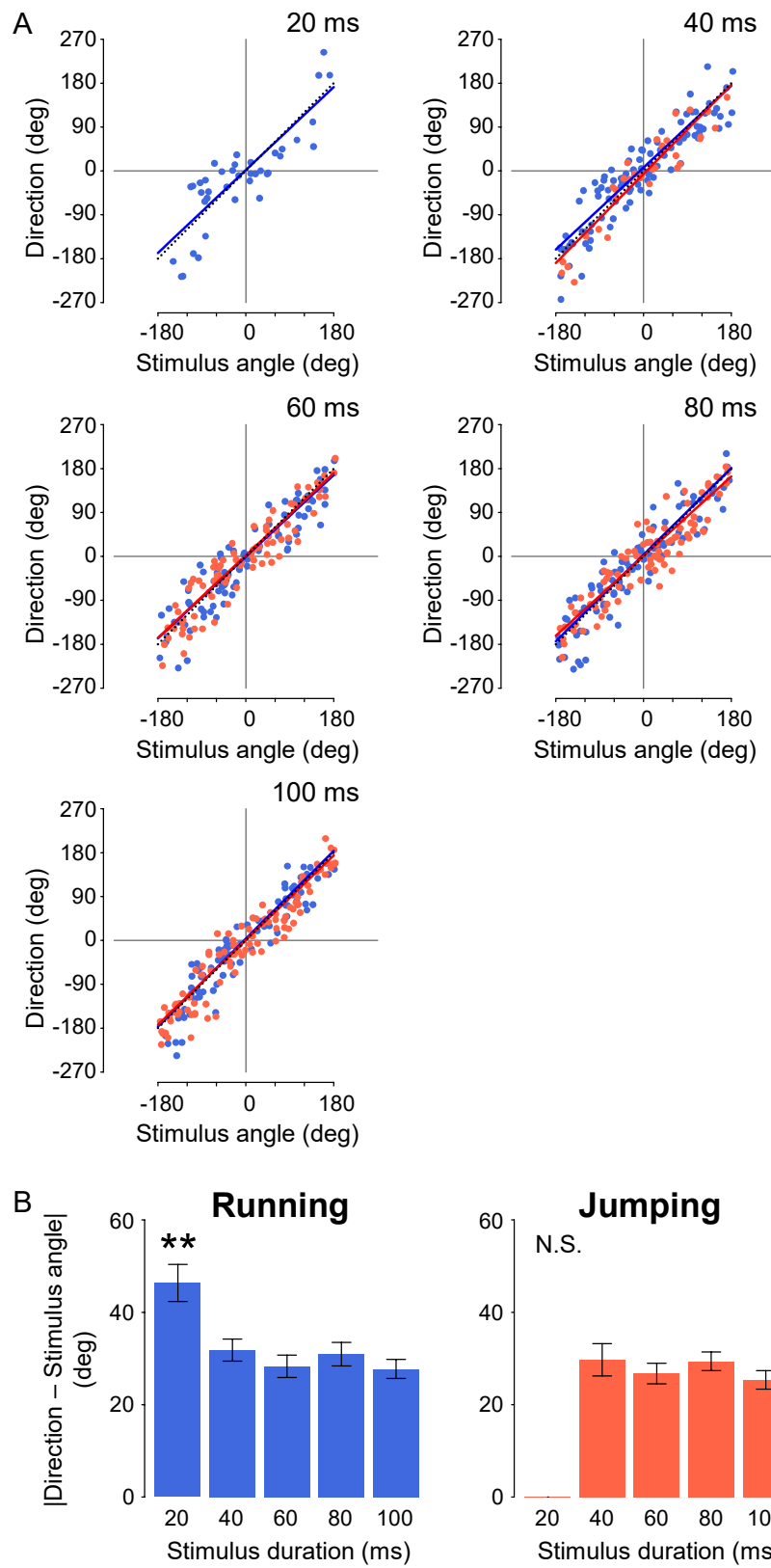


Figure 2-8. Effects of stimulus duration on control of movement direction in running and jumping. (A) Relationships between the movement direction and stimulus angle in running (blue) and jumping (red) in response to the stimuli of 20, 40, 60, 80, and 100 ms durations. No jumping was elicited by the stimulus of 20 ms duration. Colored lines represent linear regression lines for the data of running (blue) or jumping (red) with significant slope. Black dotted line indicates line of $y = x$. **(B)** Mean absolute values of the difference between the movement direction and stimulus angle in running (left) and jumping (right). Error bars indicate $\pm$ S.E.M. $**p < 0.01$, N.S. not significant, likelihood ratio test for LMMs ($\alpha = 0.05$). $n = 10$ animals.

(Figure 2-9)

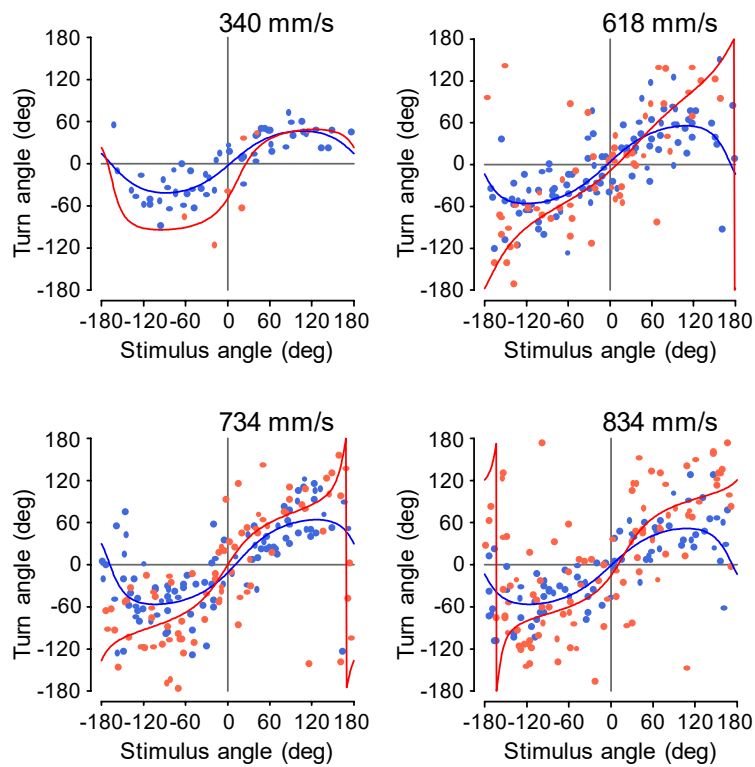


Figure 2-9. Effects of stimulus velocity on control of turn angle in running and jumping. Relationships between the turn angle and stimulus angle in running (blue) and jumping (red) in response to the stimuli of 340, 618, 734, and 834 mm/s velocities. Curves represent circular-circular regression curves.

(Figure 2-10)

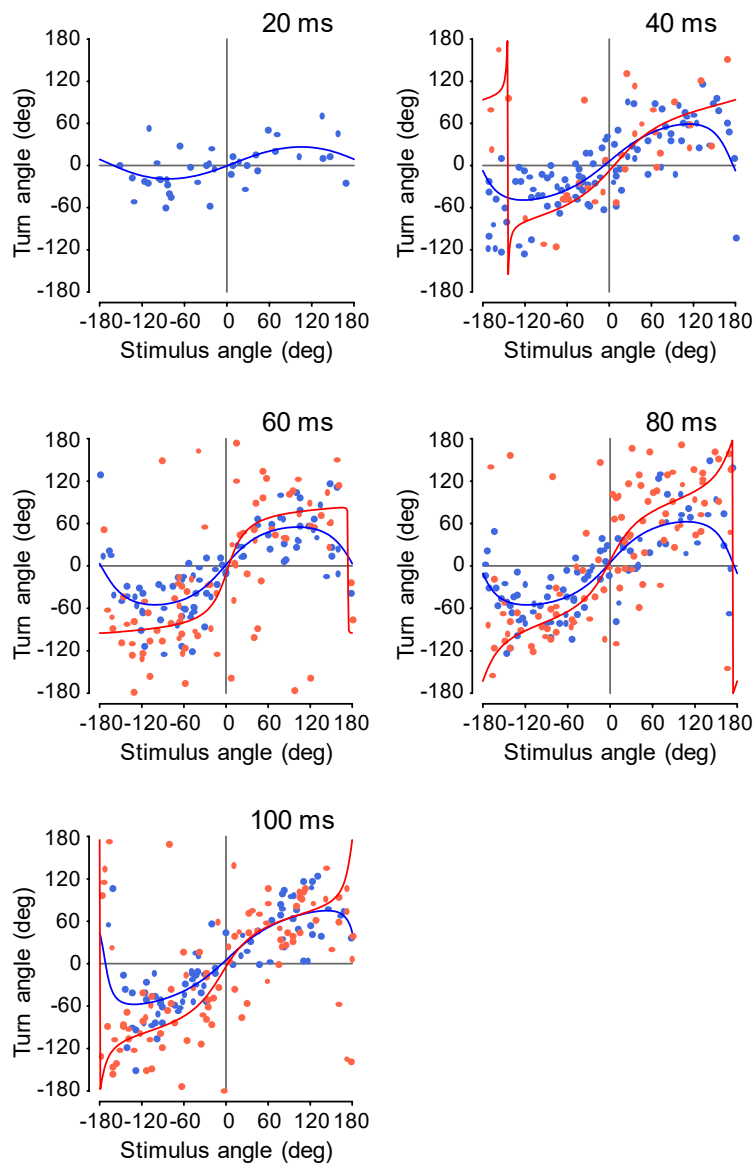


Figure 2-10. Effects of stimulus duration on control of turn angle in running and jumping elicited by the stimulus with different durations. Relationships between the turn angle and stimulus angle in running (blue) and jumping (red) in response to the stimuli of 20, 40, 60, 80, and 100 ms durations. No jumping was elicited by the stimulus of 20-ms duration. Curves represent circular-circular regression curves.

2.6 Tables

Table 2-1. Summary of the result of multiple logistic regression analysis for the effect of stimulus parameters and trial order on the response probability and selected ratio.

Experiment	Parameter	Explanatory variable	Significance of coefficient		
			Coefficient	z value	p value
“angle test”	Selection ratio	Stimulus angle	-0.269	-1.841	0.066
	(Fig. 2-1B)	Trial order	-0.002	-0.083	0.934
“velocity test”	All probability	Stimulus velocity	0.008	13.81	<0.001
	(Fig. 2-1C)	Trial order	-0.015	-0.882	0.378
	Selection ratio	Stimulus velocity	0.004	5.896	<0.001
	(Fig. 2-1D)	Trial order	0.003	0.185	0.853
“duration test”	All probability	Stimulus duration	0.072	14.57	<0.001
	(Fig. 2-1D)	Trial order	-0.014	-0.865	0.387
	Selection ratio	Stimulus duration	0.023	6.722	<0.001
	(Fig. 2-1F)	Trial order	0.006	0.473	0.636

Table 2-2. Summary of the number of trials in each response in the velocity manipulation experiment.

Stimulus velocity	Running	Jumping	No response
340 mm/s	56	7	137
618 mm/s	101	51	48
734 mm/s	98	81	21
834 mm/s	84	100	16

Table 2-3. Summary of the number of trials in each response in the duration manipulation experiment.

Stimulus duration	Running	Jumping	No response
20 ms	39	0	161
40 ms	101	33	66
60 ms	88	87	25
80 ms	90	97	13
100 ms	85	98	17

Table 2-4. Summary of the linear regression result for the movement direction and stimulus angle in “velocity test” experiment.

Stimulus velocity	Response	Significance of coefficient		
		Estimated slope	t value	p value
340 mm/s	Running	0.938	17.65	<0.001
	Jumping	0.479	1.864	0.121
618 mm/s	Running	0.868	24.42	<0.001
	Jumping	0.986	21.61	<0.001
734 mm/s	Running	0.968	31.46	<0.001
	Jumping	0.910	26.45	<0.001
834 mm/s	Running	0.909	26.44	<0.001
	Jumping	0.981	32.84	<0.001

Table 2-5. Summary of the linear regression result for the movement direction and stimulus angle in “duration test” experiment.

Stimulus duration	Response	Significance of coefficient		
		Estimated slope	t value	p value
20 ms	Running	0.946	9.727	<0.001
	Jumping	-	-	-
40 ms	Running	0.934	23.49	<0.001
	Jumping	1.015	14.66	<0.001
60 ms	Running	0.930	23.84	<0.001
	Jumping	0.938	25.05	<0.001
80 ms	Running	0.987	23.48	<0.001
	Jumping	0.908	24.81	<0.001
100 ms	Running	1.002	25.63	<0.001
	Jumping	0.965	32.33	<0.001

Chapter 3

Sensory information processing in brain was required for selection of escape responses

3.1 Introduction

For the prey animals to escape from predator's attack successfully, rapid sensorimotor transformation is required, which enables them to exhibit the appropriate and immediate response to the threat signal. Insects have a simple neural circuit consisting of small numbers of neurons that mediates quick escape responses and the simplicity is useful to address the neural mechanism (Card, 2012). For instance, the fly takes off with short or long reaction time as escape response to the visual looming stimulus (von Reyn et al., 2014). The different two take-off responses are mediated by a pair of interneurons called giant fiber and have distinct threshold of size of the looming stimulus. Since the short mode response has higher threshold than that for long mode, the short mode is elicited to the fast stimulus in which the stimulus size rapidly reached its threshold before exhibiting the long mode.

In Chapter 2, I found that the action selection of either running or jumping in cricket's wind-elicited escape behavior was changed by stimulus parameters. Furthermore, the crickets regulated the running locomotion in response to the changes in stimulus parameters. These results suggest that the neural basis involving integration of the multiple stimulus parameters mediate choosing and regulating the escape responses in crickets. The stimulus information is considered to be integrated in the brain, the most high-order region of the nervous system.

The brain is considered as the crucial part of the central nervous system to send the descending signals, which command the motor center to perform escape response. In flies and locusts, it has been reported that a pair of descending projection neurons directly mediate the escape behavior. The giant fibers in flies activate the motor neurons to move legs and wings for flight initiation responding to the visual threatening stimulus (Allen et al., 2006; Fotowat et al., 2009; Hammond and O'Shea, 2007; Levine and Tracey, 1973; Trimarchi and Schneiderman, 1995). In locusts, the descending contralateral movement detector neuron (DCMD) triggers jumping by receiving the visual stimulus information from the lobula giant motion detector (LGMD) (Gabbiani et al., 1999; Hatsopoulos et al., 1995; Rind, 1984;) and sending the command to leg motor neurons (Burrows and Rowell, 1973; Fotowat and Gabbiani, 2007; Fotowat et al., 2011; Simmons et al., 2010). Considering these previous reports, the brain would send the critical command via descending interneurons to perform wind-elicited escape behavior in crickets.

Furthermore, the directional locomotion in various behaviors are controlled by descending signals from the brain. How the descending signals are involved in controlling locomotion is addressed in insects (Borgmann and Büschges, 2015; Ritzmann and Büschges, 2007). Recent studies of flies show that particular descending neurons have role to regulate the direction of walking (Bidaye et al., 2014, 2020) or flying (Schnell et al., 2017). The turning response to the mechanosensory stimulus in cockroaches is also controlled by the descending signals from the brain (Mu and Ritzmann, 2008; Ridgel et al., 2007). In crickets' phonotaxis, which is a behavior to localize to a conspecific calling song (Hedwig, 2006; Hommaru et al., 2020), the activities of descending neurons are correlated with walking locomotion (Böhm and Schildberger, 1992; Zorović and Hedwig, 2013). Since the crickets precisely control direction in wind-elicited escape behavior, the

locomotion would be controlled by the descending signals from the brain.

The descending signals from the brain are sent to neurons in thoracic ganglia (TG) including prothoracic- (PTG), mesothoracic- (MsTG) and metathoracic ganglion (MtTG). TG is considered as the motor center in the locomotive behaviors in which the central pattern generating network (CPG) enable the rhythmic motor patterns during walking (Borgmann et al., 2009). In locusts, the thoracic interneurons are reported to coordinate the motor neuron activities directly during jumping or flying (Burrows, 1980, 1992; Burrows and Siegler, 1978; Pearson and Robertson, 1981; Robertson et al., 1982). The descending signals from the brain to TG are suggested to play important role in regulating locomotion parameters such as walking speed and direction (Bidaye et al., 2018). However, the overall picture of the central circuitry that induces and regulates escape behavior remains unclear.

It remains unknown how the ascending/descending communications between brain and TG regulate the wind-elicited escape behavior of crickets. In crickets, several ascending projection neurons, identified as Giant Interneurons (GIs), receive the direct excitatory synaptic inputs from the mechanosensory afferents and have distinct sensitivity to the direction and frequency of surrounding air currents (Miller et al., 1991; Theunissen and Miller, 1991). GIs project long and large axons through the ventral nerve cord (VNC) from the terminal abdominal ganglion (TAG) to the brain (Hirota et al., 1993), suggesting that GIs convey the information about the air currents to the brain. Considering previous reports and the result in Chapters 1 and 2, the brain would be the center of the escape behavior in which the choice of the response type and regulation of locomotion are performed based on the integration of stimulus information. In addition, previous study has shown that surgical interruption of the descending signals from the brain abolishes

the directional control in the escape running to the air-current stimulus (Oe and Ogawa, 2013), supporting the importance of the descending signals from the brain to control escape direction.

On the other hands, GIs also arborize their axon collaterals into TG on the way of main axonal shaft that extend to the cephalic ganglia (Hirota et al., 1993), suggesting synaptic contacts with the thoracic interneurons (Kanou and Shimozawa, 1985) as in cockroaches (Casagrand and Ritzmann, 1991; Ritzmann and Camhi, 1978; Ritzmann and Pollack, 1978). The thoracic neuronal circuits directly-activated by GIs may be important for “preparation” independent of the descending signals to set the quick start of leg movements. In the spontaneous walking of crayfish, the preparation of movement is mediated by descending signals from the brain (Kagaya and Takahata, 2010). If the thoracic interneurons directly-activated by GIs could prepare the escape responses in crickets, it might enable the more immediate start of escaping than the system depending on the descending signals for both preparation and initiation of the escape response.

In Chapter 3, I asked the role of the brain and TG in the wind-elicited escape behavior of crickets. The previous studies and results in Chapters 1 and 2 suggested the descending signals from the brain would be crucial for the choice and regulation of escape behavior. To address this, I partly eliminated the descending/ascending signals or only ascending signals by cutting unilateral VNC at two different positions and assessed the effects on the behavioral choice and locomotor parameters. The first was a cutting between 4th abdominal ganglion (4th AG) and TAG, so that both the brain and TG received the one side of ascending signals. In this case, the TG could receive the paired descending signals from the brain like intact crickets. The second was a cutting between subesophageal ganglion (SEG) and PTG, in which the unilateral ascending signals to the

brain was ablated like the 4th AG–TAG cutting condition, but TG received intact ascending signals from TAG. Instead, the descending signals from brain to TG were also ablated on one side. Finally, I examined the descending signals from the brain to elicit running or jumping by performing extracellular recording and monitoring the leg movement simultaneously.

3.2 Materials and Methods

Apparatus of the behavioral experiment, stimulators and video-recording procedures were the same as in Chapter 1.

Animals

I used the wild-type strains of crickets (*Gryllus bimaculatus*, Hokudai WT; Watanabe et al., 2018) which were inbred in my laboratory. Throughout the experiments, 46 laboratory-bred adult male crickets, less than 14 days after adult molting, were used. 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 26-28 °C.

VNC cutting experiment

The VNC was cut unilaterally by using micro-scissors at one of the following two locations; between 4th AG and TAG that was referred to as ‘abdomen-cut experiment’ (Fig. 3-1A, left), or between SEG and PTG referred to as ‘neck-cut experiment’ (Fig. 3-1A, center). For each of these experiment, 10 animals were severed their right nerve and other 10 animals were severed their left nerve, thus in total 20 animals were tested for each experiment.

For behavioral experiments, the air current of 834-mm/s velocity and 200-ms duration was used for all trials, as in Chapter 1. The first session of behavioral experiments was performed under intact condition (“before” in Figures). After that, the crickets were anesthetized by cooling for 20 min and fixed on a silicon platform, ventral side up. For the neck-cut experiment, the epidermal membrane of the ventral surface at the cricket’s neck was incised to expose the VNCs, and right or left VNC was cut. For the

abdomen-cut experiment, a small piece of the abdominal sternite was removed to expose VNCs and one of the VNCs was cut anterior to the TAG. After cutting, the piece of sternite cuticle was replaced on the incision. Treated crickets were left for 45 min or longer within a plastic container (138 mm × 220 mm × 135 mm) and freely accessed food and water. After resting, the second session of the experiment (“after” in Figures) was performed (Fig. 3-1B). Twenty trials were repeatedly performed in each session at inter-trial intervals of 60–90 sec as well as in Chapter 1. So, in total 40 trials were performed for each individual.

For the behavioral analysis, the definition and categorization of escape responses and measurement criteria of locomotor parameters in the initial response were the same as in Chapters 1 and 2. To assess the value difference between before and after cutting in probability of each escape response and the locomotor parameters, the ratio of the difference between the values before and after cutting to their total sum was calculated. In addition to the analysis of the locomotor parameters as in the previous chapters, the absolute value of the difference between the turn angle and movement direction was measured to assess the effects of unilateral VNC cutting on the angular controllability in the locomotion (Figs. 3-8C, 3-9C).

Electrophysiology

The descending signals from the brain evoked by air-current stimulus were recorded extracellularly in head-fixed crickets (Fig. 3-11). Crickets were anesthetized for 1 hr or longer by cooling and their antennae were removed. Then, their head and thorax were fixed dorsal side up on a semi-cylindrical platform made of a 1.5-mL plastic tube with beeswax in order to expose and cut the VNC between SEG and PTG, which was the same

position in the neck-cut experiments. The treated cricket was placed on the paper that could be flexibly bended by the hindlegs movements, so that the crickets could exhibit both running and jumping even in the head-fixed preparation. The descending signals were recorded extracellularly by using a glass suction electrode placed on the distal cut-end of a right VNC. The recorded signals were amplified by a MEG-5200 amplifier (Nihon Kohden), filtered with a bandpass of 150–3 kHz, and digitized at 20 kHz through a Powerlab 4/26 A-D converter (ADInstruments). Simultaneously to the extracellular recording, the leg movements were monitored using the high-speed digital camera that was used for behavioral experiments. Electrophysiological recording was performed more than 1 hour after the anesthetization was terminated. Descending neural activity was recorded from 6 animals.

For electrophysiological recording, the stimuli of 618 or 734 mm/s velocity and 200-ms duration were used. The stimulus was applied from the nozzle that was positioned at 100 mm to the left (intact) side of the animal on the same horizontal plane as the animal. This is because the behavioral experiments revealed that the crickets whose VNC was cut unilaterally at neck even could perform running and jumping in response to the stimulus from their intact side. Ten trials were repeated in each session of the experiment at inter-trial intervals of 60 sec. Four session, each of which consisted of 10 trials, were performed for each individual at the order of 618, 734, 618 and 734 mm/s of the stimulus velocities. Thus, 20 trials were performed for each stimulus velocity and in total 40 trials were for each individual. The crickets were allowed to rest for 5 min between different sessions in the head-fixed position.

In the behavioral analysis for the electrophysiological experiment, the locomotor parameters of the head-fixed cricket could not be measured, so just whether they jumped,

run or did not respond was determined based on its leg movement in response to the stimulus, by checking the video images frame-by-frame. If any legs began to move within 250 ms after the stimulus onset, that was the response definition period, the cricket was considered to respond to the stimulus. The behavioral onset was defined by the time of the first frame when the animals began to move any legs. The behavioral responses were categorized into running or jumping according to movement of legs, by the similar criteria used in Chapter 1. If the both hind legs kicked a paper strongly with synchronized movement during the initial response, it was defined as jumping. If the hind legs moved alternatively without synchronized kicking, the response was defined as running.

Analysis of descending neural activities

To analyze the descending signals recorded extracellularly, firstly, the spikes were detected using WaveClus (Chaure et al., 2018). Recorded data was filtered with a 300-3000 Hz band-pass and spikes were detected according to the threshold that was set to five times of standard deviation of the voltages during the recording. The firing rate was calculated as the value of spike count with each time bin with 10-ms width. To align the time course of firing rate to the behavioral onset, the time of behavioral onset was subtracted from the time of individual spikes, and then the spikes were counted in each 10-ms bin for each trial. The descending signals were compared between running and jumping, in the first spike timing, the total spike counts, the spike counts preceding the behavioral onset, and maximum spike count following the behavioral onset (Fig. 3-14). The first spike timing was defined as the time when the first spike was evoked after the stimulus onset. The total spike counts indicate the number of all evoked spikes, which were measured for two different periods; “Pre time”, the period between the stimulus

onset and behavioral onset, and “Post time”, the period after the behavioral onset. The spike count preceding the behavioral onset was measured as the number of spikes evoked between -10 and 0 ms of the time aligned to the behavioral onset. The maximum spike count was calculated as the peak value of the firing rate for which the spikes were counted in each time bin with 10-ms width at a resolution of 1ms, after the behavioral onset.

Statistical analysis

R programming software (ver. 3.6.2, R Development Core Team) was used for all statistical analyses. The analyzing methods were basically the same as that applied in Chapters 1 and 2.

In addition to the locomotor parameters analyzed in Chapters 1 and 2, the relationship between the movement direction and turn angle by using linear regression analysis as follows:

$$(Turn\ angle) = a(Movement\ direction) + b,$$

where a is the estimated coefficient and b is the intercept.

To examine the effect of VNC cutting on the all response probability (responded or not, for whole trials) and the selection ratio (running or jumping, for all responded trials) to the stimuli from ablated side and intact side, generalized linear mixed model (GLMM, the package ‘glmmML’ ver. 1.1.1 in R) was used (Figs. 3-2, 3-3). Here, the effect of cutting condition (“cut”, before or after the cutting) and stimulus side (“side”, intact or ablated side, which were divided according to the stimulus angle) were included as the explanatory variables. The animal’s IDs were considered as random effects. I supposed the GLMMs as follows:

$$(All\ response\ probability) = \beta_0 + \beta_1(cut) + \beta_2(side) + \beta_3(cut: side) + r,$$

$$(Selection\ ratio) = \beta_0 + \beta_1(cut) + \beta_2(side) + \beta_3(cut: side) + r,$$

where r was the random effect. the significance of the coefficient for each explanatory valuable and their interaction (β_1 , β_2 and β_3) was checked to assess the effect on the all response probability or selection ratio (Tables. 3-3, 3-4).

The effect of VNC cutting on the probability changes and locomotor parameters were examined using LMM (the package ‘nlme’ ver. 3.1-151 in R) was used. For the probability changes (Figs. 3-2G, 3-3G), the effect of the type of response (“response”, running or jumping) and stimulus side (“side”, intact or ablated side) were included as the explanatory variables. The animal’s IDs were considered as random effects. I supposed the LMMs as follows:

$$(Probability\ change) = \beta_0 + \beta_1(response) + \beta_2(side) + \beta_3(response: side) + r,$$

where r was the random effect. the significance of the coefficient for each explanatory valuable and their interaction (β_1 , β_2 and β_3) was checked to assess the effect on the all response probability or selection ratio (Table. 3-5). For the locomotor parameters (Figs. 3-4, 3-5, 3-7C, 3-8C, 3-9C, 3-10C), the effect of the type of response (“response”, running or jumping), cutting condition (“cut”, before or after the cutting) and stimulus side (“side”, intact or ablated side) were included as the explanatory variables. The animal’s IDs were considered as random effects. I supposed the LMMs as follows:

$$(Parameter) = \beta_0 + \beta_1(response) + \beta_2(cut) + \beta_3(side) + \beta_4(response: cut) + \beta_5(response: side) + \beta_6(cut: side) + \beta_7(response: cut: side) + r,$$

where r was the random effect. the significance of the coefficient for each explanatory valuable and their interaction (β_1 , β_2 , β_3 , β_4 , β_5 , β_6 and β_7) was checked to assess the effect on the all response probability or selection ratio (Tables. 3-6, 3-7, 3-9, 3-10). For the differences in locomotor parameters between before and after cutting (Fig. 3-6), the effect

of the type of response (“response”, running or jumping), stimulus side (“side”, intact or ablated side) and cutting location (“location”, at the abdomen or the neck) were included as the explanatory variables. The animal’s IDs were considered as random effects. I supposed the LMMs as follows:

$$\begin{aligned} (\text{Difference in parameter}) = & \beta_0 + \beta_1(\text{response}) + \beta_2(\text{side}) + \beta_3(\text{location}) + \\ & \beta_4(\text{response: side}) + \beta_5(\text{response: location}) + \beta_6(\text{side: location}) + \\ & \beta_7(\text{response: side: location}) + r, \end{aligned}$$

where r was the random effect. the significance of the coefficient for each explanatory valuable and their interaction ($\beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6$ and β_7) was checked to assess the effect on the all response probability or selection ratio (Table. 3-8).

In the analysis of descending signals, the sample size between the running and jumping in 6 individuals (Table. 3-3). Thus, LMM (the package ‘lme4’ ver. 1.1-23 in R) was adopted to compare the characteristics of the evoked descending spikes between running and jumping. The animal’s IDs were considered as random effects and I supposed the LMMs with explanatory variable of response types, which was jumping or running, as follows:

$$(\text{Parameter in spiking response}) = \beta_0 + \beta_1(\text{response type}) + r,$$

where r was the random effect. The significance of the effect of response types on the parameters in the spiking response was tested by comparing the LMMs with explanatory variable of stimulus velocity or duration and without one by the likelihood ratio test.

3.3 Results

Ablation of the ascending sensory signals diminished jumping response.

To examine the contribution of the brain and TG to the behavioral choice and regulation of locomotion in the wind-elicited escape behavior of crickets, I partly ablated the ascending/descending signals by cutting the VNC. The bilateral severing of VNCs between SEG and PTG (Fig. 3-1A, right) completely abolished escape response to the air current (Fig. 3-1C), meaning that interaction between the brain and TG was essential for the escape behavior. Then, the VNC was unilaterally severed at two different locations (Fig. 3-1A, left and center) and its effect on the action selection were tested.

First, the one side of VNC was cut between the 4th AG and TAG to ablate half of ascending signals to both TG and brain with keeping bilateral descending signals from the brain (abdomen-cut experiment, Figs. 3-1A, left, 3-2). After the cutting of VNC, the jumping was prevented or greatly reduced for the stimulus from any directions, but the running was abolished for the stimulus from the ablated side. The escape behavior in response to the stimulus from the ablated side was drastically impaired, whereas the probability of only jumping was reduced for the intact side (Fig. 3-2A-G, Table. 3-1). In addition, the jumping probability was more decreased by the cutting than the running probability (Fig. 3-2G, Table. 3-5), resulting in that the ratio of jumping in all responses was reduced (Fig. 3-2H, Table. 3-3). These results revealed that the escape responses to the stimulus from lateral side would be induced by ascending sensory signals via the VNC ipsilateral to the stimulation side and that the jumping response required bilateral ascending signals regardless of the stimulus angle.

Next, in order to ablate partly the descending signals from the brain to TG, I unilaterally cut VNC between the SEG and PTG (neck-cut experiment, Figs. 3-1A, center,

3-3). In this case, the half of the ascending sensory input to the brain was also ablated but that to TGs was intact. The one side of VNC cutting at the neck abolished only jumping in response to the stimulus from the ablated side of the crickets. In similar to the results in the abdomen-cut experiment, the response probability for the stimulus were more largely reduced for the ablated side than the intact side (Fig. 3-3A-D, Tables. 3-2 and 3-4). Differently from the results of the abdomen-cut experiment, only the probability of jumping in response to the stimulus from the ablated side was significantly reduced, and there were little effects of cutting on the running probability (Fig. 3-3E-H, Tables. 3-4 and 3-5). As a result, the ratio of jumping in all response decreased only for the ablation side (Fig. 3-3H, Table. 3-4). These results suggested that bilateral ascending signals to the brain was required to exhibit jumping and that the running could be performed by even just unilateral descending signals from the brain if the ascending sensory inputs were reached to the TG bilaterally.

The effects of VNC cutting on locomotor parameters

To test the roles of the brain and TG in regulating the locomotion in the escape responses, I firstly assessed the effects of the VNC cutting at different locations on the metric parameters including the movement distance, velocity, and reaction time. In abdomen-cut experiment, all of these parameters in both running and jumping were affected by cutting, and the effect was larger for the ablated side than that for the intact side (Fig. 3-4). The VNC cutting at the abdomen reduced the distance and velocity in both running and jumping, and the effect was larger for the ablated side (Fig. 3-4A and B, Table. 3-6). The reaction time both for running and for jumping became longer than that before abdominal VNC cutting (Fig. 3-4C, Table. 3-6). The VNC cutting at the neck also affected the all

three parameters, but the effect was not significantly different between the stimulus side except for the reaction time (Fig. 3-5). The velocity and distance drastically reduced in running and jumping for both stimulation side (Fig. 3-5A and B, Table. 3-7). The reaction time slightly increased only in the responses to the ablated-side stimuli both in running and in jumping (Fig. 3-5C, Table. 3-7).

Although all of the metric locomotor parameters were affected by both of the VNC cutting, the effects were considered to be different between the cutting location. Indeed, comparing the ablation effects between the cutting locations showed that the VNC cutting at the neck reduced the distance and velocity more largely than that at the abdomen (Fig. 3-6A and B, Table. 3-8), whereas the reaction time in the responses to the ablated-side stimuli was more increased by cutting at the abdomen than that at the neck (Fig. 3-6C, Table. 3-8). The locomotor parameters changed seriously by cutting in running rather than jumping, although the effect of response type was not significant. Taken together, the movement distance and velocity were reduced for both cutting locations, but the effects on them were larger by cutting at the neck than that at the abdomen. This indicated that bilateral ascending inputs to the brain would be needed for the high performance of escape responses and that the descending signals from the brain provided the information to regulate locomotion speed and distance. In contrast, the elongation of the reaction time for the ablated-side stimulation was larger in the abdomen-cut than that in the neck-cut experiment. Therefore, the ascending signals to TGs would be the more important factor for the start timing of response than the descending signals from the brain.

Directional control required the sensory information processing in the brain.

I also examined the effect of the VNC ablation on the directional control (Fig. 3-7). The

crickets could control their movement direction accurately in response to the stimulus from the intact side even if the half of ascending signals was ablated. The VNC cutting at the abdomen had little effects on the relationship between the stimulus angle and movement direction, as the linear regression lines was close to $y = x$ line in both running and jumping (linear regression analysis, estimated slope = 1.026, $t = 47.76$, $p < 0.001$; “before”, estimated slope = 1.028, $t = 30.10$, $p < 0.001$; “after”, in Fig. 3-7A, estimated slope = 1.003, $t = 41.80$, $p < 0.001$; “before”, estimated slope = 0.894, $t = 14.40$, $p < 0.001$; “after”, in Fig. 3-7B). The absolute difference between the movement direction and stimulus angle increased especially in the response to the stimulus from ablated side but was little affected in the response to the intact-side stimuli (Fig. 3-7C, Table. 3-9).

The VNC-cutting at neck caused more serious effects on the directional control. After VNC cutting, the crickets moved exclusively forward in both running and jumping (Fig. 3-8A and B). The distribution of plot and the histograms of the responses after cutting (Fig. 3-8A and B, right) show the drastic increase of forward movement (indicated as 0 in the movement direction), although the linear regression lines was close to $y = x$ line (linear regression analysis, estimated slope = 0.897, $t = 36.57$, $p < 0.001$; “before”, estimated slope = 0.858, $t = 23.09$, $p < 0.001$; “after”, in Fig. 3-8A, estimated slope = 1.0215, $t = 38.80$, $p < 0.001$; “before”, estimated slope = 0.846, $t = 8.228$, $p < 0.001$; “after”, in Fig. 3-8B). The backward movement (indicated as ± 180 in the movement direction) was observed in some trials, but the lateral movements were rarely elicited in both running and jumping after VNC cutting. This changes in the movement direction was also observed as the increase of the absolute difference between the direction and stimulus angle in the responses not only to the ablated-side stimuli but also to the intact side (Fig. 3-8C, Table. 3-9). Little effects of the cutting for the jumping responses to the

ablated-side stimuli would be due to that the jumping was solely elicited only by the stimulus from behind. Thus, the different effect was observed in the abdomen-cut and neck-cut experiment; crickets could move only to forward or backward and not to the lateral side after unilateral cutting of VNC at the neck, while they could control the direction after cutting at the abdomen. These results suggest the descending signals from brain to TGs would provide motor information for the directional control in the escape behaviors.

Here, I found the linear relationships between the movement direction and turn angle in the intact crickets (linear regression analysis, estimated slope = 0.598, $t = 16.68$, $p < 0.001$; “before”, in Fig. 3-9A, estimated slope = 0.395, $t = 6.373$, $p < 0.001$; “before”, in Fig. 3-9B, estimated slope = 0.557, $t = 15.77$, $p < 0.001$; “before”, in Fig. 3-10A, estimated slope = 0.440, $t = 7.462$, $p < 0.001$; “before”, in Fig. 3-10B); the crickets turned so that their body axis oriented to the movement direction at the end of the response. Thus, I checked whether this control of the turn angle against the movement direction was affected by the VNC cutting.

The VNC cutting at both locations abolished the relationships between the movement direction and turn angle (linear regression analysis, estimated slope = 0.038, $t = 1.318$, $p = 0.189$; “after”, in Fig. 3-9A, estimated slope = 0.028, $t = 0.274$, $p = 0.785$; “after”, in Fig. 3-9B, estimated slope = 0.006, $t = 0.342$, $p = 0.733$; “after”, in Fig. 3-10A, estimated slope = 0.046, $t = 0.551$, $p = 0.583$; “after”, in Fig. 3-10B). In the abdomen-cut experiment, the turn angle was still controlled for the movement toward to the intact side (Fig. 3-9A and B, right, $> 0^\circ$ of the movement direction). However, when the crickets moved toward the ablated side, most of the turn angle was negative, meaning that they moved to the opposite side from the stimulus source (intact side) but turned their body

axis toward the stimulus source in the responses to the stimulus from ablated side. This effect may be due to unbalance of left and right ascending signals. This effect on the control of the turning movement was also indicated as large increase of the difference between the turn angle and movement direction in the ablated side (Fig. 3-9C, Table. 10). In contrast, the turn angle in the neck-cut experiment were close to 0° (Fig. 3-10A and B), meaning that the crickets were not turning in any responses. Unlike the result of abdomen-cut experiment, the drastic increase in the difference between the turn angle and direction was not observed in the neck-cut experiment (Fig. 3-10C, Table. 3-10). This may be because that most of plots was also concentrated around 0° of the movement direction (Figs. 3-8, 3-10). These results suggested that the bilateral descending signals from brain was also necessary for control of turning movement.

Descending signals from the brain in running and jumping.

The VNC-cutting experiments indicated that the action selection of either running or jumping required the sensory information processing in the brain. Then, I electrophysiologically examined the descending signals leading the running or jumping, which were simultaneously monitored with a high-speed camera (Fig. 3-11). The firing rate of the descending signals were persistently higher after the peak for jumping than that for running, but there seemed to be no clear difference in the rising phase just after the stimulus onset between running and jumping (Fig. 3-12). However, when the spike timing was aligned with the behavioral onset in each response, the firing rate began to increase slightly earlier for running than for jumping (Fig. 3-13).

Then, I assessed this temporal difference in elevation of the firing rate quantitatively and found that the descending activity preceding jumping was quickly

elevated immediately before the behavioral onset compared to that preceding running (Fig. 3-14). There was not significant difference in the time for first spikes that was aligned with the stimulus onset between running and jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 2.365$, $p = 0.124$; left, in Fig. 3-14A), but those aligned with the behavioral onset was significantly earlier for running than for jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 18.55$, $p < 0.001$; right, in Fig. 3-14A), which was consistent across all recorded individuals. This meant that the jumping was started more quickly following the descending spikes than the running. The total number of spikes evoked during the period from the stimulus onset to the behavioral onset was greater in running than in jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 9.526$, $p = 0.002$; left, in Fig. 3-14B). In contrast, the spike counts for 10 ms just before the behavioral onset was more in jumping than in running (likelihood ratio test for LMMs, $X^2_{(1)} = 11.94$, $p < 0.001$; Fig. 3-14C). In addition, the descending activity during the response was higher for jumping than that for running. The total number of spikes (likelihood ratio test for LMMs, $X^2_{(1)} = 66.05$, $p < 0.001$; right, in Fig. 3-14B) and in the peak value of the firing rate between running and jumping (likelihood ratio test for LMMs, $X^2_{(1)} = 4.087$, $p = 0.043$; Fig. 3-14D) were significantly greater during jumping than during running.

3.4 Discussion

In this Chapter, I examined the roles of the brain and TG in behavioral choice and locomotion regulation in the wind-elicited escape of cricket. Firstly, bilateral VNC cutting at neck completely diminished the escape behavior including both running and jumping. Thus, neural interaction between the brain and TG was essential for the escape behavior. This result led the question how signal communications either ascending or descending signals into the brain and TG contribute to the wind-elicited escape behavior of crickets. To address this, I ablated the ascending or descending signals partly by cutting the half of VNC at the abdomen or neck and tested the effect on the escape behavior was tested.

The half ablation of the ascending inputs to brain and TG by abdominal cutting reduced response probability of both running and jumping, whereas the half ablation of ascending and descending signals to/from the brain by cervical cutting significantly reduced jumping but not running. This result meant that running required bilateral ascending inputs to TG. Meanwhile, the decrease of the jumping by VNC-cutting at both sites meant that bilateral ascending signals to the brain were necessary for jumping. Therefore, bilateral ascending sensory signals were crucial to induce the escape responses, but the essential neural circuits to process these signals might be located in different ganglia between running and jumping.

The VNC cutting at the abdomen and the neck affected the locomotor performance in escape responses, but there was some difference in the cutting effect. The cutting at the neck had more serious impacts on the distance and velocity of the escape responses than that at the abdomen, meaning that ablation of the descending signals from the brain more largely disrupted these parameters. In terms of directional control in the escape responses,

the movement direction and turn angle were less correlated with the angle of the stimulus from ablated side but were unaffected for the intact-side stimulation after the VNC cutting at abdomen. The VNC cutting at the neck, however, caused exclusively the forward movement without turning regardless of the stimulus angle. These observations suggested that the bilateral descending neural signals from brain to TG would be necessary to regulate the movements in the escape behavior. Interestingly, unlike these locomotor parameters, the reaction time became longer by cutting at the abdomen than that at the neck. Thus, bilateral ascending inputs to TG would be important to trigger the escape response.

Finally, electrophysiological recording of the descending spikes from the brain indicated that the descending activity increased more quickly and more immediately before jumping than before running. Once the response was started, the number of spikes was sustained at higher level in jumping than in running. It was demonstrated that the brain sent the descending signals differently to exhibit running or jumping as the result of the selection of the escape response.

Brain function in the behavioral choice and locomotion regulation in escape behavior

The whole results of VNC-cutting experiments at different locations indicated that the sensorimotor processing in the brain played crucial roles in the behavioral choice and regulating locomotion also for the escape behavior that requires fast movement to survive. The result suggesting that the brain implements the regulation of escape locomotion is consistent with the previous study in which the VNC-cutting experiments was conducted on the treadmill (Oe and Ogawa, 2013). In my result, the cervical VNC-cutting drastically affected not only directional control but also the distance and velocity especially in

running. This suggests that the sensory processing in the brain and the descending signals providing the processed results are essential for the crickets to run farther and faster in the escape behavior. As shown in Chapter 2, the movement velocity and distance were flexibly altered according to the stimulus parameters such as airflow speed, duration, and orientation in running (Figs. 2-2, 2-4, 2-6). The neural circuit in the brain would process and integrate these stimulus parameters to regulate the escape running.

Roles of local connection between GIs and thoracic interneurons

In addition to the critical role of the brain in mediating wind-elicited escape behavior, my result indicated that TG was also involved in some aspects of the escape behavior. Difference in the effect of VNC-ablation between the abdomen- and neck-cutting on running probability showed that the bilateral ascending signals to TG was enough to exhibit running even without half of the descending signals from the brain. Furthermore, the reaction time of running became longer by VNC cutting at the abdomen rather than by that at the neck. Therefore, the synaptic connection between the GIs and thoracic interneurons reported previously (Kanou and Shimozawa, 1985) would be also important in the wind-elicited escape behavior. While the high-speed locomotion and precise control of the movement direction in running are executed by the processing in the brain, the thoracic local circuits may contribute to immediate start of running response. Possibly, the local connection between GIs and thoracic interneurons has a role in preparing the legs so that the cricket can start to move immediately after receiving the descending command from the brain.

The descending signals triggering escape response differed for running and jumping.

The results of VNC-cutting experiments suggested that the descending signals from the brain included multiple commands for escaping such as the type of the escape response, the locomotion speed, and movement direction. The result of extracellular recording indicated that descending activity preceding the responses differed between running and jumping even if these responses were elicited by the identical stimulus. Thus, the descending signals from the brain are considered to include the commands of either running or jumping. More descending spikes began to be evoked more earlier before running than before jumping. In contrast, the descending activity increased more sharply before the response onset of jumping. This suggested that the descending command signals to jump was sent more immediately before the cricket started jumping, which was consistent with that the reaction time for jumping was shorter than that for running shown in Chapter 1 (Fig. 1-2).

In several species of animals, the descending neurons of which excitation directly trigger the specific escape response were identified, such as the Mauthner-cell in fish (Kohashi and Oda, 2008), DCMD in locusts (Fotowat and Gabbiani, 2007; Simmons et al., 2010) and the giant fiber in crayfish (Edwards et al., 1999) and flies (Allen et al., 2006; von Reyn et al., 2014). The jumping in the cricket escape behavior is possibly triggered by the command signals provided by such descending neurons preceding the response start. However, one point to be noted is that the cricket's jumping was also precisely controlled in their movement direction, which was demonstrated throughout my experiments. The jumping in the cricket is relatively stereotypic compared with running, but possibly a more controllable response than other animals' escape behaviors such as C-turn in fish and jumping in locusts. Considering these points, triggering the jumping response in the cricket may involve more complicated neural circuits. It is likely that

jumping speed and direction are dominated by multiple descending neurons rather than single command neuron. Unfortunately, it was technically difficult to identify the spike units specific to the jumping or running from the extracellular recording. In crickets, nearly 200 descending brain neurons are contained in one VNC (Staudacher, 1998) and respond to various modality of sensory stimuli including air current (Staudacher, 2001). It remains unknown which descending neurons are responsible to command the running or jumping and to regulate locomotion speed and direction in the escape behavior. Further physiological investigation of the brain neurons by intracellular recording or optical imaging during escape behavior will allow us to identify the descending neurons conveying the command signals.

3.5 Figures

(Figure 3-1)

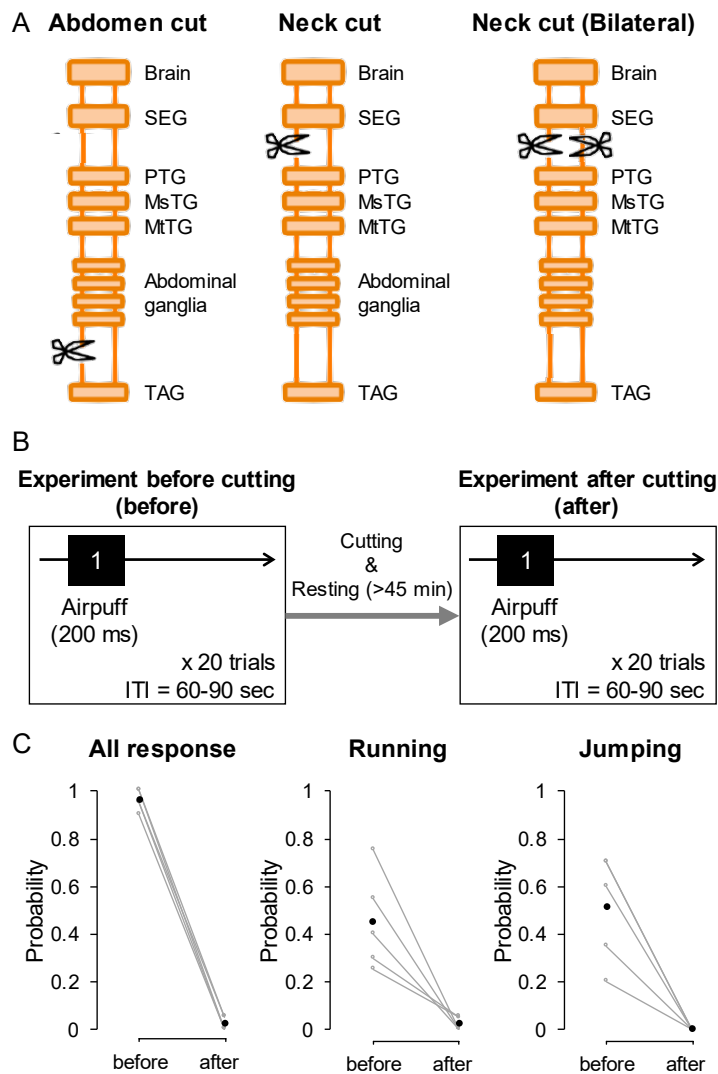


Figure 3-1. VNC-cutting experiments at different locations. (A) Diagram showing the locations of VNC-cutting. Half of the VNC was cut between 4th AG and TAG (left, abdomen-cut experiment) or between SEG and PTG (middle, neck-cut experiment). Both sides of VNC were also cut between SEG and PTG in the bilateral neck-cut experiment (right). **(B)** The temporal scheme in the VNC-cutting experiments. In the same individuals, the behavioral experiments were performed before and after cutting. **(C)** Response probabilities in the bilateral neck-cut experiment.

(Figure 3-2)

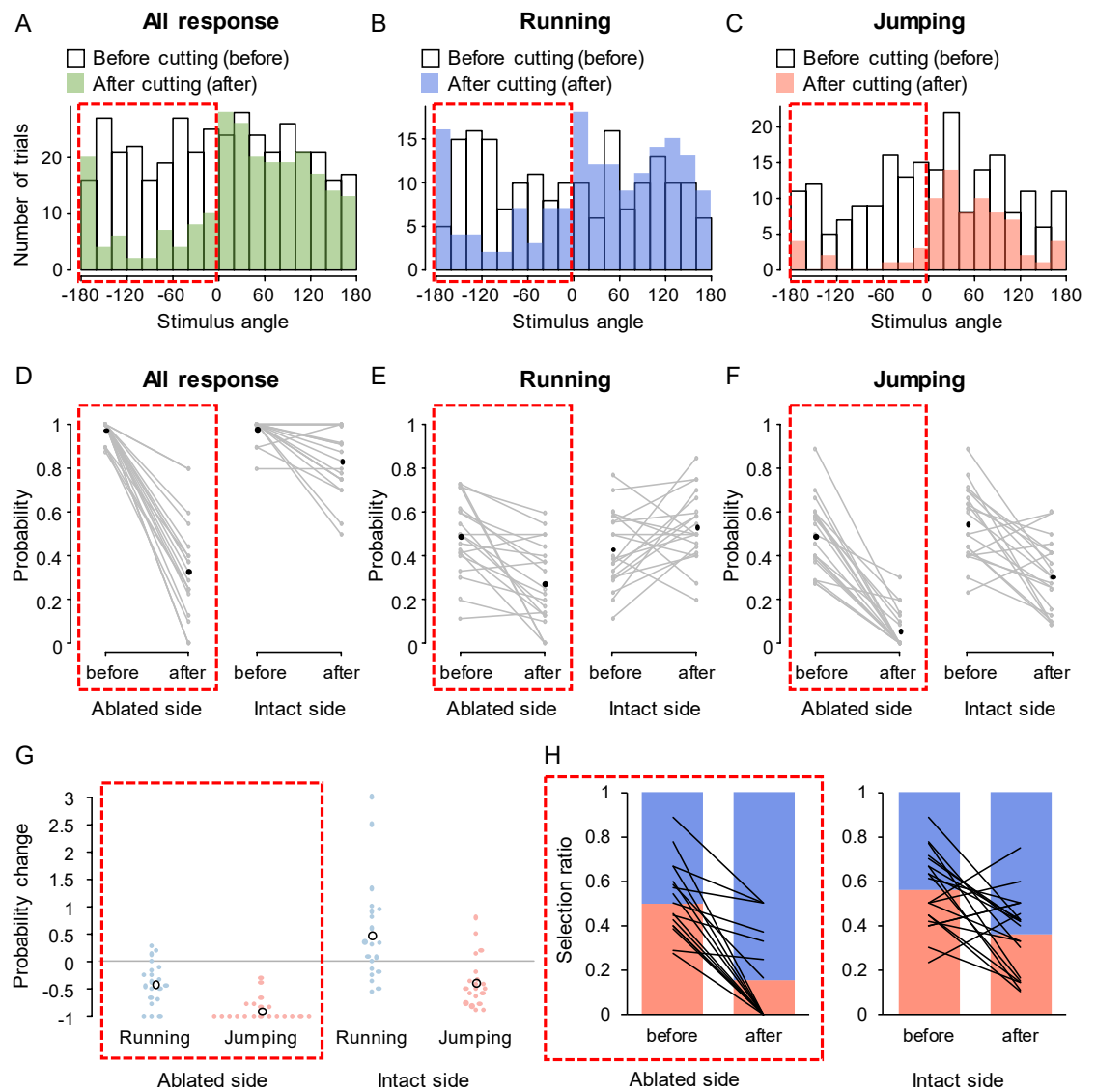


Figure 3-2. Effects of VNC cutting at the abdomen on the action selection. (A–C)

Distributions of the number of responding trials for all responses including running and jumping (A), for running response (B), and for jumping response (C) against the stimulus angle. Histograms represent the data for the range of every 20° of the stimulus angles. Black open bars indicate the data before cutting (before) and colored ones indicate that after cutting (after). Red dotted squares indicate the angular range of the stimuli from ablated side. **(D–E)** Probabilities of all responses (D), running (E) and jumping (F) before and after VNC cutting. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Gray open circles represent the response probabilities for each individual, and black filled circles represent the mean of the probabilities for all individuals. GLMM was adopted in (D) (see Materials and Methods) ($\alpha = 0.05$). **(G)** Changes in the probabilities of running and jumping. Colored filled circles represent the probability changes for each individual, and black open circles represent the mean of the probabilities for all individuals. LMM was adopted (see Materials and Methods) ($\alpha = 0.05$). **(H)** The selection ratio of the running (blue) to jumping (red) for ablated side (left, enclosed by a red dotted square) and for intact side (right). Black lines connect the mean values of the running and jumping responses for each individual. GLMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-3)

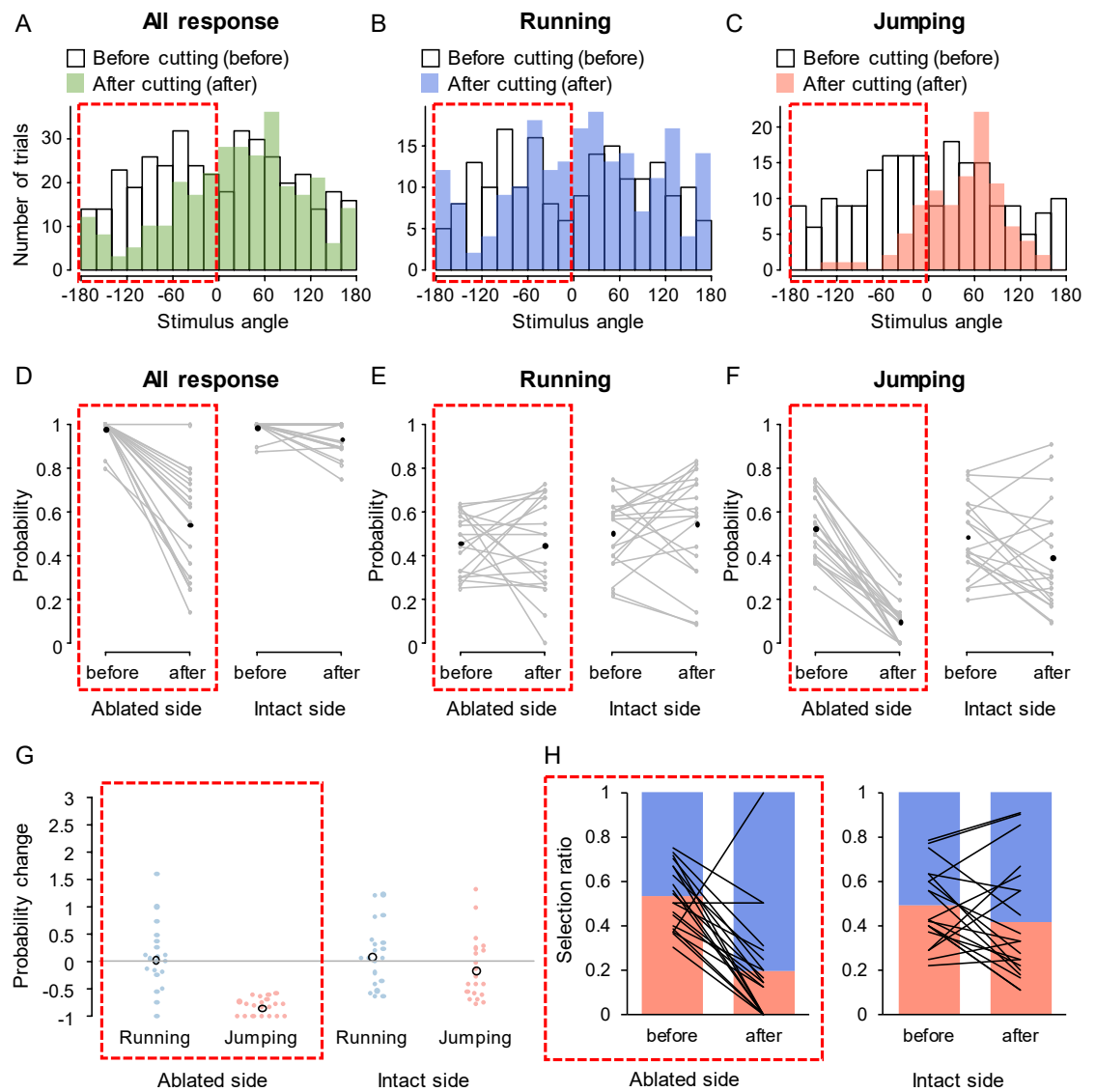


Figure 3-3. Effect of VNC cutting at the neck on the action selection. (A–C)

Distributions of the number of responding trials for all responses including running and jumping (A), for running response (B), and for jumping response (C) against the stimulus angle. Histograms represent the data for the range of every 20° of the stimulus angles. Black open bars indicate the data before cutting (before) and colored ones indicate that after cutting (cut). Red dotted squares indicate the angular range of the stimuli from ablated side. **(D–E)** Probabilities of all responses (D), running (E) and jumping (F) before and after VNC cutting. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Gray open circles represent the response probabilities for each individual, and black filled circles represent the mean of the probabilities for all individuals. GLMM was adopted in (D) ($\alpha = 0.05$). **(G)** Changes in the probabilities of running and jumping. Colored filled circles represent the probability changes for each individual, and black open circles represent the mean of the probabilities for all individuals. LMM was adopted ($\alpha = 0.05$). **(H)** The selection ratio of the running (blue) to jumping (red) for ablated side (left, enclosed by a red dotted square) and for intact side (right). Black lines connect the mean values of the running and jumping responses for each individual. GLMM was adopted ($\alpha = 0.05$). n = 20 animals.

(Figure 3-4)

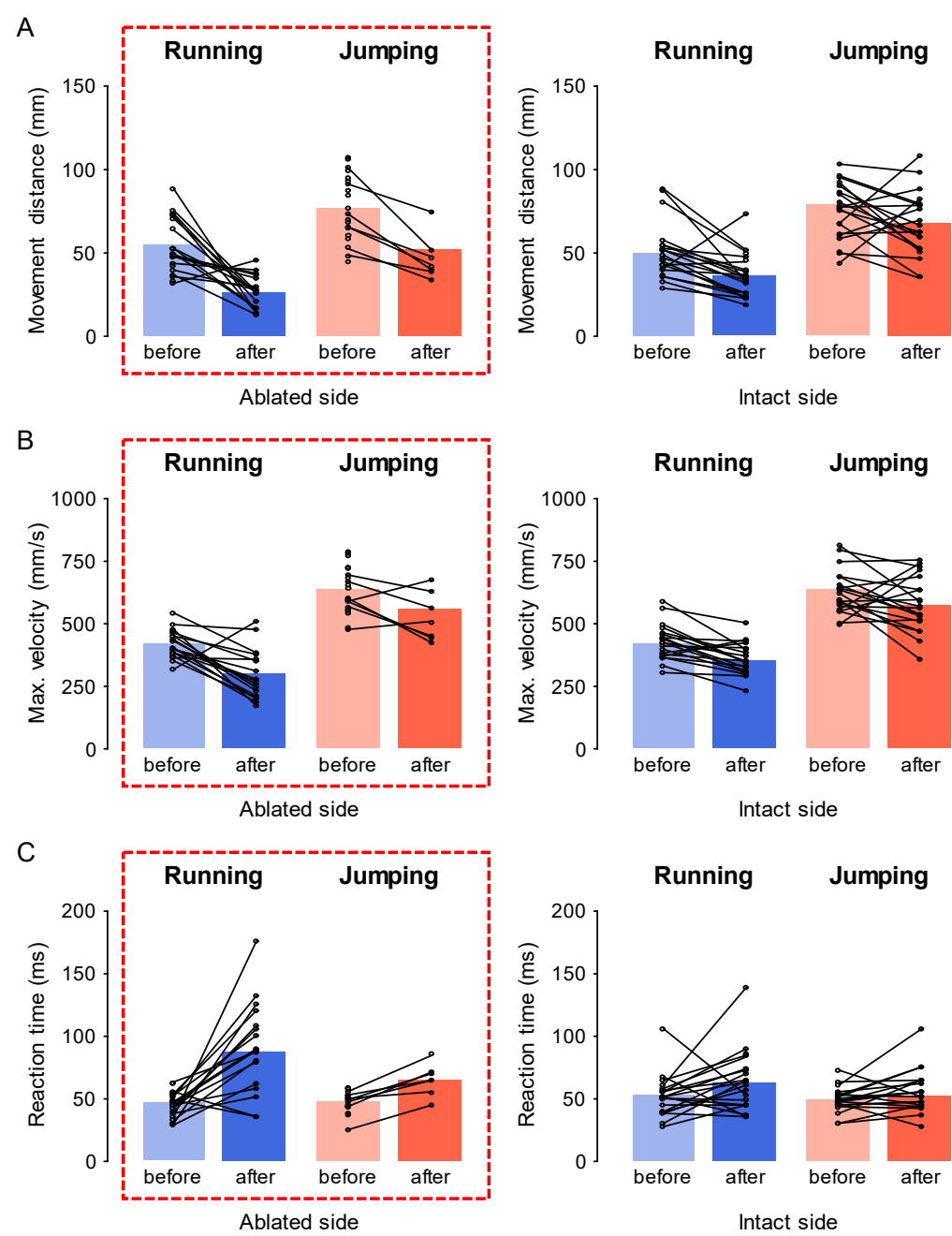


Figure 3-4. Effect of VNC cutting at the abdomen on the metric locomotor parameters in the escape responses. (A–C) Movement distance (A), maximum translational velocity (B), and reaction time (C) before and after VNC cutting. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). n = 20 animals.

(Figure 3-5)

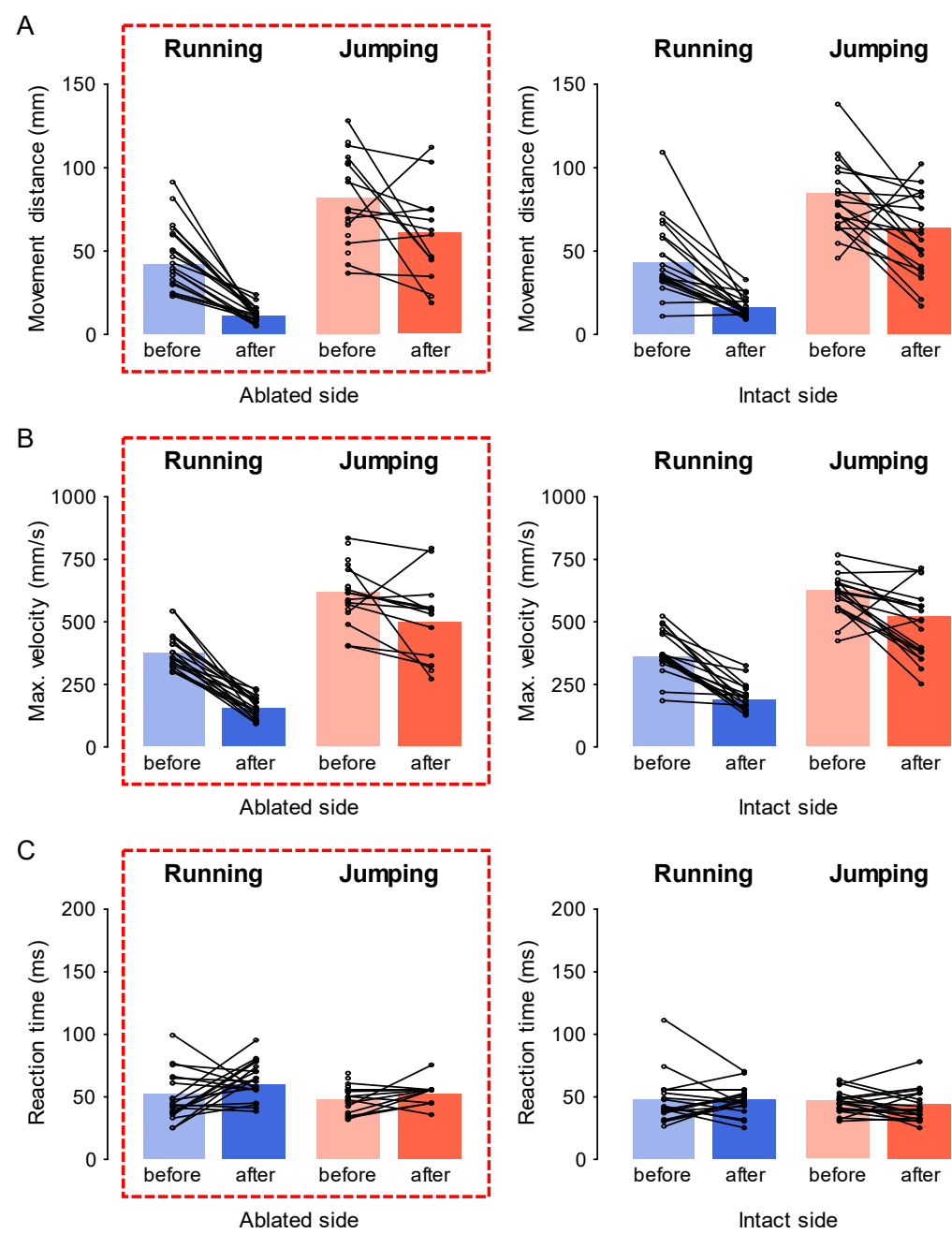


Figure 3-5. Effect of VNC cutting at the neck on the metric locomotor parameters in the escape responses. (A–C) Movement distance (A), maximum translational velocity (B), and reaction time (C) before and after VNC cutting. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-6)

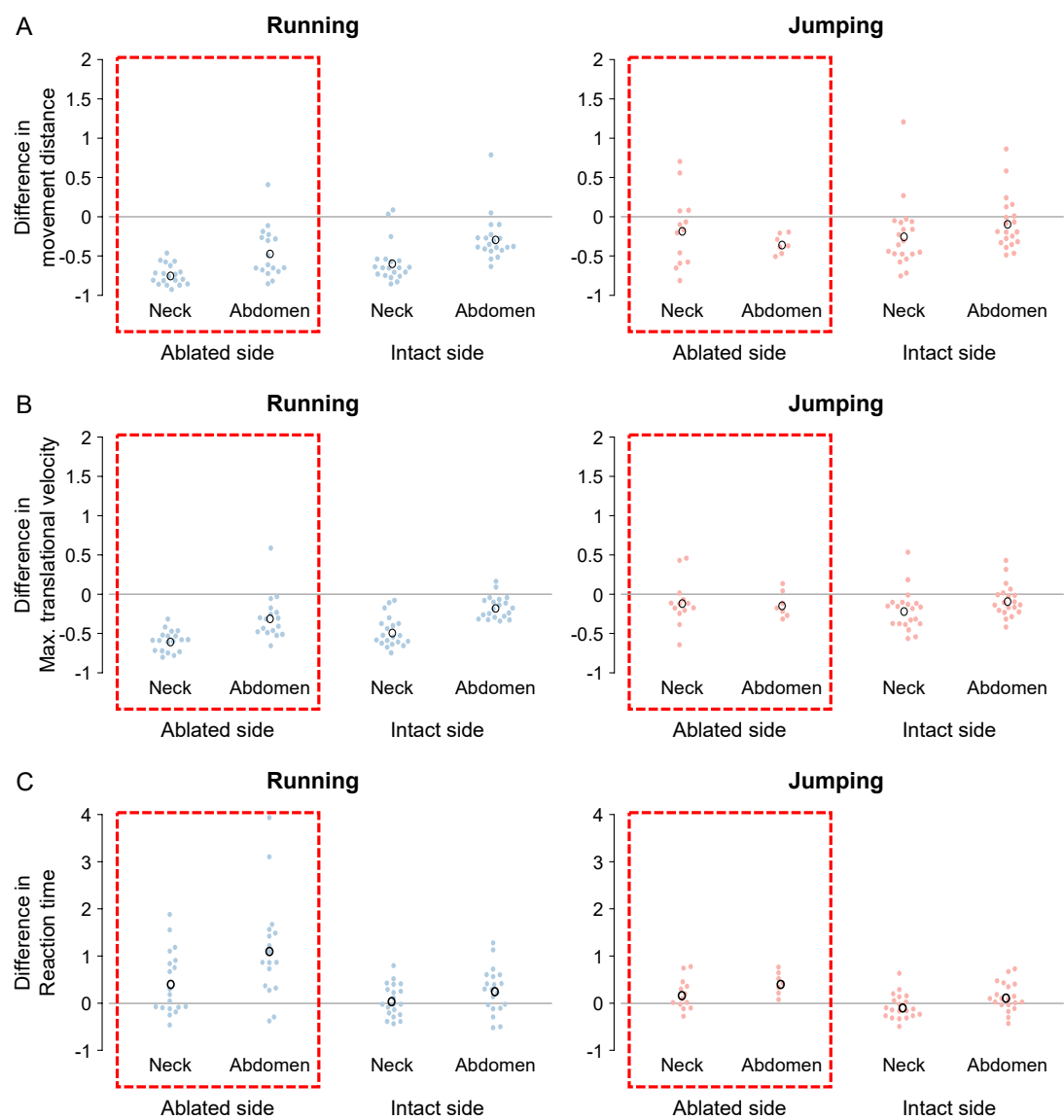


Figure 3-6. Difference in the metric locomotor parameters in the VNC cutting experiment. (A–C) Difference in the movement distance (A), maximum translational velocity (B), and reaction time (C) between before and after VNC cutting. The data are divided to the responses to the stimuli from the ablated side (enclosed by a red dotted square) and from the intact side. Colored filled circles represent the probability changes for each individual, and black open circles represent the mean of the probabilities for all individuals. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-7)

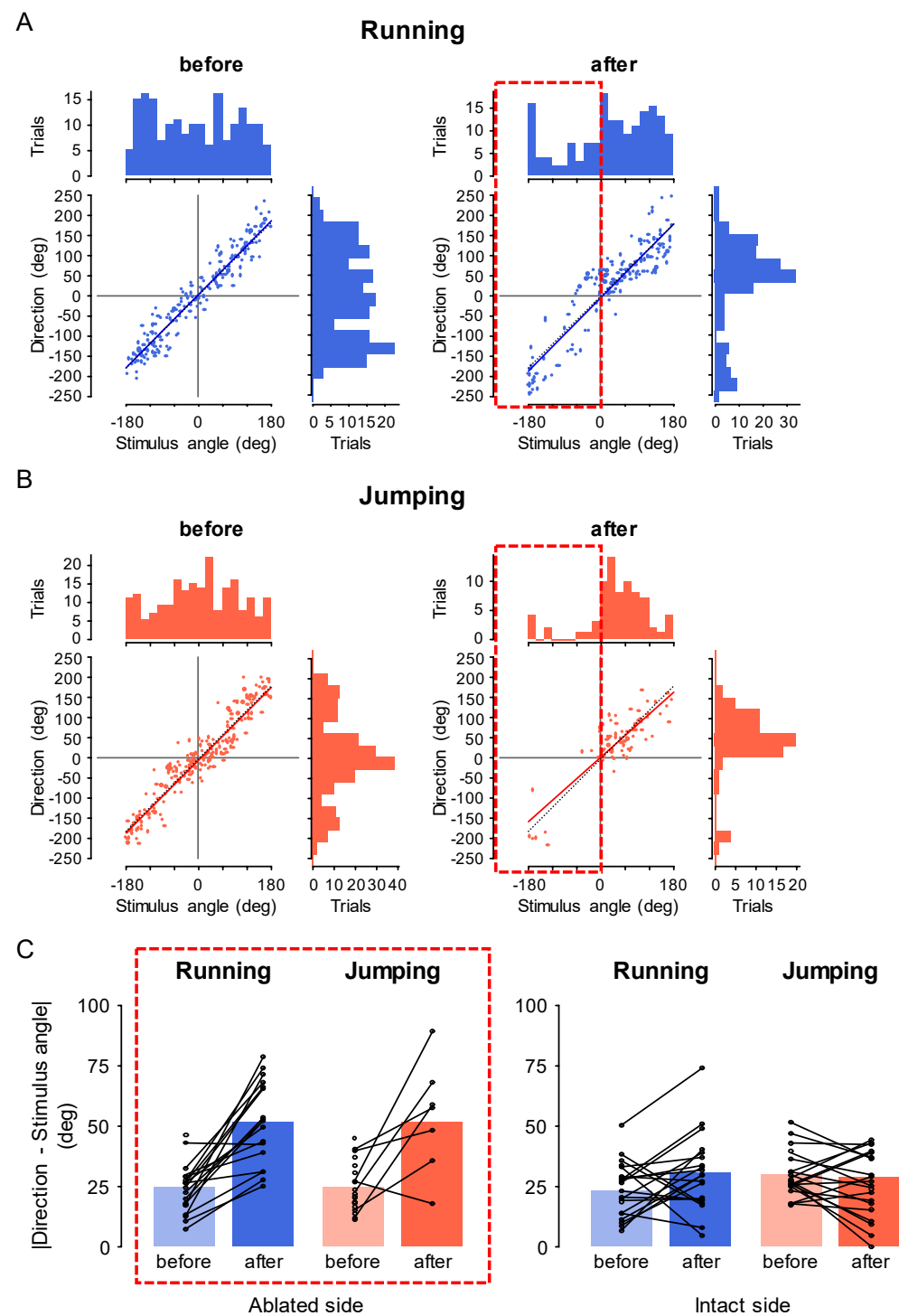


Figure 3-7. Effect of VNC cutting at the abdomen on the movement direction. (A,B)

Relationships between the movement direction and stimulus angle before (left, before) and after cutting (right, after) in running (A) and jumping (B). The colored lines are linear regression lines that were significant and dotted lines represent $y = x$ lines. Histograms show the distributions of the data plotted against stimulus angle (above) or against movement direction (right side). Red dotted square indicates the angular range of the stimuli from ablated side. (C) Effects on the absolute value of the difference between the movement direction and stimulus angle. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-8)

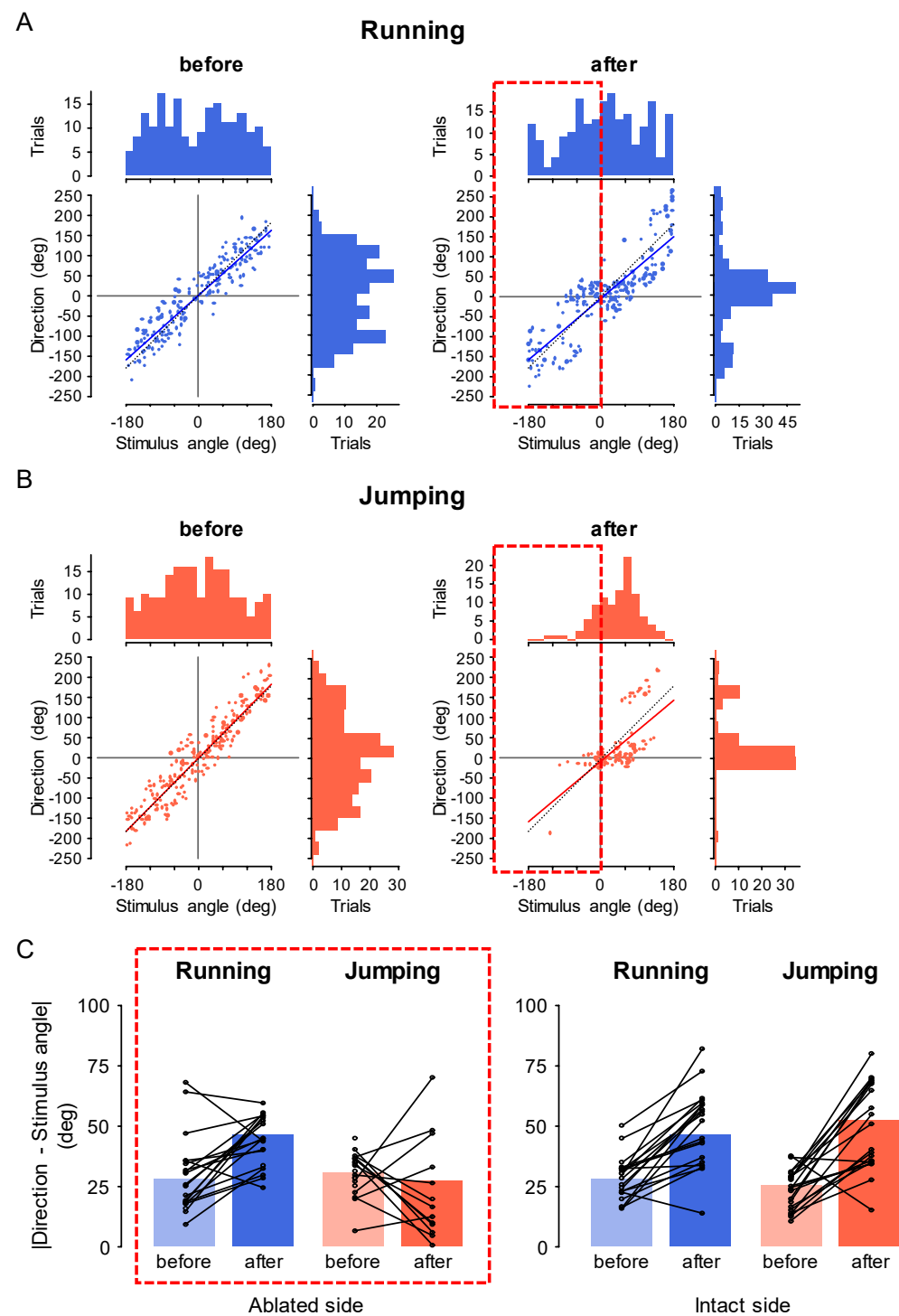


Figure 3-8. Effect of VNC cutting at the neck on the movement direction. (A,B)

Relationships between the movement direction and stimulus angle before cutting (left, before) and after cutting (right, after) in running (A) and jumping (B). The colored lines are linear regression lines that were significant and dotted lines represent $y = x$ lines. Histograms show the distributions of the data plotted against stimulus angle (above) or against movement direction (right side). Red dotted square indicates the angular range of the stimuli from ablated side. (C) Effects on the absolute value of the difference between the movement direction and stimulus angle. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right) in the neck-cut experiment. Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-9)

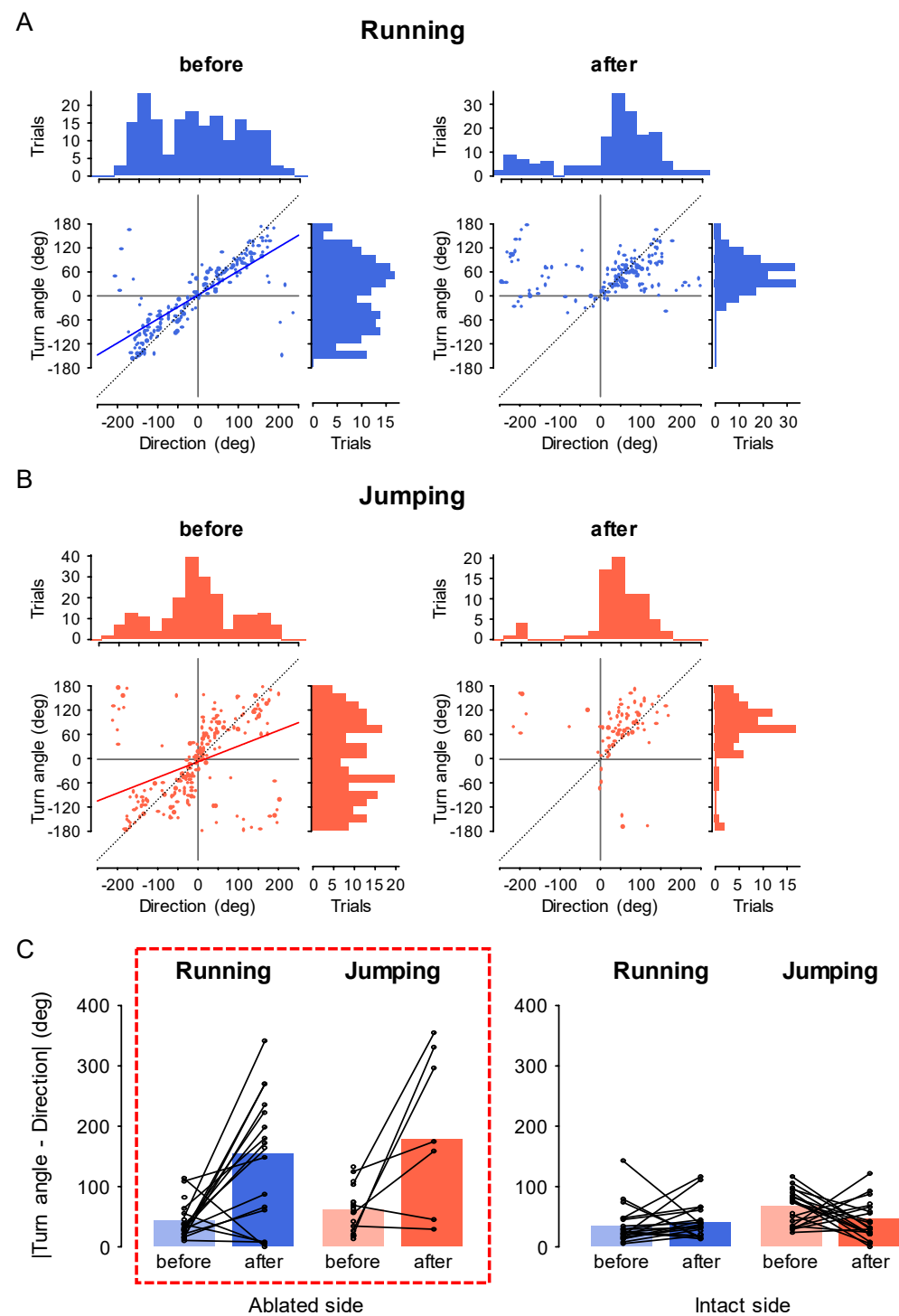


Figure 3-9. Effect of VNC cutting at the abdomen on the turn angle. (A,B)

Relationships between the turn angle and movement direction before cutting (left, before) and after cutting (right, after) in running (A) and jumping (B). Colored lines indicate linear regression lines that were significant. Dotted lines represent $y = x$ lines. Histograms show the distributions of the data plotted against movement direction (above) or against turn angle (right side). (C) Effects on the absolute value of the difference between the turn angle and movement direction. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right). Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-10)

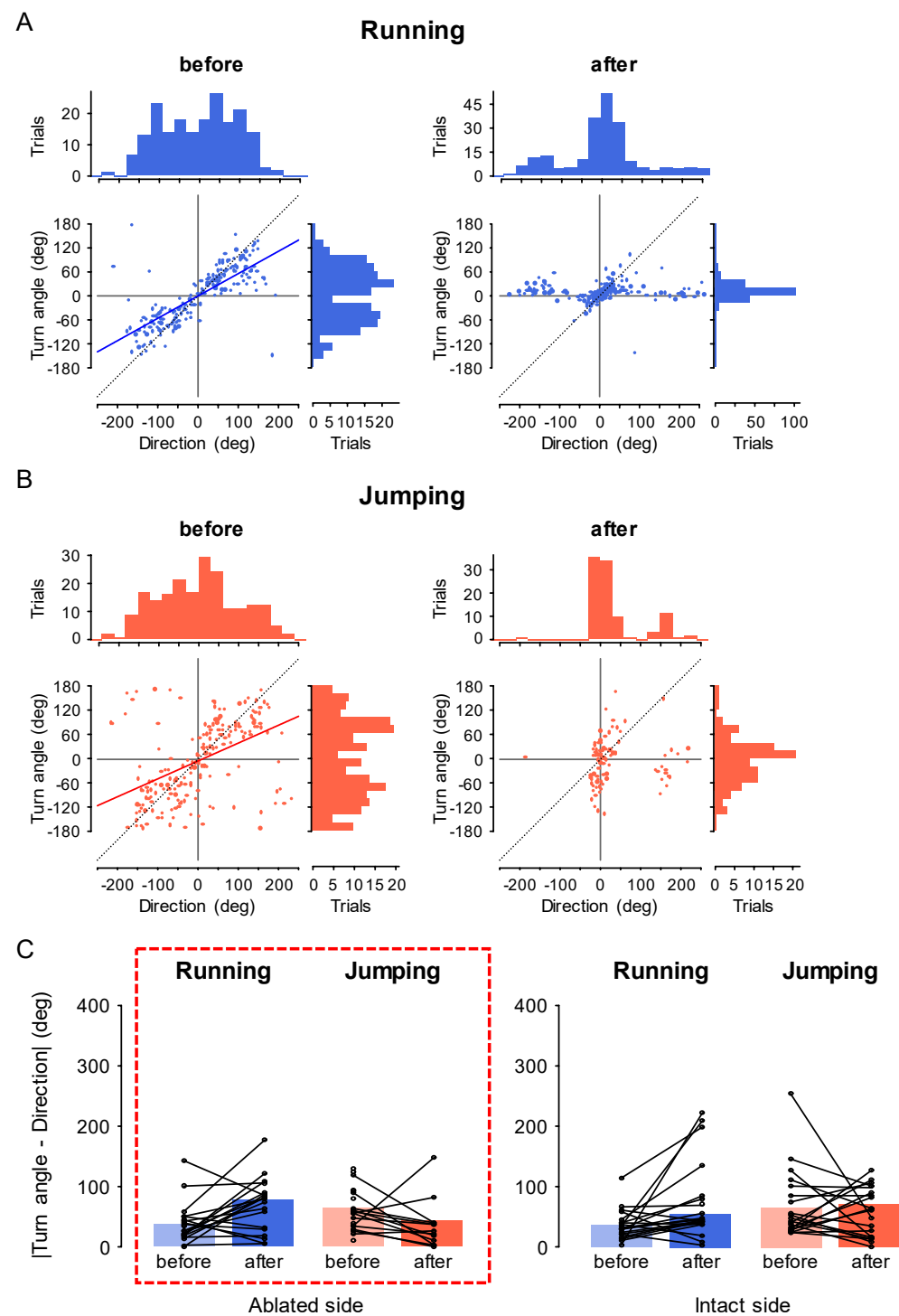


Figure 3-10. Effect of VNC cutting at the neck on the turn angle. (A,B) Relationships between the turn angle and movement direction before cutting (left, before) and after cutting (right, after) in running (A) and jumping (B). Colored lines indicate linear regression lines that were significant. Dotted lines represent $y = x$ lines. Histograms show the distributions of the data plotted against movement direction (above) or against turn angle (right side). **(C)** Effects on the absolute value of the difference between the turn angle and movement direction. The data are divided to the responses to the stimuli from the ablated side (left, enclosed by a red dotted square) and from the intact side (right) in the neck-cut experiment. Blue bars indicate the mean value for all responses in running, and red ones indicate that in jumping. Black open circles connected with lines indicate the mean value for each individual. LMM was adopted ($\alpha = 0.05$). $n = 20$ animals.

(Figure 3-11)

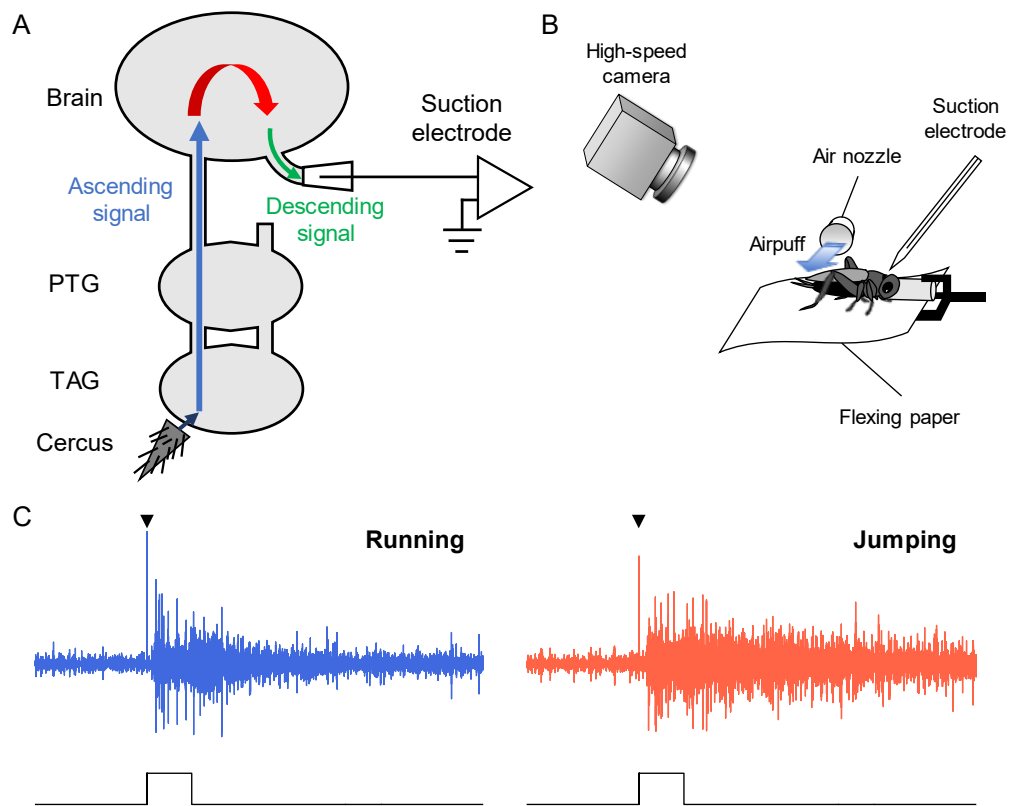


Figure 3-11. Electrophysiological recording of descending activity during the escape behavior. (A) Diagram showing the extracellular recording of the descending activities. Descending spikes was recorded using a suction electrode from distal cut-end of right VNC between SEG and PTG. (B) Experimental apparatus for simultaneous recording of descending neural activities and cricket's movement. A cricket was fixed at its head and thorax with a semi-cylindrical tube and placed on the paper so that it could freely move its legs. Air-current stimulus, of which velocity was 618 or 734 mm/s and duration was 200ms, was applied from a nozzle on contralateral side to the recording (blue arrow). The movement of cricket's legs was monitored with a high-speed digital camera at 120 fps from behind. (C) Typical firing responses of descending neural activities in running (left) and jumping (right), which were recorded from the same individual. Lower traces indicate the air-current stimulus, of which onset was marked by black arrowheads.

(Figure 3-12)

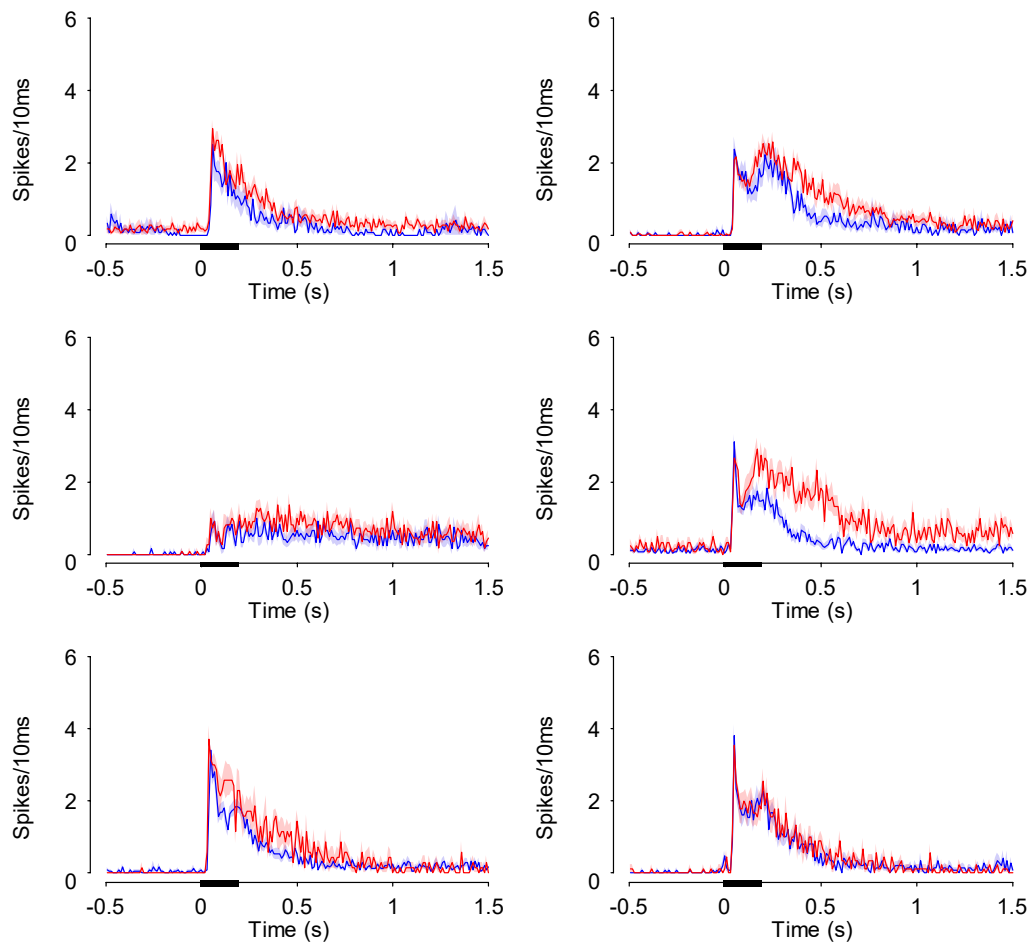


Figure 3-12. Time courses in firing rate of descending spikes in running and jumping.

The lines indicate mean of the number of spikes during 10-ms time bin for running (blue traces) and jumping (red traces) in each individual. Shaded areas represent range of $\pm$ SEM. Black bars indicate the stimulation, of which the start time was indicated as 0 on the time axes. Six panels indicate the data recorded from different individuals.

(Figure 3-13)

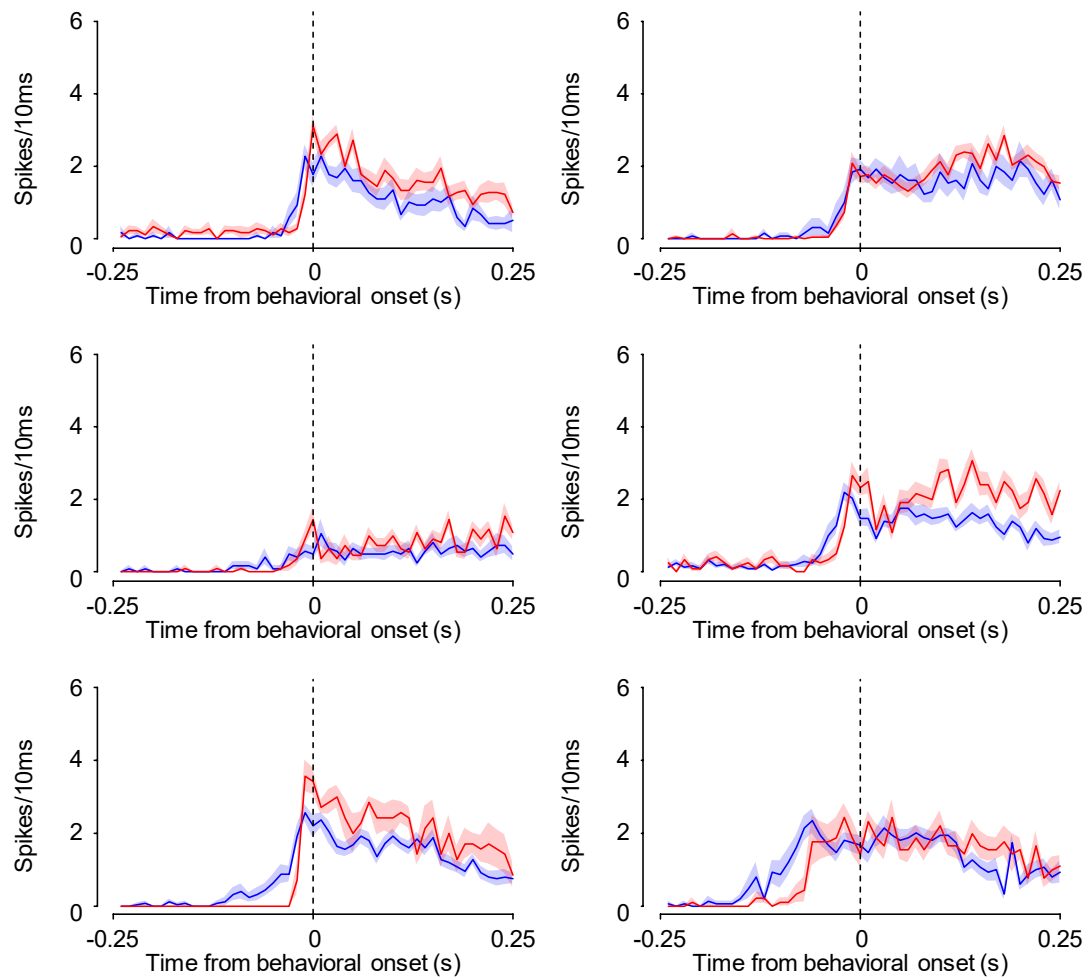


Figure 3-13. Time courses in firing rate of descending spikes in running and jumping aligned with behavioral onset. The lines indicate mean of the number of spikes during 10-ms time bin, which were aligned with the start of leg movement for running (blue traces) and jumping (red traces) in each individual. Shaded areas represent range of $\pm$ SEM. Black dotted lines indicate the behavioral onset, which are also indicated as 0 on the time axes. Six panels indicate the data recorded from different individuals.

(Figure 3-14)

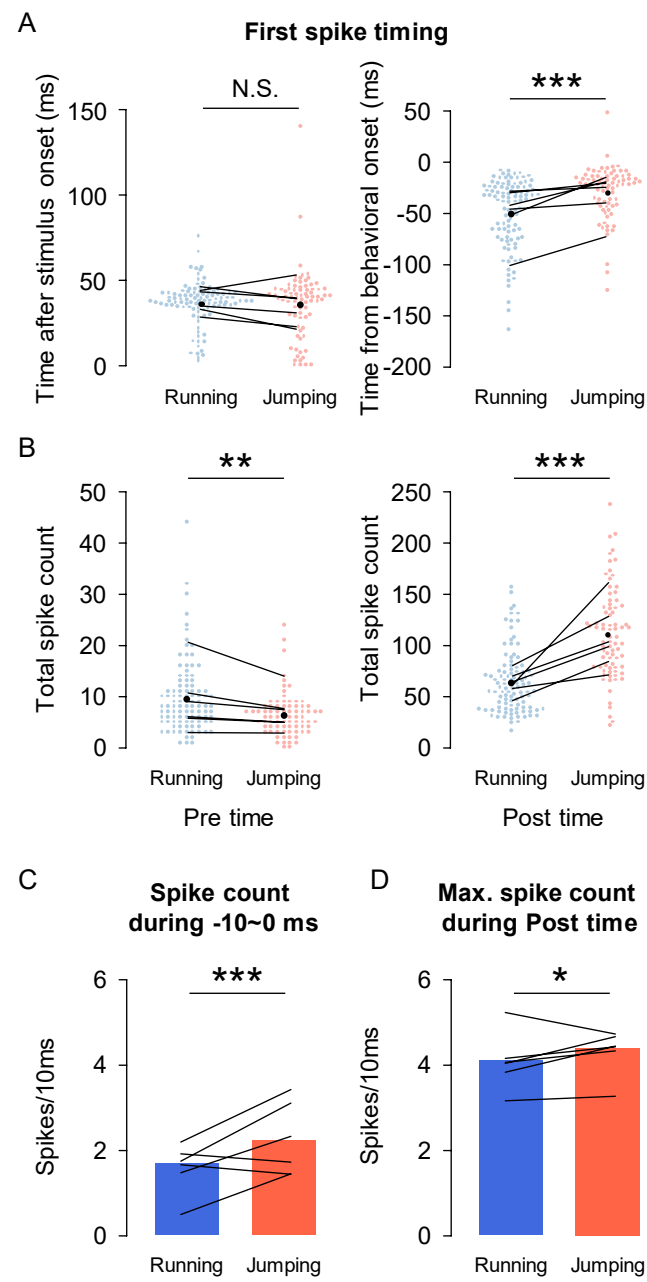


Figure 3-14. Differences in the descending activities between running and jumping.

(A) Time of the first spikes relative to the stimulus onset (left) or to the behavioral onset (right) in running (blue) and jumping (red) responses. (B) Total numbers of spikes evoked before (left) or after (right) the start of running (blue) and jumping (red) movements. (C) The number of spikes evoked during 10 ms just before the start of running (blue) and jumping movement (red). (D) The maximum value of the spike counts for each 10-ms bin after the start of running (blue) and jumping movement (red). Black filled circles in (A), (B) and bars in (C), (D) represent the mean value for all responses. Colored plots in (A) and (B) represent the data for each response. Black lines connect the mean values of the running and jumping responses for each individual. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, likelihood ratio test for LMMs ($\alpha = 0.05$). $n = 6$ animals.

3.6 Tables

Table 3-1. Summary of the number of trials in each response in abdomen-cut experiment.

Condition	Stimulus side	Running	Jumping	No response
Before cutting	Ablated side	97	97	4
	Intact side	88	110	4
After cutting	Ablated side	52	11	126
	Intact side	113	64	34

Table 3-2. Summary of the number of trials in each response in neck-cut experiment.

Condition	Stimulus side	Running	Jumping	No response
Before cutting	Ablated side	93	105	4
	Intact side	98	98	2
After cutting	Ablated side	88	19	84
	Intact side	116	79	14

Table 3-3. Summary of the GLMM result for response probability in abdomen-cut experiment.

Explanatory variable	Significance of coefficients			
	All response probability (Fig. 3-2D)		Selection ratio (Fig. 3-2H)	
	Z value	p value	Z value	p value
cut	-4.315	<0.001	-3.736	<0.001
side	-0.055	0.956	-1.104	0.269
cut:side	-3.371	<0.001	-1.826	0.068

Table 3-4. Summary of the GLMM result for response probability in neck-cut experiment.

Explanatory variable	Significance of coefficients			
	All response probability (Fig. 3-3D)		Selection ratio (Fig. 3-3H)	
	Z value	p value	Z value	p value
cut	-2.596	<0.001	-1.843	0.065
side	-0.883	0.377	0.644	0.520
cut:side	-2.095	0.036	-3.820	<0.001

Table 3-5. Summary of the LMM result for probability change.

Explanatory variable	Significance of coefficients					
	Probability change in abdomen-cut (Fig. 3-2G)			Probability change in neck-cut (Fig. 3-3G)		
	df	t value	p value	df	t value	p value
response	57	-4.933	<0.001	57	-1.536	0.130
side	57	-5.083	<0.001	57	-0.331	0.742
response:side	57	1.576	0.121	57	-2.781	0.007

Table 3-6. Summary of the LMM result for locomotor parameters in abdomen-cut experiment.

Explanatory variable	Significance of coefficients								
	Distance (Fig. 3-4A)			Velocity (Fig. 3-4B)			Reaction time (Fig. 3-4C)		
	df	t	p	df	t	p	df	t	p
response	605	6.841	<0.001	605	12.01	<0.001	605	-0.496	0.620
cut	605	-4.243	<0.001	605	-3.855	<0.001	605	3.105	0.002
side	605	0.924	0.356	605	0.060	0.952	605	-1.619	0.106
response:cut	605	1.039	0.299	605	0.212	0.832	605	-1.406	0.160
response:side	605	-0.610	0.542	605	0.251	0.802	605	0.774	0.439
cut:side	605	-2.508	0.012	605	-2.082	0.038	605	5.688	<0.001
response:cut:side	605	-0.050	0.960	605	0.618	0.537	605	-1.400	0.162

Table 3-7. Summary of the LMM result for locomotor parameters in neck-cut experiment.

Explanatory variable	Significance of coefficients								
	Distance (Fig. 3-5A)			Velocity (Fig. 3-5B)			Reaction time (Fig. 3-5C)		
	df	t	p	df	t	p	df	t	p
response	669	9.555	<0.001	669	13.13	<0.001	669	-0.502	0.616
cut	669	-7.199	<0.001	669	-10.01	<0.001	669	0.399	0.690
side	669	-0.263	0.793	669	0.488	0.626	669	1.220	0.223
response:cut	669	1.438	0.151	669	2.733	0.006	669	-1.503	0.133
response:side	669	0.050	0.960	669	-0.164	0.870	669	-0.410	0.682
cut:side	669	-1.169	0.243	669	-1.819	0.069	669	2.785	0.006
response:cut:side	669	0.327	0.744	669	0.824	0.410	669	0.044	0.965

Table 3-8. Summary of the LMM result for differences in locomotor parameters.

Explanatory variable	Significance of coefficients								
	Distance (Fig. 3-6A)			Velocity (Fig. 3-6B)			Reaction time (Fig. 3-6C)		
	df	t	p	df	t	p	df	t	p
response	109	1.949	0.054	109	1.286	0.201	109	-0.858	0.393
side	109	-1.696	0.093	109	-1.847	0.068	109	4.938	<0.001
location	109	-3.000	0.003	109	-4.584	<0.001	109	-1.291	0.200
response:side	109	-0.478	0.634	109	0.667	0.506	109	-1.738	0.085
response:location	109	0.996	0.322	109	1.922	0.057	109	0.049	0.961
side:location	109	0.170	0.866	109	0.142	0.887	109	-2.061	0.042
response:side: location	109	1.332	0.186	109	0.898	0.371	109	1.018	0.311

Table 3-9. Summary of the LMM result for absolute difference between the movement direction and stimulus angle.

Explanatory variable	Significance of coefficients					
	Direction in abdomen-cut			Direction in neck-cut		
	(Fig. 3-7C)			(Fig. 3-8C)		
	df	t	p	df	t	p
response	605	2.129	0.034	669	-0.932	0.352
cut	605	2.455	0.014	669	6.048	<0.001
side	605	0.369	0.712	669	0.100	0.920
response:cut	605	-1.911	0.057	669	1.825	0.068
response:side	605	-1.593	0.112	669	1.101	0.271
cut:side	605	4.168	<0.001	669	-0.201	0.841
response:cut:side	605	1.055	0.292	669	-3.710	<0.001

Table 3-10. Summary of the LMM result for absolute difference between the turn angle and movement direction.

Explanatory variable	Significance of coefficients					
	Turn angle in abdomen-cut (Fig. 3-9C)			Turn angle in neck-cut (Fig. 3-10C)		
	df	t	p	df	t	p
response	605	2.985	0.003	669	2.684	0.007
cut	605	0.576	0.565	669	1.856	0.064
side	605	0.625	0.532	669	-0.156	0.876
response:cut	605	-1.640	0.102	669	-0.928	0.354
response:side	605	-0.784	0.434	669	-0.105	0.916
cut:side	605	6.146	<0.001	669	1.553	0.121
response:cut:side	605	0.961	0.337	669	-1.933	0.054

Table 3-11. Summary of the trial numbers of each response in extracellular recording.

Individuals	Running	Jumping
ID081901	12	18
ID092002	12	11
ID092302	25	7
ID102302	13	22
ID101504	25	12
ID102302	15	9

General Discussion

Trade-off in motor performance between running and jumping

In Chapter 1, quantitative analysis of the locomotor parameters revealed the locomotion characteristics of running and jumping and what behavioral benefits in their performances were traded off. The cricket could move faster and farther in jumping than in running, indicating that the jumping had advantages in the locomotion speed. On the other hand, the crickets could respond to the additional stimulus more frequently during running than during jumping, thus the running enables the crickets to change their movement more flexibly than the jumping. These results indicated the trade-off between the locomotion speed and behavioral flexibility for the escape responses of the cricket.

Noteworthy results were that the movement direction in jumping was controlled as precisely as in running. So far, in the animals that have multiple escape responses, the faster response was reported to be less controllable in the movement direction than slower response (Bhattacharyya et al., 2017; Card and Dickinson, 2008). However, the crickets jumped even laterally or backwards, which enabled them to move to opposite direction to the stimulus from any direction, unlike locusts (Santer et al., 2005; Simmons et al., 2010). Locusts have long hind legs relative to their body length and escape exclusively by jumping (Tauber and Camhi, 1995). On the other hand, crickets move usually by running and exhibit jumping only for escape behavior. High controllability of jumping direction in the cricket may result from their ecological and kinematic characteristics. Possibly, an exquisite coordination in movements of the two hind legs controls the balance in power and timing of kicking, allowing the crickets to jump laterally or backwards. Further research focusing the kinematics of legs could elucidate the mechanism of the jumping with precise directional control in crickets.

Action selection based on stimulus velocity and duration

The results in Chapter 2 demonstrated that the velocity and duration of the air-current stimulus affected the action selection of either running or jumping. The jumping was more likely chosen when the cricket received faster and longer stimulus, indicating the larger predator approaching with higher speed. Therefore, the neural system including the integration process of the multiple stimulus parameters is suggested to underlie this action selection of the crickets. In addition, I found the different effects of stimulus parameters on the locomotor performance in running and jumping. The movement distance and velocity were altered depending on the stimulus parameters in running but not in jumping, also indicating the flexibility of running as an additional advantage.

Since the stimulus triggering the escape behavior give the prey animal the information how the predator is approaching, perception of multiple stimulus characteristics and selection of appropriate response based on the stimulus information would be crucial for the successful escape. Previous studies reveal that the stimulus velocity affects the action selection between distinct escape responses (Bhattacharyya et al., 2017; von Reyn et al., 2014), and specific projection neurons send the information of stimulus velocity to the giant fiber triggering escape response in flies (von Reyn et al., 2017). The cricket GIs show different sensitivity to the velocity and direction of the air-current stimulus (Miller et al., 1991; Ogawa et al., 2008; Tanaka, in Master thesis 2018). Further investigation on the postsynaptic circuit of GIs in the cricket brain would provide the clue to understand the neural basis of the action selection depending on the multiple stimulus parameters.

Neural circuits underlying the action selection of either running or jumping

I addressed the roles of the brain and TG in two different escape responses, running and jumping in Chapter 3. Ablation experiments of one side of VNC at the abdomen or the neck demonstrated that the sensory information processing in the brain was required for jumping whereas bilateral ascending sensory input to TG was necessary for running. The partial ablation affected the locomotor performance such as the movement distance, velocity and directional control. This indicated that these locomotor parameters were regulated by the descending signals from the brain. Finally, I found that the brain provided different descending signals preceding the start of running and jumping.

Previously, the interneurons involved in the wind-elicited escape running has been examined in cricket's brain and TG, and some descending interneurons of which activity is correlated with the speed and direction of escape running are identified in the brain (Hörner, 1992). However, the key interneurons that send descending command to trigger the specific escape response like DCMD in locusts has not been reported in the crickets. In locusts, the DCMD directly elicits jumping responding to the visual looming stimulus (Bennet-Clark, 1975; Gabbiani et al., 1999; Heitler and Burrows, 1977). Interestingly, ablation of unilateral descending signals of DCMD by cutting VNC, similar to my neck-cut experiment, has little effects on the jumping unlike my result in Chapter 3. This means that half of descending command of DCMD is enough to elicit jumping in locust (Santer et al., 2008; Fotowat et al., 2011). In crickets, the half ablation of ascending and descending signals to and from the brain drastically reduced the jumping and diminished the controllability of the movement direction. Considering the following two conclusions, (1) the movement direction was precisely controlled even in the jumping response and (2) the action selection of either running or jumping was affected by several

stimulus parameters, more complicated neural mechanism that involves multiple interneurons would underlie the escape behavior of the crickets than those reported in the other animals. Further study focusing on integration process of the several stimulus parameters would improve the understanding of the neural basis underlying the action selection.

I found the brain function to choose and regulate the escape responses, but it remains unknown what neural circuits conducts information processing for this function in the cricket escape behavior. One of the conceivable brain regions involved in the action selection and locomotor regulation is the central-body complex (CX). The CX is a higher center to integrate the preprocessed sensory information and to send outputs to premotor regions where the descending neurons receive synaptic inputs (Bender, 2010; Martin et al., 1999; Strauss, 2002; Strauss and Heisenberg, 1993). In cockroaches, the neural activities of central complex have been reported to control movement direction and turning during walking (Guo and Ritzmann, 2013; Martin et al., 2015). Considering precise control of the movement direction against the stimulus angle in the cricket, the CX would be involved in escape locomotion. Further physiological studies focusing on the CX activity during escape behavior may elucidate the neural mechanism for the action selection and locomotion regulation in the escape behavior.

References

- Allen, M. J., Godenschwege, T. A., Tanouye, M. A. and Phelan, P. (2006). Making an escape: Development and function of the *Drosophila* giant fibre system. *Semin. Cell Dev. Biol.* **17**, 31–41.
- Baba, Y. and Ogawa, H. (2017). Cercal system-mediated antipredator behaviors. In H.W. Horch, T. Mito, A. Popadic, H. Ohuchi, and S. Noji (Eds.), *The cricket as a model organism* (pp. 211–228). Springer.
- Baba, Y. and Shimozawa, T. (1997). Diversity of motor responses initiated by a wind stimulus in the freely moving cricket, *Gryllus bimaculatus*. *Zool. Sci.* **14**, 587–594.
- Bender, J. A., Pollack, A. J. and Ritzmann, R. E. (2010). Neural activity in the central complex of the insect brain is linked to locomotor changes. *Curr. Biol.* **20**, 921–926.
- Bennet-Clark, H. (1975). The energetics of the jump of the locust *Schistocerca Gregaria*. *J. Exp. Biol.* **63**, 53–83.
- Betley, J. N., Xu, S., Cao, Z. F. H., Magnus, C., Yu, Y. and Sternson, S. M. (2015). Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* **521**, 180–185.
- Bhattacharyya, K., McLeen, D. L. and MacIver, M. A. (2017). Visual threat assessment and reticulospinal encoding of calibrated responses in larval zebrafish. *Curr. Biol.* **27**, 2751–2762.
- Bidaye, S. S., Bockemühl, T. and Büschges, A. (2018). Six-legged walking in insects:

- how CPGs, peripheral feedback, and descending signals generate coordinated and adaptive motor rhythms. *J. Neurophysiol.* **119**, 459–475.
- Bidaye, S. S., Laturney, M., Chang, A. K., Liu, Y., Bockemühl, T., Büschges, A. and Scott, T. (2020). Two brain pathways initiate distinct forward walking programs in *Drosophila*. *Neuron* **108**, 469–485.
- Bidaye, S. S., Machacek, C., Wu, Y. and Dickson, B. J. (2014). Neuronal control of *Drosophila* walking direction. *Science* **344**, 97–101.
- Böhm, H. and Schildberger, K. (1992). Brain neurones involved in the control of walking in the cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **166**, 113–130.
- Borgmann, A. and Büschges, A. (2015). Insect motor control: methodological advances, descending control and inter-leg coordination on the move. *Curr. Opin. Neurobiol.* **33**, 8–15.
- Borgmann, A., Hooper, S. L. and Büschges, A. (2009). Sensory feedback induced by front-leg stepping entrains the activity of central pattern generators in caudal segments of the stick insect walking system. *J. Neurosci.* **29**, 2972–2983.
- Briggman, K. L., Abarbanel, H. D. and Kristan, W. B. (2005). Optical imaging of neuronal populations during decision-making. *Science* **307**, 896–901.
- Bulbert, M. W., Page, R. A. and Bernal, X. E. (2015). Danger comes from all fronts: predator-dependent escape tactics of túngara frogs. *PLoS ONE* **10**, e0120546.
- Burrows, M. (1980). The control of sets of motoneurons by local interneurons in the locust. *J. Physiol.* **298**, 213–233.

- Burrows, M. (1992). Local circuits for the control of leg movements in an insect. *Trends Neurosci.* **15**, 226–232.
- Burrows, M. and Rowell, C. H. F. (1973). Connections between descending visual interneurons and metathoracic motoneurons in the locust. *J. Comp. Physiol.* **85**, 221–234.
- Burrows, M. and Siegler, M. V. S. (1978). Graded synaptic transmission between local interneurons and motor neurons in the methathoracic ganglion of the locust. *J. Physiol.* **285**, 231–255.
- Byers, J., Hebets, E. and Podos, J. (2010). Female mate choice based upon male motor performance. *Anim. Behav.* **79**, 771–778.
- Card, G. M. (2012). Escape behaviors in insects. *Curr. Opin. Neurobiol.* **22**, 180–186.
- Card, G. and Dickinson, M. (2008). Performance trade-offs in the flight initiation of *Drosophila*. *J. Exp. Biol.* **211**, 341–353.
- Casagrand, J. L., Guzik, A. L. and Eaton, R. C. (1999). Mauthner and reticulospinal responses to the onset of acoustic pressure and acceleration stimuli. *J. Neurophysiol.* **82**, 1422–1437.
- Casagrand, J. L. and Ritzmann, R. E. (1991). Localization of ventral giant interneuron connections to the ventral median branch of thoracic interneurons in the cockroach. *J. Neurobiol.* **22**, 643–658.
- Casas, J. and Dangles, O. (2010). Physical ecology of fluid flow sensing in arthropods. *Annu. Rev. Entomol.* **55**, 505–520.

- Catania, K. C. (2009). Tentacled snakes turn C-starts to their advantage and predict future prey behavior. *Proc. Natl. Acad. Sci. USA*. **106**, 11183–11187.
- Chaure, F. j., Rey, H. G. and Quiroga, R. Q. (2018). A novel and fully automatic spike-sorting implementation with variable number of features. *J. Neurophysiol.* **120**, 1859–1871.
- Combes, S. A., Rundle, D. E., Iwasaki, J. M. and Crall, J. D. (2012). Linking biomechanics and ecology through predator–prey interactions: flight performance of dragonflies and their prey. *J. Exp. Biol.* **215**, 903–913.
- Cromwell, H. C. and Schultz, W. (2003). Effects of expectations for different reward magnitudes on neuronal activity in primate striatum. *J. Neurophysiol.* **89**, 2823–2838.
- Dangles, O., Ory, N., Steinmann, T., Christides, J.-P. and Casas, J. (2006). Spider’s attack versus cricket’s escape: velocity modes determine success. *Anim. Behav.* **72**, 603–610.
- De Franceschi, G., Vivattanasam, T., Saleem, A. B. and Solomon, S. G. (2016). Vision guides selection of freeze or flight defense strategies in mice. *Curr. Biol.* **26**, 2150–2154.
- Dickinson, A. and Balleine, B. (1994). Motivational control of goal-directed action. *Anim. Learn. Behav.* **22**, 1–18.
- Domenici, P. (2010). Context-dependent variability in the components of fish escape response: integrating locomotor performance and behavior. *J. Exp. Zoo.* **313A**,

59–79.

Domenici, P., Blagburn, J. H. and Bacon, J. P. (2011a). Animal escapology I: theoretical issues and emerging trends in escape trajectories. *J. Exp. Biol.* **214**, 2463–2473.

Domenici, P., Blagburn, J. M. and Bacon, J. P. (2011b). Animal escapology II: escape trajectory case studies. *J. Exp. Biol.* **214**, 2474–2494.

Domenici, P., Booth, D., Blagburn, J. M. and Bacon, J. P. (2008). Cockroaches keep predators guessing by using preferred escape trajectories. *Curr. Biol.* **18**, 1792–1796.

Doya, K. (2008). Modulators of decision making. *Nature Neurosci.* **11**, 410–416.

Dunn, T. W., Gebhardt, C., Nauman, E. A., Riegler, C., Ahrens, M. B., Engert, F. and Del Bene, F. (2016). Neural circuits underlying visually evoked escapes in larval zebrafish. *Neuron* **89**, 613–628.

Dupuy, F., Casas, J., Body, M. and Lazzari, C. R. (2011). Danger detection and escape behaviour in wood crickets. *J. Insect Physiol.* **57**, 865–871.

Eaton, R. C., Lee, R. K. K. and Foreman, M. B. (2001). The Mauthner cell and other identified neurons of the brainstem escape network of fish. *Prog. Neurobiol.* **63**, 476–485.

Edwards, D. H., Heitler, W. J. and Krasne, F. B. (1999). Fifty years of a command neuron: the neurobiology of escape behavior in the crayfish. *Trends Neurosci.* **22**, 153–161.

Eilam, D. (2005). Die hard: a blend of freezing and fleeing as a dynamic defense—

- implications for the control of defensive behavior. *Neurosci. Biobehav. Rev.* **29**, 1181–1191.
- Evans, D. A., Stempel, A. V., Vale, R. and Branco, T. (2019). Cognitive control of escape behaviour. *Trends Cogn. Sci.* **23**, 334–348.
- Evans, D. A., Stempel, A. V., Vale, R., Ruehle, S., Lefler, Y. and Branco, R. (2018). A synaptic threshold mechanism for computing escape decisions. *Nature* **558**, 590–594.
- Fadok, J. P., Krabbe, S., Markovic, M., Courtin, J., Xu, C., Massi, L., Botta, P., Bylund, K., Müller, C., Kovacevic, A., Tovote, P. and Lüthi, A. (2017). A competitive inhibitory circuit for selection of active and passive fear responses. *Nature* **542**, 96–100.
- Fehr, E. and Rangel, A. (2011). Neuroeconomic foundations of economic choice—recent advances. *J. Econ. Perspect.* **25**, 3–30.
- Fotowat, H., Fayyazuddin, A., Bellen, H. J. and Gabbiani, F. (2009). A novel neuronal pathway for visually guided escape in *Drosophila melanogaster*. *J. Neurophysiol.* **102**, 875–885.
- Fotowat, H. and Gabbiani, F. (2007). Relationship between the phases of sensory and motor activity during a looming-evoked multistage escape behavior. *J. Neurosci.* **27**, 10047–10059.
- Fotowat, H., Harrison, R. R. and Gabbiani, F. (2011). Multiplexing of motor information in the discharge of a collision detecting neuron during escape behaviors. *Neuron*

69, 147–158.

Fukutomi, M. and Ogawa, H. (2017). Crickets alter wind-elicited escape strategies depending on acoustic context. *Sci Rep.* **7**, 15158.

Fukutomi, M., Someya, M. and Ogawa, H. (2015). Auditory modulation of wind-elicited walking behavior in the cricket *Gryllus bimaculatus*. *J. Exp. Biol.* **218**, 3968–3977.

Gabbiani, F., Krapp, H. G. and Laurent, G. (1999). Computation of object approach by a wide-field, motion-sensitive neuron. *J. Neurosci.* **19**, 1122–1141.

Gazzola, M., Van Rees, W. M. and Koumoutsakos, P. (2012). C-start: optimal start of larval fish. *J. Fluid. Mech.* **698**, 5–18.

Gold, J. I. and Shadlen, M. N. (2007). The neural basis of decision making. *Annu. Rev. Neurosci.* **30**, 535–574.

Gras, H. and Hörner, M. (1992). Wind-evoked escape running of the cricket *Gryllus bimaculatus*: I. Behavioral analysis. *J. Exp. Biol.* **171**, 189–214.

Gross, C. T. and Canteras, N. S. (2012). The many path to fear. *Nature Rev. Neurosci.* **13**, 651–658.

Guo, P. and Ritzmann, R. E. (2013). Neural activity in the central complex of the cockroach brain is linked to turning behaviors. *J. Exp. Biol.* **216**, 992–1002.

Hale, M. E. (2002). S- and C-start escape responses of the muskellunge (*Esox masquinongy*) require alternative neuromotor mechanisms. *J. Exp. Biol.* **211**, 1819–1828.

- Hammond, S. and O'Shea, M. (2007). Escape flight initiation in the fly. *J. Comp. Physiol. A* **193**, 471–476.
- Hatsopoulos, N., Gabbiani, F. and Laurent, G. (1995). Elementary computation of object approach by a wide-field visual neuron. *Science* **270**, 1000–1003.
- Hedwig, B. (2006). Pulses, patterns and paths: neurobiology of acoustic behaviour in crickets. *J. Comp. Physiol. A* **192**, 677–689.
- Heitler, W. J. and Burrows, M. (1977). The locust jump I. the motor programme. *J. Exp. Biol.* **66**, 203–219.
- Herberholz, J. and Marquart, G. D. (2012). Decision making and behavioral choice during predator avoidance. *Front. Neurosci.* **6**, 1–15.
- Hirota, K., Sonoda, Y., Baba, Y. and Yamaguchi, T. (1993). Distinction in morphology and behavioral role between dorsal and ventral groups of cricket giant interneurons. *Zool. Sci.* **10**, 705–709.
- Hommaru, N., Shidara, H., Ando, N. and Ogawa, H. (2020). Internal state transition to switch behavioral strategies in cricket phonotaxis. *J. Exp. Biol.* **223**, currently being compiled.
- Jacobs, G. A., Miller, J. P. and Aldworth, Z. (2008). Computational mechanisms of mechanosensory processing in the cricket. *J. Exp. Biol.* **205**, 2005–2016.
- Jacobs, G. A. and Theunissen, F. E. (1996). Functional organization of a neural map in the cricket cercal sensory system. *J. Neurosci.* **16**, 769–784.
- Jacobs, G. A. and Theunissen, F. E. (2000). Extraction of sensory parameters from a

- neural map by primary sensory interneurons. *J. Neurosci.* **20**, 2934–2943.
- Kanou, M. and Shimozawa, T. (1985). Responses of cricket leg motoneurons to air-current stimuli: velocity dependent inhibition and acceleration dependent excitation. *Zool. Sci.* **2**, 629–639.
- Kavaliers, M. and Choleris, E. (2001). Antipredator responses and defensive behavior: ecological and ethological approaches for the neurosciences. *Neurosci. Biobehav. Rev.* **25**, 577–586.
- Kohashi, T. and Oda, Y. (2008). Initiation of Mauthner- or non-Mauthner-mediated fast escape evoked by different modes of sensory input. *J. Neurosci.* **28**, 10641–10653.
- Korn, H. and Faber, D. S. (2005). The Mauthner cell half a century later: a neurobiological model for decision-making? *Neuron* **47**, 13–28.
- LeDoux, J. and Daw, N. D. (2018). Surviving threats: neural circuit and computational implications of a new taxonomy of defensive behavior. *Nat. Rev. Neurosci.* **19**, 269–282.
- Levine, J. and Tracey, D. (1973). Structure and function of the giant motorneuron of *Drosophila melanogaster*. *J. Comp. Physiol.* **87**, 213–235.
- Lima, S. L. and Dill, L. M. (1990). Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640.
- Lind, J. and Cresswell, W. (2005). Determining the fitness consequence of antipredation behavior. *Behav. Ecol.* **16**, 945–956.
- Liu, Y. C. and Hale, M. E. (2017). Local spinal cord circuits and bilateral Mauthner cell

- activity function together to drive alternative startle behaviors. *Curr. Biol.* **27**, 697–704.
- Martin, J. P., Guo, P., Mu, L., Harley, C. M. and Ritzmann, R. E. (2015). Central-complex control of movement in the freely walking cockroach. *Curr. Biol.* **25**, 2795–2803.
- Martin, J. -R., Raabe, T. and Heisenberg, M. (1999). Central complex substructures are required for the maintenance of locomotor activity in *Drosophila melanogaster*. *J. Comp. Physiol. A* **185**, 277–288.
- Matsuura, T., Kanou, M. and Yamaguchi, T. (2002). Motor program initiation and selection in crickets, with special reference to swimming and flying behavior. *J. Comp. Physiol. A* **187**, 987–995.
- Miller, J. P., Jacobs, G. A. and Theunissen, F. E. (1991). Representation of sensory information in the cricket cercal sensory system. I. Response properties of the primary interneurons. *J. Neurophysiol.* **66**, 1680–1689.
- Miller, J. P., Krueger, S., Heys, J. J. and Gedeon, T. (2011). Quantitative characterization of the filiform mechanosensory hair array on the cricket cercus. *PLoS ONE* **6**, e27873.
- Mu, L. and Ritzmann, R. E. (2008). Interaction between descending input and thoracic reflexes for joint coordination in cockroach: I. Descending influence on thoracic sensory reflexes. *J. Comp. Physiol. A* **194**, 283–298.
- Oe, M. and Ogawa, H. (2013). Neural basis of stimulus-angle-dependent motor control of wind-elicited walking behavior in the cricket *Gryllus bimaculatus*. *PLoS ONE* **8**, e80184.

- Ogawa, H., Cummins, G. I., Jacobs, G. A. and Miller, J. P. (2006) Visualization of ensemble activity patterns of mechanosensory afferents in the cricket cercal sensory system with calcium imaging. *J. Neurobiol.* **66**, 293–307.
- Ogawa, H., Cummins, G. I., Jacobs, G. A. and Oka, K. (2008). Dendritic design implements algorithm for synaptic extraction of sensory information. *J. Neurosci.* **28**, 4592–4603.
- Pearson, K. G. and Robertson, R. M. (1981). Interneurons coactivating hindleg flexor and extensor motoneurons in the locust. *J. Comp. Physiol. A* **144**, 391–400.
- Preuss, T. and Faber, D. S. (2003). Central cellular mechanisms underlying temperature-dependent changes in the goldfish startle-escape behavior. *J. Neurosci.* **23**, 5617–5626.
- Preuss, T., Osei-Bonsu, P. E., Weiss, S. A., Wang, C. and Fabor, D. S. (2006). Neural representation of object approach in a decision-making motor circuit. *J. Neurosci.* **26**, 3454–3464.
- Rangel, A., Camerer, C. and Montague, P. R. A. (2008). framework for studying the neurobiology of value-based decision making. *Nat. Rev. Neurosci.* **9**, 545–556.
- Ridgel, A. L., Alexander, B. E. and Ritzmann, R. E. (2007). Descending control of turning behavior in the cockroach, *Blaberus discoidalis*. *J. Comp. Physiol. A* **193**, 385–402.
- Rind, F. C. (1984). A chemical synapse between two motion detecting neurones in the locust brain. *J. Exp. Biol.* **110**, 143–167.

- Ritzmann, R. E. and Büschges, A. (2007). Adaptive motor behavior in insects. *Curr. Opin. Neurobiol.* **17**, 629–636.
- Ritzmann, R. E. and Camhi, J. M. (1978). Excitation of leg motor neurons by giant interneurons in the cockroach *Periplaneta Americana*. *J. Comp. Physiol. A.* **125**, 305–316.
- Ritzmann, R. E. and Pollack, A. J. (1978). Identification of thoracic interneurons that mediate giant interneuron-to-motor pathways in the cockroach. *J. Comp. Physiol. A.* **159**, 639–654.
- Robertson, R. M., Pearson, K. G. and Reichert, H. (1982). Flight interneurons in the locust and the origin of insect wings. *Science* **217**, 177–179.
- Santer, R. D., Yamazaki, Y., Rind, F. C. and Simmons, P. J. (2005). Motor activity and trajectory control during escape jumping in the locust *Locusta migratoria*. *J. Comp. Physiol. A.* **191**, 965–975.
- Santer, R. D., Yamazaki, Y., Rind, F. C. and Simmons, P. J. (2008). Preparing for escape: an examination of the role of the DCMD neuron in locust escape jumps. *J. Comp. Physiol. A.* **194**, 69–77.
- Sato, N., Shidara, H. and Ogawa, H. (2017). Post-molting development of wind-elicited escape behavior in the cricket. *J. Insect. Physiol.* **103**, 36–46.
- Schnell, B., Ros, I. G. and Dickinson, M. H. (2017). A descending neuron correlated with the rapid steering maneuvers of flying *Drosophila*. *Curr. Biol.* **27**, 1200–1205.
- Schultz, W. (2006). Behavioral theories and the neurophysiology of reward. *Annu. Rev.*

- Psychol.* **57**, 87–115.
- Shang, C., Liu, Z., Chen, Z., Shi, Y., Wang, Q., Liu, S., Li, D. and Cao, P. (2015). A parvalbumin-positive excitatory visual pathway to trigger fear responses in mice. *Science* **348**, 1472–1477.
- Shimozawa, T. and Kanou, M. (1984). Varieties of filiform hairs: range fractionation by sensory afferents and cercal interneurons of a cricket. *J. Comp. Physiol. A* **155**, 485–493.
- Simmons, P. J., Rind, F. C. and Santer, R. D. (2010). Escapes with and without preparation: The neuroethology of visual startle in locusts. *J. Insect Physiol.* **56**, 876–883.
- Soma, M. and Garamszegi, L. Z. (2011). Rethinking birdsong evolution: meta-analysis of the relationship between song complexity and reproductive success. *Behav. Ecol.* **22**, 363–371.
- Staudacher, E. (1998). Distribution and morphology of descending brain neurons in the cricket *Gryllus bimaculatus*. *Cell. Tissue. Res.* **294**, 187–202.
- Staudacher, E. M. (2001). Sensory responses of descending brain neurons in the walking cricket, *Gryllus bimaculatus*. *J. Comp. Physiol. A* **187**, 1–17.
- Stewart, W. J., Cardenas, G. S. and McHenry, M. J. (2013). Zebrafish larvae evade predators by sensing water flow. *J. Exp. Biol.* **216**, 388–398.
- Strauss, R. (2002). The central complex and the genetic dissection of locomotor behavior. *Curr. Opin. Neurobiol.* **12**, 633–638.

- Strauss, R and Heisenberg, M. (1993). A higher control center of locomotor behavior in the *Drosophila* brain. *J. Neurosci.* **13**, 1852–1861.
- Takagi, S., Cocanougher, B. T., Niki, S., Miyamoto, D., Kohsaka, H., Kazama, H., Fetter, R. D., Truman, J. W., Zlatić, M., Cardona, A. and Nose, A. (2017). Divergent connectivity of homologous command-like neurons mediates segment-specific touch responses in *Drosophila*. *Neuron* **96**, 1373–1387.
- Tauber, E. and Camhi, J. M. (1995). The wind-evoked escape behavior of the cricket *Gryllus bimaculatus*: integration of behavioral elements. *J. Exp. Biol.* **198**, 1895–1907.
- Theunissen, F. E. and Miller, J. P. (1991). Representation of sensory information in the cricket cercal sensory system. II. Information theoretical calculation of system accuracy and optimal tuning-curve width of four primary interneurons. *J. Neurophysiol.* **66**, 1690–1703.
- Trimarchi, J. R. and Schneidman, A. M. (1995). Different neural pathways coordinate *Drosophila* flight initiations evoked by visual and olfactory stimuli. *J. Exp. Biol.* **198**, 1099–1104.
- Turesson, H., Satta, A. and Domenici, P. (2009). Preparing for escape: anti-predator posture and fast-start performance in gobies. *J. Exp. Biol.* **212**, 2925–2933.
- Turner, H. N., Amengol, K., Patel, A. A., Himmel, N. J., Sullivan, L., Iyer, S. C., Bhattacharyya, S., Iyer, E. P. R., Landry, C., Galko, M. J. and Cox, D. N. (2016). The TRP channels Pkd2, NompC, and Trpm act in cold-sensing neurons to mediate unique aversive behaviors to noxious cold in *Drosophila*. *Curr. Biol.* **26**,

3116–3128.

von Reyn, C. R., Breads, P., Peek, M. Y., Zheng, G. Z., Williamson, W. R., Yee, A. L., Leonardo, A. and Card, G. M. (2014). A spike-timing mechanism for action selection. *Nat. Neurosci.* **17**, 962–970.

von Reyn, C. R., Nern, A., Williamson, W. R., Breads, P., Wu, M., Namiki, S. and Card, G. M. (2017). Feature integration drives probabilistic behavior in the drosophila escape response. *Neuron* **94**, 1190–1204.

Walker, J. A., Ghalambor, C. K., Griset, O. L., McKenny, D. and Reznik, D. N. (2005). Do faster starts increase the probability of evading predators? *Funct. Ecol.* **19**, 808–815.

Wang, L., Chen, I. Z. and Lin, D. (2015). Collateral pathways from the ventromedial hypothalamus mediate defensive behaviors. *Neuron* **85**, 1344–1358.

Watanabe, T., Ugajin, A. and Aonuma, H. (2018). Immediate-early promoter-driven transgenic reporter system for neuroethological research in a hemimetabolous insect. *eNeuro* **5**.

Ydenberg, R. C. and Dill, L. M. (1986). The economics of fleeing from predators. *Adv. Study. Behav.* **16**, 229–249.

Zorović, M. and Hedwig, B. (2013). Descending brain neurons in the cricket *Gryllus bimaculatus* (de Geer): auditory responses and impact on walking. *J. Comp. Physiol. A* **199**, 25–34.

Research Achievement

Publication

1. **Sato, N.**, Shidara, H. and Ogawa, H. (2019) Trade-off between motor performance and behavioural flexibility in the action selection of cricket escape behaviour. *Scientific Reports* 9: 18112.

Presentations

International (All presentations were poster)

1. **Sato, N.**, Shidara, H. and Ogawa, H. Trade-off between speed, directional accuracy and behavioral flexibility in action selection of the escape behavior in the cricket. *48th annual meeting of the Society for Neuroscience* [152.06/QQ4] San Diego, USA. November 2018.
2. **Sato, N.** and Ogawa, H. Action selection between walking and jump in the wind-elicited escape behavior of the cricket. *47th annual meeting of the Society for Neuroscience* [156.03/LL6] Washington, D.C., USA. November 2017.

Domestic

1. **佐藤 和**, 設樂 久志, 小川 宏人. コオロギ気流逃避行動における行動選択の神経基盤. 日本動物学会第 91 回大会 [H-09] Online, 2020 年 9 月 (ポスター発表)
2. **佐藤 和**, 設樂 久志, 小川 宏人. コオロギの生得的逃避行動における異なる戦略間のトレード・オフ. 日本動物学会第 90 回大会 [1I1300] 大阪, 2019 年 9 月 (口頭発表)
3. **佐藤 和**, 小川 宏人. Action selection between running and jump in the wind-

elicited escape behaviors of the cricket. 日本比較生理生化学会第 39 回大会 [P-069] 福岡, 2017 年 11 月 (ポスター発表)

4. 佐藤 和, 小川 宏人. コオロギ気流誘発性逃避行動における刺激パラメータ依存的行動選択. 日本動物学会第 88 回大会 [1G0930] 富山, 2017 年 9 月 (口頭発表)
5. 佐藤 和, 小川 宏人. コオロギ気流誘発性逃避行動における行動選択. 第 40 回日本神経科学大会 [3P-154] 幕張, 2017 年 7 月 (ポスター発表)

Achievements of other research

Publication

1. **Sato, N.**, Shidara, H. and Ogawa, H. (2017) Post-molting development of wind-elicited escape behavior in the cricket. *Journal of Insect Physiology* 103: 36–46.

Presentations

1. 佐藤 和, 小川 宏人. 成虫コオロギにおける成虫脱皮後の気流誘発性逃避行動の経時的発達変化. 日本動物学会北海道支部第 61 回大会 [O-12] 旭川, 2016 年 8 月 (口頭発表)
2. 佐藤 和, 小川 宏人. コオロギ気流誘発性逃避行動における成虫脱皮後の経時的変化. 第 39 回日本神経科学大会 [P1-063] 横浜, 2016 年 7 月 (ポスター発表)

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