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Masaki Sakai *Editor*

Death-Feigning in Insects

Mechanism and Function of Tonic Immobility

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Entomology Monographs

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Preface

The so-called death-feigning is a well-known behavior because the term is commonly used in daily life. Death-feigning means taking an irresponsive attitude toward others by appearing to be dead. In biology, death-feigning means the behavior of an animal assuming an immobile posture as if had fainted. Death-feigning is often called tonic immobility (TI) and this term is used hereafter.

TI is a peculiar but not so rare behavior. Many animal species from invertebrates to vertebrates show TI. In the natural habitat, TI occurs when the prey is attacked by a predator. The prey lies down in a paralyzed manner and remains immobile sometimes for up to several hours.

TI has been studied since a long time ago and there are lots of reports on TI. However, there are not so many researchers who have interests in mechanism and function. One reason is probably TI seems to be a kind of physiologically abnormal state and another reason is that there is not a suitable way to demonstrate the function of TI in the natural habitat. Knowledge of TI is informally used in laboratory procedures and animal husbandry, for example, where techniques of TI induction are needed to quiet large animals like a sheep and a rabbit for injections and blood samples in stock farming. In fishery, they need to quiet large fish such as sharks and rays quickly after capturing on the ship.

On the other hand, some researchers are interested in TI behavior itself. Here I introduce my experience. I found that the cricket *Gryllus bimaculatus,* a popular insect bred in the laboratory, showed distinct TI. This finding led me to investigate its neural mechanism since I had an interest in narcolepsy in cats. I speculated that TI in crickets might be a model of sleep paralysis in cats. This model seemed promising because the nervous systems of insects are much simpler than those in mammals. However, during the course of my searching the central nervous system for neural correlates with TI, I gradually realized that it was still premature to associate the mechanism of TI in crickets with that of narcolepsy in cats. Since then, a quarter of a century has passed, but it seems that not much progress has been made in the study of the TI mechanism.

I was asked by Prof. Hideharu Numata, the series editor of *Entomology Monographs* in Springer, to make a book on TI (May 2017). At first, I hesitated to accept it because TI occupies only a small portion of behavioral science. However, there are several researchers in Japan who have studied TI by different approaches. Besides, almost no monographs on TI had been published. This led me to believe that a book on TI would gain "scarcity value" in entomology.

I first discussed this matter with Hiroshi Nishino, who was my former collaborator, and then asked two Japanese researchers Atsushi Honma and Takahisa Miyatake to participate in the project. Atsushi Honma is a field worker on TI research and Takahisa Miyatake is a specialist in the functional significance of TI. At the same time, I requested three German researchers, Hans-Joachim Pflüger, Ansgar Büschges and their mentor Ulrich Bässler, to take part in the project. This is because experimental studies on insect TI have been conducted mainly in Germany and a neurophysiological study on insect TI was performed for the first time by Bässler's group at Kaiserslautern University.

After the main plan for the book had been established, another topic, freezing came to issue. Freezing is a behavior that is similar in some ways but distinctly different from TI. Freezing occurs when the prey encounters a predator, being earlier in the sequence of a predator's attack. The prey stops all the movements and usually assumes a crouching posture so as not to be spotted by the predator and waits for a chance to escape. Freezing has been well investigated as one of the fear responses including flight and fight, and its neural mechanism has been established in mammals. On the other hand, TI is placed peripherally in the context of fear response and its neural mechanism remains uncertain. What is worse still, TI is sometimes mixed up with freezing. I, therefore, decided to incorporate freezing behavior to clarify both behaviors. I asked Ryo Nakano, Takuma Takanashi, Wataru Kojima, and Shigehiro Namiki who are specialists of insect freezing to join us. Finally, Masayuki Yoshida, a specialist in fish behavior, was asked to support us. This is because studies on TI and freezing have been done more in vertebrates than in invertebrates. As a result, one chapter was set up for vertebrates to present examples of not only preceding but also advanced studies on TI and freezing.

Neurobiology in insects has been rapidly developing, and the gap between the nervous structures in insects and vertebrates has been narrowing. Insects are easy to handle and suitable for the use of new technologies such as molecular and genetic analyses. Therefore, it is expected that studies on TI and freezing in insects will progress rapidly and that knowledge of the neural mechanism and function will make an important contribution to TI study in vertebrates. In this trend, this book covering (1) two defense immobility behaviors (TI and freezing), (2) their two main issues (mechanism and function) in insects, and (3) immobility behavior in vertebrates is unique and valuable. I hope that this book will be read by many students and researchers and will help them to understand immobility behavior.

I would like to thank Dr. Hideharu Numata for his offer of making this book and Fumiko Yamaguchi and Selvakumar Rajendran in Springer for their great help in editing.

Okayama, Japan Masaki Sakai

Contents

Chapter 1
Freezing and Tonic Immobility: Their Definitions and Naming

Masaki Sakai

Abstract Defensive immobility behaviors, the so-called freezing and tonic immobility (death-feigning), are discussed. Freezing occurs when the prey encounters a predator, while tonic immobility occurs at the time of the predator's attack. Since these two immobility reactions have some similarity in appearance, they have sometimes been used incorrectly in reports. To clarify the difference between these two immobility reactions, their characteristics, definitions, mechanisms, and functions are summarized. As for the naming of the two immobility reactions, the term "freezing" has no obvious problem for usage because what it indicates is easily understood. On the other hand, "tonic immobility (TI)" is a bit complicated because many different terms including TI have been used to indicate the same or a similar immobility reaction. In this book, the most appropriate naming is considered.

Keywords Freezing · Tonic immobility · Insects · Mechanism · Function · Definition · Naming

1.1 Introduction

This book is about defensive immobility behavior when animals are threatened or attacked by predators. This immobility behavior includes two reactions: freezing and tonic immobility (TI). Freezing occurs when the prey encounters a predator, while TI occurs at the time of the predator's attack. Thus, in a situation in which both reactions occur, freezing comes first and then TI occurs. Both are known to be useful for the prey's survival (Edmunds 1974; Ruxton et al. 2018). There is some confusion regarding these two immobility reactions. Freezing is sometimes included in TI, while TI is sometimes classified in freezing. This is due to the fact that the two

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immobility reactions have some similarity in gross appearance. However, freezing and TI are distinctly different (Marx et al. 2008; Roelofs 2017; Humphreys and Ruxton 2018): The former is a state of attentive immobility to avoid detection and to enhance perception, while the latter is a state of a kind of paralysis caused by profound motor inhibition (Misslin 2003; Roelofs 2017). In this book, the definition and characteristics of both reactions are explained in detail depending on the animals referred to in each chapter. Here various aspects of freezing and TI including their characteristics, definitions, mechanisms, and functions are described comprehensively, and the information should be useful for understanding each chapter (see also Chaps. 3 and 11). In Chap. 1, immobility reaction in mammals is described in some detail despite the fact that this book is about immobility reaction in insects. This is because immobility reaction in mammals has been more extensively studied and is more familiar to readers. The appropriateness of the naming of freezing and tonic immobility (TI) is also considered.

1.2 Freezing and TI

To facilitate an understanding of the concepts of freezing and TI, these two reactions are shown with a comparison between mammals and insects (Fig. 1.1A, B).

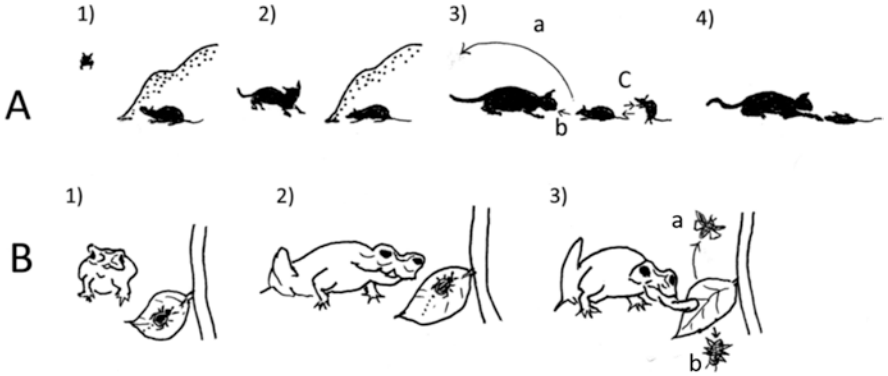

Fig. 1.1 Freezing and TI. (A) A rat and a cat. 1–4 correspond to Step 1–Step 4 in the text. In 3, (a) shows an escape jump, (b) shows a defense attack, and (c) shows standing on guard. (B) A beetle and a toad. 1–3 correspond to Step 1–Step 3 in the text. In 3, (a) shows escape flight and (b) shows falling

1.2.1 A Rat and a Cat

1.2.1.1 Step 1 (Encounter, Fig. 1.1A-1)

A rat encounters a cat in the garden. Before the cat notices the rat, the rat becomes aware of the presence of a cat by its sound or smell. When there is some distance between them, the rat quickly hides in nearby shade and becomes motionless. This immobility reaction is called freezing here. The stationary rat may intermittently try to determine the location of the cat by changing its posture and position. This is called orienting response (Chap. 11). Although the orienting response should not be mixed up with freezing since the former has been distinguished from the latter by behavioral tests (Hagenaars et al. 2014), orienting response is included in freezing (in a wide sense) here for simplicity.

1.2.1.2 Step 2 (Approach, Fig. 1.1A-2)

The cat comes closer to the rat and notices it. The threat is rapidly increasing for the rat. The rat must decide whether to run away or to stay there. The rat will flee if there is an escape route or access to a shelter. However, if neither is available, the rat has no choice but to remain motionless. The tension increases further and some conflict, i.e., to stay or to flee, may occur. This state is also called freezing here. The reactions in Step 1 and Step 2 are called attentive immobility (Volchan et al. 2017).

1.2.1.3 Step 3 (Imminent Attack, Fig. 1.1A-3)

Finally, the cat spots the rat and orients towards it. The rat crouches as it is or temporarily stands on its hind legs (Fig. 1.1A-3c). These reactions are also called freezing (in a wide sense) here, though these reactions have been named “immobility under attack” (Volchan et al. 2017). Then as soon as the cat moves to attack, the rat makes a jump (escape jump, Fig. 1.1A-3a) or snaps at the cat’s nose (defense attack, Fig. 1.1A-3b) (Blanchard et al. 1986). However, the rat may continue to assume a crouching posture without doing anything.

1.2.1.4 Step 4 (Attack, Fig. 1.1A-4)

Immediately after Step 3, the cat seizes the rat with its foreleg. In this state, when the posture of the rat is not greatly different from that of freezing, it may be a continuation of freezing. However, if the rat falls sideways and becomes immobile as if it has fainted, this reaction is called tonic immobility (TI). During TI, the rat makes no response to perturbation by the cat. If the rat remains immobile, the cat loses interest in the rat and throws it away. After a while, the rat carefully stands up while making sure that there is no predator nearby and runs away without serious injury.

As described above, in a state of freezing, all body movements except for respiration and eye movement cease with the entire body stiffened. It has been shown that the internal state in freezing differs depending on the situation and the individual (Eilam 2005). This is because the degree of threat differs depending on the predator's strength, distance between the prey and predator (Eilam 2005; Nishiumi and Mori 2015), and presence or absence of an escape route (Eilam 2005). The degree of threat also depends on the lineage, individual character, and experience of the rat (Bolles 1970; Blanchard et al. 1986). Thus, to describe freezing behavior, consideration of the prey's internal state in addition to its apparent state may be needed.

On the other hand, as shown in Fig. 1.1A-4, TI is elicited by contact and/or restraint. During TI, the rat shows a kind of paralysis: it does not move its limbs or change its posture at all. The rat remains lying on the ground defenselessly as the "last resort" (Humphreys and Ruxton 2018). However, this behavior does in fact offer a good chance of survival.

1.2.2 A Beetle and a Toad

1.2.2.1 Step 1 (Encounter, Fig. 1.1B-1)

A toad comes near a small tree with a potato beetle walking on a leaf. Before the toad notices the beetle, the beetle becomes aware of the presence of danger by the shadow, vibration or wind. The beetle suddenly stops all movements including movements of its limbs, antennae, and palpi. This is called freezing here.

1.2.2.2 Step 2 (Approach, Fig. 1.1B-2)

Later, the beetle begins to move while the toad remains stationary. The toad now notices the beetle and orients towards it. If the beetle had stopped moving when there was stimulation evoked by the toad, the toad might not have known the location of the beetle because the toad pays attention only to moving objects. However, the beetle keeps moving.

1.2.2.3 Step 3 (Attack, Fig. 1.1B-3)

The toad then ejects its tongue at the moving beetle. The beetle is instantly caught in the toad's mouth. However, if the toad ejects its tongue in a slightly wrong direction, the beetle might fly off the leaf in response to a moderate wind or/and vibration (escape flight, Fig. 1.1B-3a) or it might slip off the leaf in response to a strong shock or/and vibration (falling, Fig. 1.1B-3b). If the beetle falls to the ground, it will lie motionless in a stiffened posture. During the immobility, the beetle will not respond to any perturbation. As a result, the toad becomes unaware of the location of the beetle. This reaction is called tonic immobility (TI; death-feigning).

As was the case for the rat, the beetle shows two steps of immobility response: freezing and TI. In insects, freezing may not be greatly different from a resting state in gross appearance. However, at the time of freezing, all movements of an insect including movements of the head, abdomen, limbs, and appendages stop immediately and the insect remains completely motionless. This reaction serves as an instant disappearance from the sight of the predator. Freezing should function for either reinforcing hiding or preparing to flee. However, it cannot be judged which is the case from only the external appearance (It may be tested by giving a gentle touch to the freezing insect.).

On the other hand, TI in insects is clearly different from either a resting state or freezing. Although some insects assume the normal resting posture during TI, most insects assume an unnatural posture on their back with their limbs irregularly flexed or extended so that they cannot maintain their normal posture. While insects may show a response to even weak mechanical stimulation during freezing, they are basically unresponsive during TI, though it has been shown that some insects respond to some sensory stimulation and terminate TI (Fabre 1900; Holmes 1906; Godden 1972). It is surprising that some insects such as water stick insects (Holmes 1906) and stick insects (Bässler 1983) maintain TI for a while even after their body has been cut into two pieces.

1.3 Mechanisms of Freezing

Freezing is one of the so-called fear responses that an animal shows when it is facing a threat, naturally a predator. As the fear response consisting of flight, fight, and freeze has been studied extensively, neural mechanisms of freezing are described here in the framework of fear response.

1.3.1 Vertebrates

Fear response has been studied mainly in mammals as representative higher vertebrates since the publication by Darwin in 1872 with some studies in birds, reptiles, amphibians, and fish (Chap. 11). The results have been summarized in many review articles (LeDoux 2000; Misslin 2003; Eilam 2005; Roelofs 2017). There are two kinds of fear response, an unconditioned fear response and a conditioned fear response which differ in procedures to induce the response. In either case, animals show a characteristic posture and facial expression as the response of skeletal muscles. For the autonomic system, the sympathetic nervous system is activated, resulting in various phenomena including dilatation of the papilla, increase in blood pressure, and erection of hair muscles. Among the three reactions of fear response freezing is caused by tonic muscle activation to maintain crouching accompanying bradycardia by activation of the parasympathetic nervous system following initial activation of the sympathetic nervous system (Roelofs 2017).

The center for the fear response is located in the amygdala, and its central nucleus is responsible for the pivotal function. The afferent input from the cerebral cortex and thalamus comes into the central nucleus via the lateral nucleus. The efferent output of the central nucleus is sent to the midbrain and hypothalamus, which produce the patterns of fear response. The periaqueductal gray in the midbrain, especially the ventromedial part of it, plays a critical role in the expression of freezing (Roelofs 2017).

1.3.2 Insects

Insects, as representative higher invertebrates, also show a fear response (LeDoux 2000). The fear response in question here is freezing, that is, an instant cessation of ongoing activity such as cleaning and walking. This response is normally elicited by alarm stimuli of predators such as sound (Roeder 1962), vibration (Dambach 1989; Friedel 1999), and looming light (Card 2012). Instant cessation is also evoked by a safe stimulus such as shade for hiding when a cockroach is running away (Okada and Toh 1998). In some cases, freezing occurs in the air. Flying moths (Roeder 1962) and lacewings (Miller and Olesen 1979; Miller 1983) show nose diving (natural fall) in response to ultrasound pulses mimicking the voice of a bat. This diving is caused by temporary cessation of wing movement. Interestingly, this defensive immobility reaction seems to have diverted to unexpected behavior (Chap. 5). An immobility reaction to vibration is also used for avoiding an inconvenient encounter between the same species (Chap. 6).

Many developments have been made in neuroethology of insects in the past 50 years (Bässler 1983; Huber et al. 1989; Burrows 1996). Neural circuits underlying behavior have been analyzed in detail in various studies including studies on navigation (Honkanen et al. 2019) and even studies at the genetic level on courtship (Yamamoto and Koganezawa 2013). However, there has been little study on freezing at a neural level. This is partly because immobility behavior has not been as attractive as dynamic behaviors such as jumping, flying, and calling, and partly because it is difficult to search the central nervous system for neurons correlated with an immobile state. Since insects, however, have a simple nervous system and are easy to handle, rapid progress in the study of mechanisms of freezing is expected (Gibson et al. 2015, Chap. 10). More recently, it has been reported that fear reaction in the fly *Drosophila* has some behavioral characteristics common to those of mammals, and it was suggested that there may be a basic neural structure, “primitive motives,” underlying fear (Anderson and Adolphs 2014; see also Chap. 10). In addition to studies on mechanisms, progress has also been made in studies on adaptive and functional aspects of freezing (Chaps. 5 and 6).

1.4 Mechanisms of TI

TI is an immobility reaction that the prey assumes when attacked by a predator. The study of TI has a long history and there have been a lot of related reports. About 800 reports on TI have been available (Maser and Gallup 1977). Here, animals that show TI and the history of TI studies are briefly described first, and then methods by which TI is elicited, characteristics of TI, involvement of the nervous system in TI and some hypotheses underlying TI are summarized.

1.4.1 Animals

TI is seen in a wide variety of animal species including both invertebrates and vertebrates. Invertebrates in which TI is observed include mollusks, arachnids, crustaceans, and insects, and vertebrates include fish, amphibians, reptiles, birds, and mammals (Ratner 1967; Humpherys and Ruxton 2018). However, there are some species in which TI never occur (Fabre 1900), possibly because those species do not need TI for their protection. Interestingly, domestic animals such as dogs, cats, and even rats have a tendency not to assume TI (Klemm 1971).

1.4.2 Brief History of TI Studies

TI is not observed as frequently as freezing. However, TI has attracted attention for many years because it is a curious and intriguing behavior. In the seventeenth century, Schwenter (1636) in the Netherlands and Kircher (1646) in Germany conducted experiments on TI using chickens (Völgyesi 1966). In the late eighteenth century to early nineteenth century, with the enthusiasm over animals and humans being able to be hypnotized and mentally treated, TI was very popular in France as mesmerism (animal magnetism) (Völgyesi 1966). Scientific study of TI was established in Germany before World War II (Mangold 1920; Bleich 1928; Steiniger 1936; see Chap. 2). However, it was after World War II that the real physiological study of TI began. There were many experimental studies on TI, and excellent reviews on TI based mainly on results for mammals and birds were published in the late 1960s to early 1970s (Völgyesi 1966; Ratner 1967; Chertok 1968; Klemm 1971; Gallup 1974). However, studies on TI declined soon thereafter. This may be partly due to technical limitations and partly due to loss of interest in TI since possible hypotheses for TI had already run out.

1.4.3 General Features and Neural Mechanisms of TI

1.4.3.1 Vertebrates

TI can be induced by various methods including repetitive stimulation, pressing, restraining, and turning of the subject. Thus, tactile, proprioceptive, equilibrium, auditory, and visual stimuli are all effective for elicitation of TI (Foley 1938; Gilman and Marcuse 1949). However, these stimuli are necessary but not sufficient for TI because a certain stimulus is effective in some animals but not in others (Ratner 1967; Klemm 1977). The motor system is not functional during TI as seen in the loss of righting reflex. Catalepsy is commonly observed during TI. Catalepsy is a phenomenon in which a limb that has been passively moved remains temporarily at the same position to which it was moved (Chaps. 2, 8 and 9). Simultaneous activation of two opposite types of muscle tonus, tension and relaxation, which is called plastic tonus or waxy flexibility, may underlie the induction of catalepsy (Hoagland 1928). In contrast, the sensory system is still functional to some extent during TI. There is considerable evidence showing that animals continue to monitor the environment and process information during TI (Gallup 1974). However, the effect of a strong stimulus that would otherwise cause pain has been shown to be weakened (analgesia) during TI, indicating that sensory inhibition is certainly exerted. It was shown that a subject abruptly awakes and that the activity that was ongoing before interruption recovers. The role of the autonomic nervous system in TI is still controversial because results of experimental studies have not been inconsistent (Ratner 1967; Hagenaars et al. 2014). See Chap. 11 for more information.

It should be pointed that some higher vertebrates including opossums (Weidron 1954) and hognose snakes (Edgren and Edgren 1955) show remarkably intricate TI as if they are appealing to be dead to predators, and these animals are often introduced in articles as if they are representative animals for TI. However, their immobility reaction is rather exceptional because it seems to occur somewhat intentionally (Chap. 11), unlike TI in most other animals, which occurs almost as a reflex.

1.4.3.2 Hypotheses of TI Based on Vertebrate Study

There are various views and theories regarding the mechanisms of TI in vertebrates. Proposed mechanisms include reflex, cerebral inhibition, hypnosis, sleep, confusion of space sensation, and paralysis caused by fear (Gilman and Marcuse 1949). Each may well explain the results of experiments conducted in a certain species by a certain method, but they are not necessarily applicable to other experiments using different species and different methods (Ratner 1967). Among the many studies that have been conducted on TI mechanisms, Klemm's work in rabbits is the most substantial and his theory is persuasive. He concluded that TI is a kind of reflex and that the center of TI is located in the posterior part of the reticular formation in the brain stem, which inhibits the motor system in the spinal cord during TI (Klemm 1971, 1977, 2001).

Pavlov's theory mainly consists of cerebral inhibition, hypnosis, and sleep (Edmonston 1967). The theory was based on the results of experiments with repetitive sound stimulation in dogs that were very different from experiments with the commonly used methods, that is, a sudden restriction or overturning using a variety of animal species. Hypnosis or/and sleep caused by hypothetical cerebral inhibition was indeed in immobile state in Pavlov's dogs, but his theory cannot be applied to most of the results of TI studies. Ratner (1967) wrote: However, little explanatory power is achieved by likening one process that is not understood to another process that is not understood.

Regarding the hypothesis of confusion of space sensation, Chertok reported that TI is a phenomenon that is caused by the separation of sensory-motor and motivation systems from the environment under the condition in which paralyzed animals cannot maintain their normal posture (Chertok 1968). He suggested that TI is a kind of regressive behavior that is caused by the evolutionally old nervous structure when the cerebral cortex becomes dysfunctional. It explains well some aspects of TI, but his hypothesis needs to be verified by strict experiments.

Finally, there is the fear hypothesis. This hypothesis was proposed by Preyer (1881) according to Gilman and Marcuse (1949). Fear is merely an explanatory concept to provide an integrative and heuristic perspective (Gallup 1974). Nevertheless, the fear hypothesis has been gaining support because of the fact that it is difficult to induce TI in tamed animals but easy to be induced in timid animals (Gallup 1974). It was reported that TI is similar to the defenseless state of a raped woman with fear, suggesting that fear is certainly an important factor for TI (Suarez and Gallup 1979; Marx et al. 2008). In experimental conditions with animals, however, TI can be elicited by a sudden overturning irrespective of fear. This fact indicates that fear is not a direct cause of TI but plays a role in modifying the center of TI via the limbic system (Klemm 1971; Woodruff 1977).

Here, the functional aspect of TI should be mentioned. TI seems to make the predator lose their interest in the prey or divert their attention so that it yields a chance for the prey to escape with minimum injury, and if this is not the case, the prey may be seriously wounded due to useless resistance even if it narrowly escaped (Sargent and Eberhardt 1975). However, this would be difficult to demonstrate because there are very few chances for encountering TI in a natural habitat (see Chap. 3). On the other hand, in experimental conditions in which the predator is allowed to attack the prey, there is the problem of cruelty. Thus, such experiments would be difficult to carry out for ethical reasons (Gallup 1974).

1.4.3.3 Insects

In insects, it is known that TI is elicited by contact, restraint, and substrate vibration. A number of examples are shown in the book Souvenir Entomologiques (Fabre 1900) and in some old reviews (Robertson 1904; Holmes 1906; Rabaud 1919;

Mangold 1920; Bleich 1928; Steiniger 1936; see Chaps. 2 and 7). The modern study of TI was established by Bässler who clarified the behavioral definition and introduced new physiological techniques using stick insects (Bässler 1983). The activities of muscles and motor units in stick insects were recorded during catalepsy (Godden 1972; Bässler 1983), and the properties of motoneurons and interneurons underlying catalepsy were analyzed (Driesang and Büschges 1993, Chap. 9). Later the sensory and motor systems responsible for the elicitation and maintenance of TI were intensively studied at the behavioral (Nishino and Sakai 1996, Chap. 7) and neural levels in crickets (Nishino and Sakai 1996, Chap. 8).

In general, it seems that researchers dealing with insects do not have an interest in proposing a hypothesis like hypotheses that have been proposed for vertebrates to explain TI mechanisms uniformly with one or a few principles but only have an interest in analyzing neural circuits underlying TI in a certain species. In the near future, freezing and TI in insects may be explained by differential controls over a common neural circuit depending on the stimulus condition and the internal state. Elucidation of the neurophysiology of TI in insects may provide important clues for studies in vertebrates. In addition, much progress has been made in functional study of TI in insects because of the easy handling of insects with no ethical restrictions (Chaps. 3 and 4). Even genetic analysis of TI has already been performed in insects (Chap. 4).

1.5 Naming

1.5.1 Freezing

The term freeze has a meaning of "crouch" in English. It is an intense and motionless posture. Freezing is commonly used in the context of a defensive reaction, though it is occasionally used in different contexts (Nieh 1993, see Chap. 6). The term freezing used in this book is a reaction (except TI) in which the prey stops all movements upon encountering a predator or danger and the absence of movement is maintained until the threat is gone. Although the definition of freezing (in a wide sense) is somewhat ambiguous because it sometimes includes a qualitatively different response such as orienting response, the term freezing represents well the state of immobility reaction in the context of defense behavior. To indicate that reaction, there does not seem to be better term than freezing. Thus, we use the term "freezing."

1.5.2 TI

The definition of TI is clear. However, an immobility reaction (except for freezing) that is the same as or similar to TI has been given various names including death-feigning, animal hypnosis, thanatosis, tonic immobility, catalepsy, cataplexy, fascination, entrancement, mesmerism, fright paralysis, and proximal inhibition (Gallup 1974; Cheyne 2016). There are about 30 different names (Gallup 1974). Some comments are given below to avoid confusion.

The terms death-feigning, animal hypnosis, thanatosis, and tonic immobility have been used most commonly in reports dealing with immobility behavior other than freezing. These terms seem to come from the appearance of an animal in an immobile state. The first three terms have some anthropomorphic nuance. To eliminate this nuance, the terms "immobility reflex" (mechanism-oriented) (Klemm 1977) and "contact defensive immobility" (function-oriented) (Woodruff 1977) were proposed, but neither of these terms has been used frequently. Although the term catalepsy is used, it is not a behavioral term but one of the attributes of the immobility reaction. Here, the appropriateness of each of the above four terms is considered.

Death-Feigning This term was first used by Darwin (1900). The meaning of the term is playing dead. It is intuitively easy to understand but at the same time somewhat misleading. That is, except for a few cases, the prey does not intentionally play dead and actually the prey in an immobile posture does not necessarily look dead. On the other hand, it is not known whether the predator assumes that the prey is dead. Therefore, the term death-feigning is scientifically not appropriate. However, it has some utility value because it contains the meaning of survival value in preys (Ratner 1967).

Animal Hypnosis The term hypnosis comes from "sleep" in Greek. The meaning of this term is also easy to understand, and animal hypnosis has therefore often been used in reports. However, hypnosis indicates a state of shallow sleep in humans, and animal hypnosis might therefore give the impression of human hypnosis, which is very different both in the method of induction and in the state of immobility from animal hypnosis (Klemm 1977). Thus, animal hypnosis is not appropriate for describing animal behavior.

Thanatosis The word thanatosis also comes from Greek. The meaning is "death." It is used in almost the same meaning as that of death-feigning and animal hypnosis. Although this single word is convenient for usage, it is not a common word and has a strong association of death. Thus, thanatosis is also not appropriate.

Tonic Immobility According to Hoagland (1928), the term tonic immobility (TI) was first used by Crozier (1923). If TI is taken simply to mean a continuous motionless state in animals, it can have many meanings including rest, sleep, hide, defense, ambush, alert, orient, and freeze. It was suggested that TI is not appropriate because the duration of TI is actually not so long in some cases (Klemm 1977). Nevertheless, TI has now become assimilated into the field of behavioral science in that it represents the same meaning as the meanings of the terms (death-feigning, animal hypnosis, and thanatosis) that have been used in academic reports (Gallup 1974). The term TI has recently been more commonly used (Marx et al. 2008; Volchan et al. 2017; Humphreys et al. 2018). For these reasons, TI is recommended in this book, but other terms are not necessarily excluded. Other terms are used depending on the context.

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Chapter 2
Historical Review on Thanatosis with Special Reference to the Work of Fritz Steiniger

Hans-Joachim Pflüger, Ansgar Büschges, and Ulrich Bässler

Abstract Fritz Hermann Steiniger (1908–1985) was among the first to study in detail thanatosis and catalepsy in insects, in particular stick insects and water striders. He looked at these phenomena from a behavioral point of view and tried to unravel underlying neuronal mechanisms. In particular, he examined which kind of sensory stimuli are required to induce such an akinetic condition in insects. His studies also led him to explain and define many of the different terms of akinetic and immobile states which existed at that time and ranged from death feigning, tonic immobility to reaction inhibition, and even animal hypnosis.

Keywords Catalepsy · Flexibilitas cerea · Thanatosis · Tonic immobility · Reflex activation · Central nervous system · Protective reflex

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2.1 Introduction

Fritz Steiniger[1] was one of the first authors, who dealt with catalepsy in stick insects[2] in detail. His doctoral thesis was concerned with the phenomenon of catalepsy in stick insects and water striders. He published his main results in *Zeitschrift für Morphologie und Ökologie der Tiere* (*Journal of Morphology and Ecology of Animals*; Steiniger 1932/1933). The phenomenon of "catalepsy," particularly known from stick insects and water striders, is generated by a mechanical stimulus leading to akinesis and "Flexibilitas cerea" ("waxous motility") and according to earlier studies was also believed relating to the protective stick posture (Schleip 1911; Schmidt 1913). Explanations ranged from cataleptic state being the outcome of an "inner motivation" (Schmidt 1913) to being induced by light resulting in a cataleptic posture ("akinesis") whereas movements of these nocturnal animals only occur in darkness. Steiniger gives the following definitions: Akinesis (immobility); Catalepsy, where muscles are still with a tone; Animal Hypnosis (immobility), increasing sensory thresholds, painless state, no correction of postural displacement. After Heymons and v. Lengerken (1926) the opposite to hypnosis is thanatosis, the latter being an active reaction of the animal to avoid predation. What is a cataleptic state in the stick insect? A passively displaced leg stays apparently motionless in this new posture but in fact returns very slowly to the previous set point. The return motion is so slow that animals and humans alike will not notice it. Bässler vividly compared cataleptic movements of the stick insect with the velocity of the hour hand of a watch (Bässler 1983).

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[1] Fritz Steiniger (1908 to 1985) was a scientist during politically difficult times in Germany. He did his doctoral studies at the University of Greifswald with Prof. Günther Just. He remained there as a private docent and later, in 1945, was promoted to apl-Professor (extraordinary professor). He seemed to have been very close to his supervisor as Steiniger and Just made similar career decisions and took similar career paths. Steiniger was a member of the NSDAP (National Sozialistische Deutsche Arbeiter Partei, National Socialist German Workers' Party, commonly known as the Nazi-party) and chose to become a civil servant like his former supervisor Just. During this period, he served in various functions in the Reichskommissariat Ostland (Administration of Eastern provinces) that comprised tasks like "pest control and racial policy." In fact, Steiniger and Just worked in the field of hereditary science and anthropology in the most prominent institute in Berlin. The work of this institute was devoted to the scientific justification of the racial policies of the Nazi-regime. Judged from today's perspective this was a task that certainly would have resulted in such a person not being allowed to continue with his career after the outcome of the second world war and the atrocities of the Nazi-regime. It should, however, also be noted here that reports exist, that Steiniger had rescued individual Jews. This may have aided him in continuing with his career after the war as a civil servant. Finally, he lectured at the Tierärztliche Hochschule Hannover (University of Veterinary Science). For his former supervisor Just, this clearance took longer, but in the end, he became a professor at the University of Tübingen after the war.

[2] Many of the experiments cited in the following article have been performed in the stick insect *Carausius morosus*. This species may be called a laboratory animal, because today it only lives in laboratories. All cultured *Carausius morosus* are descendants of a few animals that were found 1897 in India (Leuzinger et al. 1926). Since *Carausius morosus* has obligatory parthenogenesis, all animals are females which exclusively propagate by parthenogenesis. This was perhaps the reason, why it was kept in private houses hundred years ago, because children would not ask "how descendants are made.".

2.2 Which Kind of Stimuli Induce or Abolish the Cataleptic State?

Light influences a cataleptic state and the stick posture and, therefore, a circadian rhythm is thought to underlie these states. It is also noted that stick insects have two periods of feeding activity: one after sunset and the other before sunrise. Interestingly, approx. 10% of tested animals also reveal catalepsy during a quiescent resting period around midnight. A behavioral condition abolishing a cataleptic state is hunger and corresponding to this, the presentation of food odors yields the same result. Strong mechanosensory stimuli, for example, to antennae or abdomen, also abolish the cataleptic state and induce escape movements. However, animals show great variability in their responses to mechanical stimuli and in as much as they induce or abolish a cataleptic state. However, Steiniger dismisses suggestions by Rabaud (1919) who made a difference between induced and spontaneous catalepsy and described the latter as related to sleep. The cataleptic state is also influenced by the age of the animals with young animals moving much more than old animals. This may suggest that the cataleptic state is “not yet fully” developed. However, Steiniger notes that nymphs are sensitive to light like adults and that they also have a protective stick posture. Steiniger also mentions that the cataleptic state is accompanied by analgesic conditions as in deep catalepsy a very high threshold to nociception seems to exist.

2.3 Localization of “Center of Cataleptic State”

The cataleptic state is associated with the central nervous system as destruction of the cerebral ganglion (“brain”) completely abolishes the cataleptic state. Similar observations were already made by previous authors like Schmidt (1913) and Reisinger (1928). Schmidt (1913) cut both connectives between the first and second thoracic segment and could induce catalepsy only in the prothoracic leg which still had a connection to the cerebral ganglion. Reisinger (1928) however found that the severed posterior part had still the ability to show catalepsy which was not observed in Steiniger’s experiments and therefore, he agrees to the findings of Schmidt. Without a cerebral ganglion (decerebrated animals), the gnathal ganglia (former suboesophageal ganglion) are not capable to induce a cataleptic state (Reisinger 1928). Within the cerebral ganglion the most important area is the Central Complex (CC), as an intact CC is necessary for the cataleptic state (Steiniger 1932/1933). Steiniger suggests that the state of catalepsy may vary in its intensity with the deepest cataleptic state being the stick posture and the weakest cataleptic state being “full” movement capability. In this view all transitional states are possible, and the actual degree of catalepsy depends on how all external and internal influences (stimuli) contribute to it. Thus, catalepsy is the net result of “catalepsy-promoting and catalepsy-inhibiting” influences. Very similar conclusions were found for the other insects, water striders, which show similar behavioral conditions of catalepsy with the deepest cataleptic state corresponding to the animals taking a protective posture spontaneously. As most water striders touch the underground with their ventral side this was also considered as Thigmotaxis. In the cataleptic state water striders do not react to optical stimuli which they usually do. In addition, cold temperatures seem to favor the cataleptic state in water striders.

2.4 The Ecological Importance of the Cataleptic State

The stick posture was considered as a mimesis and protective against attacks by birds and lizards as was supposed for the conspicuous rocking movements of stick insects (Rupprecht 1971; Pflüger 1977). Both catalepsy and rocking movements serve the task of hiding the insect in its environment from predators.

2.5 Evolutionary Considerations

In comparing stick insects and water striders, Steiniger asks whether catalepsy is an inherent trait of more ancient insects, for example, the common ancestors of stick insects and water striders, or whether it has evolved as a kind of “stick mimicry”? Steiniger mentions that there may have been an increased likelihood of similar mutations in the Orthopterans and Hemipterans due to their common origin and, perhaps, a stick posture was formed under similar selection constraints in Mantids, Phasmids, Acridids, and Hemipterans. He argues that a stick-like body-morphology will favor catalepsy and, dependent on the length of the body, the cataleptic state will increase in intensity as will be a tendency for a mimetic stick posture. This is supported by studies on Proscopiids, an insect group, which has evolved twig mimesis independently of phasmids (Wolf et al. 2001; see below).

2.6 Definitions of Various Animal Immobilities by Steiniger

In his large article on “animal hypnosis,” Steiniger (1936, Fig. 2.1) gives the definitions for various aspects discussed at the time. Those are (with some modifications by the authors of this article): “Animal hypnosis” (*Tierische Hypnose*), in analogy to human hypnosis not a valuable term according to Steiniger; Akinesis (*Bewegungslosigkeit*), no movements, motionless; Reflex immobility, immobilization (*Reflektorische Bewegungslosigkeit*), only inducible by an external stimulus to which no reaction (movement) follows; Thanatosis, death feigning (*sich totstellen*), protective posture to avoid predation (Steiniger does not like this explanation and completely dismisses it); “Mechanohypnosis,” a special case of hypnosis, following

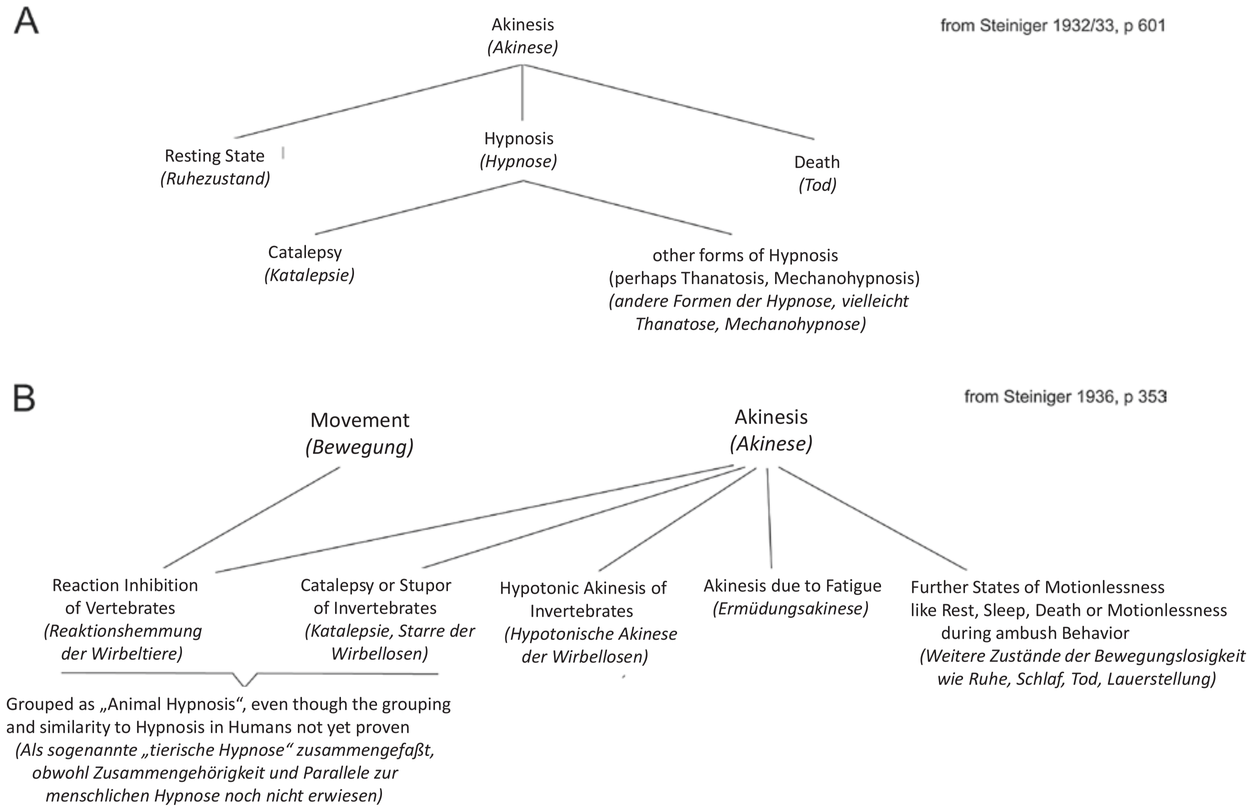

Fig. 2.1 (**a**) Steiniger’s attempt to put catalepsy into a context of other forms of motionlessness in his 1932/1933 article in “Zeitschrift für Morphologie und Ökologie der Tiere (*Journal of morphology and ecology of animals*)”. His understanding of catalepsy is: akinesis with increased muscle tone and flexibilitas cerea, and additional observation of decreased reflex excitability, analgesy and lacking ability for correcting position in space. (**b**) Extended version of the relationship of terms (and behaviors) discussed in his 1936 article in” Ergebnisse der Biologie (*Results in Biology*)”

a mechanical stimulus a movement reaction is lacking; Reaction-inhibition of vertebrates (*Reaktionshemmung*), exertion of movement is prevented (this is what Steiniger favors to the term hypnosis); Cataplexy (*Schreckstarre*; Preyer 1878, cited in Steiniger 1936): equivalent to stupor (daze) (as a result of fear, "Angst"), Catalepsy (*Katalepsie*); here defined as a particular state of the musculature leading to *flexibilitas cerea (wächserne Biegsamkeit)*, Analgesia (*Analgesie*); absent feeling of pain during normally painful actions (in the state of "hypnosis" also reduced pain sensation); Hypnotic akinesis (*Hypnotische Akinese*), muscle tone more relaxed than during catalepsy (but rather weak definition as differences between vertebrates and invertebrates are not well explained); Stupor (*Starre*), a result of many different conditions such as tetanus, toxins, death (rigor mortis), or cataleptic conditions (?); Akinesis due to fatigue (*Ermüdungsakinse*), a particular muscular condition. Tonic reflexes (*Tonische Reflexe*), long lasting (tonic) contractional state of vertebrate musculature.

2.7 Conditions for Inducing Immobility in an Animal

The initiation of the "hypnotic state" is very similar in many different phyla: after the animal is quickly turned on its back it often remains motionless in this posture (but not necessarily). A good example is when a chicken is pushed to the ground and its head stretched and pushed to the ground as well and held for some time, it may remain in this motionless posture for a while. Steiniger mentions that some sensory and motor reactions persist, including picking and vocalization. As some animals close their eyes during this period, some authors have described the inhibition of reaction as related to sleep, and corresponding to this, a black head cover renders many birds motionless. Some authors described the cause of such absence of motion as an instinct released by fear, particularly in squirrels and opossums. In mice and rats immobile postures induced by fear, mainly by the sight of a predator, were named "freezing."

2.8 Problems with the Term "Animal Hypnosis"

Steiniger is very critical as far as the word "hypnosis" is concerned and takes a very cautious approach to the claims of previous authors who suggested to have hypnotized vertebrate animals by fixing their eyes and holding them tightly or forcing them to gaze on a light spot or object. He also reports on horses that apparently were "hypnotized" by special hand movements and eye contact. The best correspondence to human hypnosis should occur in primates and indeed there are corresponding reports. Steiniger concludes, however, that the experiments do not allow to distinguish true hypnosis, in the human sense, from sleep or inhibition of reaction (*Reaktionshemmung*). The latter term is preferred by him. Particularly enlightening is Steiniger's response to the book of F. Völgyesi on Human and Animal Hypnosis (Völgyesi 1938). In his reply (Steiniger 1940), he clearly makes a difference between people like Völgyesi who look at hypnosis not from a scientific and physiological point of view but rather regard it as unveiling some more mysterious and psychic forces unexplained by natural sciences, and those relying more on science-based explanations. Accordingly, Völgyesi misinterpreted all examples where animals where immobilized by some hypnotic action, often with added ingredients of *charlatanry*, as true hypnosis, although explanations such as reaction inhibition or training of animals could not be excluded and, according to Steiniger, would have been more appropriate. Correctly, Steiniger pointed out that Völgyesi completely misinterpreted the work of Pavlov who looked at nervous systems and behavior from a physiological and exclusively scientific point of view (Pavlov 1926).

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Chapter 3
The Function of Tonic Immobility: Review and Prospectus

Atsushi Honma

Abstract Death-feigning (also called tonic immobility [TI]) is defined as a specific immobile defensive state elicited during the later stage of the attack sequence of predation events. Although TI has long been of interest to biologists, there is little research on its function. In general, the study of predator avoidance behavior is associated with technical and ethical difficulties. This chapter identifies two other reasons impeding research on the function of TI: (1) confusion with similar concepts and (2) implicit assumptions due to the meanings contained in the terms. This chapter emphasizes the importance of properly defining TI and discussing TI in the context of behavioral interaction between predator and prey to avoid these problems. Then, several case studies, where the revealed functions of TI were not what would normally be expected, are introduced. Future developments in the study of TI are also discussed.

Keywords Tonic immobility · Function · Anti-predator defense · Stages of predation · Secondary defense

3.1 Introduction

Death-feigning or tonic immobility (TI) has long been reported in many taxonomic groups and has been studied physiologically, mainly in vertebrates (see Chap. 1); however, behavioral and evolutionary studies have been limited and anecdotal. Ruxton et al. (2004) noted that very little research on the function of TI had been undertaken, which has promoted recent studies on the function of TI (e.g., Humphreys and Ruxton 2018a; Ruxton et al. 2018).

In general, the study of predation and predator avoidance behavior is associated with technical and ethical difficulties, making it difficult to directly observe

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predatory events, especially in the field. It might be relatively easy to observe such events indoors; however, we do not know whether these are natural (e.g., Are proper predators used? Do predators and prey behave the same way as in the field?). In addition, conducting predation experiments for direct observation of predatory events raises ethical issues (especially in vertebrates). There are two other problems specific to research on the function of TI: (1) confusion with similar concepts and (2) misunderstandings due to the meanings contained in the terms.

In this chapter, I will first clarify these two issues that should be considered when studying the function of TI. Then, examples of recent studies on the functions of the three types of TI conducted in insects are presented. Finally, future directions in TI research are discussed.

3.2 Problems in the Study of the Function of TI

3.2.1 Confusion with Similar Concepts

TI is a type of prey immobility behavior that occurs during predator–prey behavioral interactions. A similar immobility behavior is called freezing, with which TI has often been confused (see Chap. 1). However, the two can be distinguished from each other using multiple features.

To begin with, these two behaviors are activated during different stages of predation. Edmunds (1974) categorized predator avoidance behaviors into two categories: primary defense invoked before the predator finds the prey and secondary defense invoked after the predator has started attacking the prey (Fig. 3.1). Endler (1991) further refined the steps of predatory behaviors into six stages, organizing each predator avoidance behavior as a tactic that inhibits the predator from moving

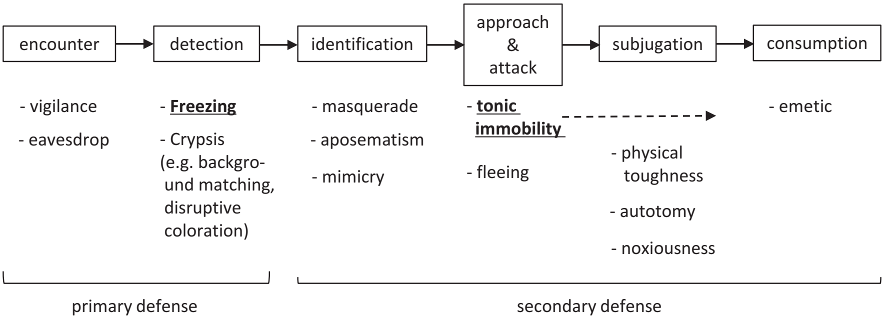

Fig. 3.1 The six stages of predatory behavior and anti-predator behavior at each stage (based on Endler 1991). TI is triggered during the approach and attack, or subjugation stages, whereas freezing is triggered during the detection stage. Freezing is one of the primary defenses performed before the predator detects the prey, whereas TI is one of the secondary defenses performed after the predator detects the prey (Edmunds 1974)

to the next stage (Fig. 3.1). These organizational methods are also valid for distinguishing between freezing and TI (Fig. 3.1). Freezing is triggered during the early stages of a predator encounter, detection, and identification, and is believed to prevent the predator from attacking (primary defense, Fig. 3.1). TI is triggered by direct contact or indirect stimulation by a predator attack and may function to prevent subjugation and consumption (secondary defense, Fig. 3.1). Generalized predation methods are used during the early steps in the predation sequence whereas specialized methods are used during the later stages (Endler 1991). Thus, it would be expected that the defensive mechanisms of TI are more specialized than those of freezing (but see a specialized defensive mechanism of the frog to a snake predator in Nishiumi and Mori (2020)).

The physiological conditions at the time of these behaviors also make it possible to distinguish between freezing and TI. A freezing animal remains immobile without changing its posture. In contrast, because TI is accompanied by rigidity, animals in the TI state are generally unable to maintain a self-standing state and a change in posture occurs. Physiological changes, such as a decrease in respiratory frequency, may also occur in TI (see Chap. 1).

3.2.2 Misunderstandings Due to the Meanings Contained in the Terms

In this book, the term TI, which is more neutral, is used as a general term for death-feigning (see Chap. 1), but other terms have also been used for this behavior, e.g., feign death, play dead, playing possum, thanatosis, and animal hypnosis. The first three of these mean “to pretend to be dead, to pretend to be a possum, an animal that pretends to be dead,” and thanatosis also has the meaning of death. Animal hypnosis has mainly been used in physiological studies with vertebrates and has not usually been used in the context of predator avoidance. Essentially, TI is behavior defined by behavioral and physiological mechanisms, and thus its function is not self-evident (see also Humphreys and Ruxton 2018a). However, the use of terms containing death or pretending death leads to the implicit assumption that its function must also be to increase the probability of survival in a state where the prey appears to be dead from the point of view of the predator. This implicit assumption may lead one to conclude that any phenomenon that does not conform to the assumption cannot be called death-feigning (i.e., TI). The author experienced this twist when I submitted a paper on the function of TI by a grasshopper toward frogs (see Sect. 3.3.1). A reviewer of the journal commented, “To begin with, death-feigning involves the behavior of blinding or tricking a predator by becoming immobile. Therefore, this study did not involve death-feigning.” However, it is unreasonable to assume that the function of TI is to escape attack from a predator by becoming immobile as if it were dead, if one takes the point of view of the predator (Ruxton 2006). TI is triggered when a predator approaches or makes contact with a prey to capture it. In that case, the predator is considered to have already detected and recognized the prey as food. Therefore, for the predator avoidance behavior as described above to work, we must assume a situation in which the predator abandons the food it caught because it has just died. There are cases such as opossums who disguise their poor quality as a food item by emitting a rotten odor during TI; however, in many other cases, it must be assumed that the predator remains maladaptive and abandons its prey because it is only just now dying, although the predator will kill the prey to consume it.

Humphreys and Ruxton (2018a) claim that the behavioral functions of TI should be unique to it and do not consider those that are thought to have functions in common with other predator avoidance behaviors to be functions of TI. However, I think that this thinking might lead to the same type of twist as above, whereby TI, which is originally defined in terms of behavioral and physiological aspects, is excluded because its functions do not fit the assumed functions. In this chapter, I presume that TI has been clearly defined in terms of behavioral and physiological aspects and includes a wide variety of functions (including those that overlap with other predator avoidance behaviors). In the following, the functions of TI in invertebrates that have been revealed so far will be introduced with examples from the author's own research.

3.3 Case Studies of the Function of TI

3.3.1 Specialized TI Posture for Gape-Limited Predators

Criotettix japonicus is a large (15–20 mm in body length) pygmy grasshopper species that inhabits the ground surface of paddy fields and other wetlands. It has morphological features such as an extremely long, hard pronotum with a pair of lateral spines (Fig. 3.2). The pygmy grasshopper exhibits TI when attacked by sympatric predators, e.g., frogs. If a frog attempts to swallow the grasshopper in its mouth,

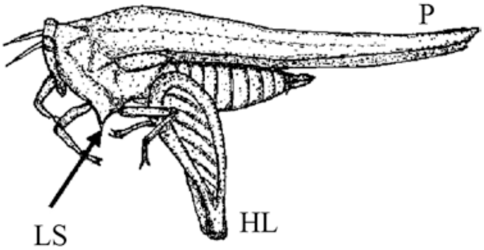

Fig. 3.2 The TI posture of the pygmy grasshopper. During TI, the grasshopper bends its hind legs (HL) downward to form a T-shape with the pronotum (P). The grasshopper has a pair of lateral spines on its side of thorax (LS). This figure is reprinted from Honma et al. (2006) with permission by the Royal Society London

then the grasshopper initiates TI and if the frog spits out the grasshopper, it continues TI for a time.

3.3.1.1 Defensive Mechanism of TI

During TI, the pygmy grasshopper adopts a characteristic posture in which it bends its hind legs downward (Fig. 3.2), which is distinguishable from freezing (i.e., when the frog turns around in response to the grasshopper's movements to eat food or drink water, the grasshopper becomes immobile for a time, but in this case of freezing the grasshopper's posture remains the same). The grasshopper only initiates TI after the frog has caught it and just before being swallowed. At this point, there is no requirement for the grasshopper to trick the frog by pretending to be dead and there is no reason for the frog to give up swallowing the food. Therefore, it is assumed that TI was preventing the frog from feeding for some other reason. It was thought that the posture assumed by the grasshopper during TI (Fig. 3.2) was physically preventing the frog from swallowing the grasshopper. Therefore, the hind legs of the grasshopper were tied to the pronotum with a very thin fishing line so that the frog could not assume the TI posture. When the frog attacked the tied grasshopper, the grasshopper was easily swallowed by the frog. In this study, the author and colleagues discovered that the direction in which the grasshopper was held in the frog's mouth changed when its legs were tied to its pronotum (Honma et al. 2006). The frog swallowed the grasshopper in the TI posture in a lateral position (Fig. 3.3a–c; see movie at Honma 2004a); however, if the grasshopper could not assume the TI posture, the predator changed the direction of the prey to the dorsal position (Fig. 3.3d) and easily swallowed it up (Fig. 3.3e, f; see movie at Honma 2004b). When the grasshopper was held sideways in the frog's mouth (i.e., the lateral position; Fig. 3.3a), the spines on the side of the grasshopper's thorax moved to a position where they could be propped up against the frog's upper and lower jaws (Fig. 3.3b). As a frog swallows its prey, it pumps the prey into the back of its throat by pushing it with its upper and lower jaws, which is thought to make the grasshopper's spines more likely to stick.

3.3.1.2 Effectiveness of the Defense

The experiments have shown that the inhibition of swallowing in the TI posture is particularly effective for small frogs (Honma et al. 2006). Therefore, is the effectiveness of this TI limited? To address this question, we collected 316 individuals of *Pelophylax nigromaculatus* (snout-ventral length [SVL] 20.70–71.28 mm) in the field and used a non-invasive method to extract their stomach contents (Hirai and Matsui 1999) to investigate how often the pygmy grasshopper was being fed on by frogs. We found grasshoppers in the stomach contents of only three frogs, which measured 42.42, 43.31, and 38.66 mm in SVL (Fig. 3.4). It was estimated that the maximum that a new adult frog can grow in a year is approximately 40 mm and

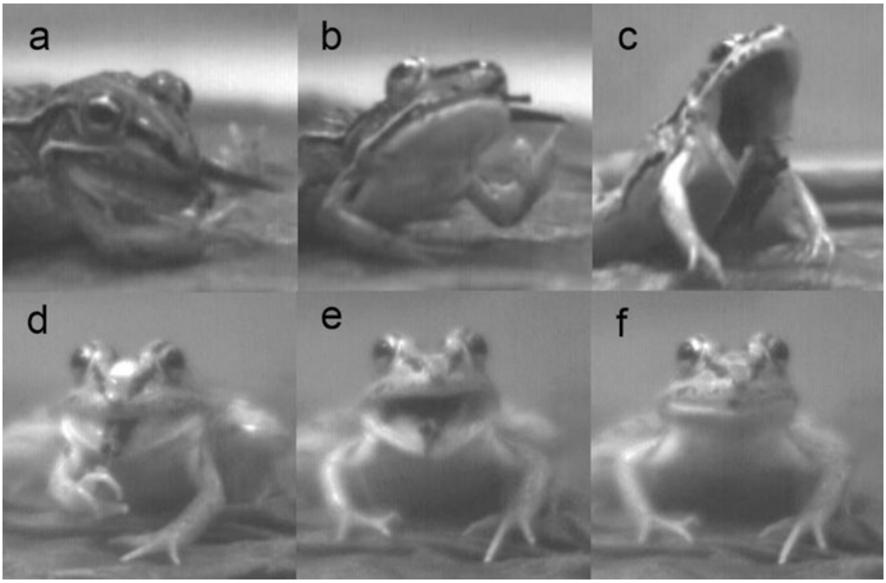

Fig. 3.3 Inhibition of swallowing by TI of the pygmy grasshopper. The grasshopper assumes the TI posture when sucked by the Japanese pond frog, so the frog bites the grasshopper on its side (**a**). The lateral spines of the grasshopper pierce the upper and lower jaws of the frog's mouth (**b**), and the frog spits the grasshopper out (**c**). The grasshopper remains in the TI posture for a while, but recovers when the frog undergoes a shudder that moves in response to other prey. If the hind legs are tied to the pronotum to prevent the grasshopper from assuming the TI posture, then the frog adjust the direction of the grasshopper so that it can easily swallow it (i.e., the direction in which the spines do not pierce it), and then swallows it (**d**–**f**)

>80% of individuals collected at this time were <40 mm in length (Fig. 3.4). Therefore, TI is very effective as a predator avoidance behavior for the frog, considering that the density of the grasshoppers at the study site was high (1.63/m^2). The experimental results indicated that TI was not very effective in inhibiting swallowing for frogs with larger body sizes. However, such large frogs did not exploit the grasshoppers as food in the field. This might be because the frogs select a size of food that is more efficient in foraging according to their own body size (Hirai 2002). Therefore, TI might be effective as a predator avoidance behavior, even if it is expected to have a defensive effect only against small frogs that use the pygmy grasshopper as food.

3.3.1.3 Predator Specificity of TI Defense

Honma and colleagues confirmed that this immobile behavior only occurred for frogs among potential sympatric predators. When they conducted predation experiments with predators such as Japanese quail, *Coturnix japonica* (a model avian predator), Japanese pond frog, *P. nigromaculatus*, praying mantis, *Tenodera*

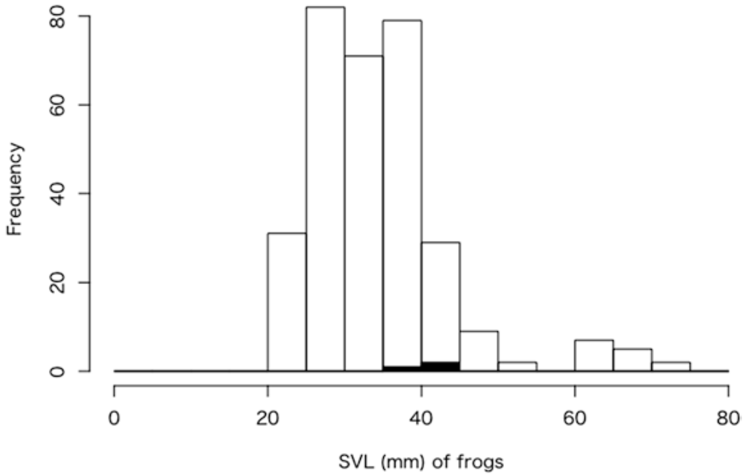

Fig. 3.4 Frequency distribution of the snout-ventral length (SVL) of the black-spotted pond frog, *Pelophylax nigromaculatus* collected in the study field ($n = 316$). A sharp decrease is seen beyond 40 mm. The pygmy grasshopper was detected from the stomach contents of only three frogs (filled bars)

Table 3.1 Response of the pygmy grasshopper, *Criotettix japonicas,* to four types of predators (Japanese quail, *Coturnix japonica*, Japanese pond frogs, *Pelophylax nigromaculatus*, praying mantis, *Tenodera angustipennis*, and wolf spiders, *Pardosa pseudoannulata*). Irrespective of the level of danger of each predator, the grasshoppers exhibited TI only to the frog. Different lowercase letters indicate a significant difference (Fisher's exact test with Bonferroni correction, $p < 0.001$)

Predator	Attack	Tonic immobility	Predation success
Bird	10	0^b	2
Frog	20	17^a	4
Mantis	9	0^b	8
Spider	20	0^b	0

This table has been reproduced from Honma et al. (2006), with permission by the Royal Society of London

angustipennis, and wolf spider, *Pardosa pseudoannulata*, all the grasshoppers attacked by the praying mantis were preyed upon; however, the grasshoppers exhibited TI only to the frog (Table 3.1). Therefore, TI of the pygmy grasshopper is a specialized defensive behavior against frog predators.

3.3.1.4 Generality of Function

The function of TI observed in the pygmy grasshopper is to inhibit predation by the swallowing predator by stiffening up in a characteristic rigid posture. However, this function of TI is not limited to the grasshopper. When attacked by rainbow trout (*Oncorhynchus mykiss*), nymphs of the stonefly, *Pteronarcys dorsata*, assume TI by curling up in a posture with their cerci to project outward-like spines. This TI is thought to be a specific predator avoidance behavior for fish predators because the stonefly nymph avoided predation by crayfish by releasing repellent substances (Moore and Williams 1990). When the stonefly nymph assumed a TI posture, the rainbow trout could not swallow it, but when given a truly dead individual, it easily swallowed it (Moore and Williams 1990). Therefore, the TI-inhibited predation by the rainbow trout, not because of its apparent deadness, but because its characteristic rigid posture, inhibited predation by the gape-limited predators. It has also been reported that when the cerci of a stonefly nymph were excised and presented to rainbow trout, the stonefly nymph was easily preyed upon (Otto and Sjöström 1983).

In many cases of TI by invertebrates, the rigidity of the posture during TI is different from the deflated posture assumed by truly dead individuals and partial body prostration is often observed. This characteristic rigidity posture of TI has been treated only as an indicator that the animal is in the state of TI; however, if the animal is essentially “pretending” to be dead, its posture should also resemble that of the dead individual (indeed, this is the case for the opossum). The results of Honma et al. (2006) at least partially resolved this discrepancy by providing a case in which the rigid posture itself has a function of predator avoidance.

3.3.2 Instantaneous Switching of Defensive Coloration

The wood tiger moth, *Parasemia plantaginis*, is a member of the family Arctiidae, which is widely distributed in the Palearctic region and has warning colors. It is often observed on foliage in the field, where it is highly conspicuous owing to the high contrast between its body coloration and the background (Fig. 3.5a). When approached by humans, they often exhibit TI and drop to the ground. Under normal conditions, the moths have their wings folded in a roof-shape (Fig. 3.5a); however,

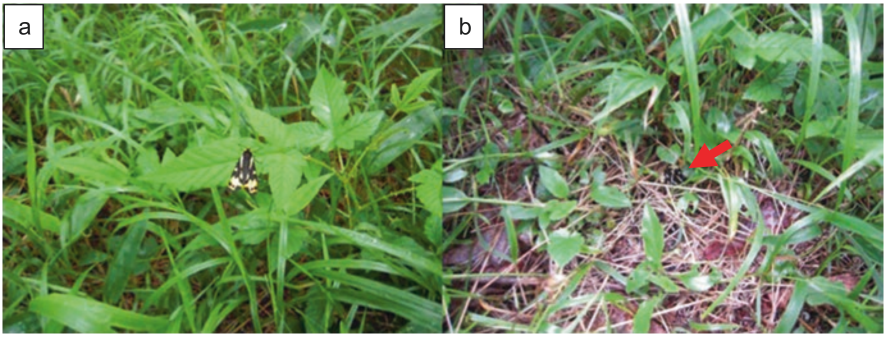

Fig. 3.5 The wood tiger moth often basks on foliage and is very conspicuous (**a**), but when approached, it assumes TI and drops to the ground. Dead grass and soil provide a background for this species, which, combined with the fragmented coloration of the forewings, makes it very difficult to spot (**b**). The photograph shows an individual perched on grass (**b**) that fell to the ground after TI (the arrow in **b**). This figure is reprinted from Honma et al. (2015) with permission by John Wiley & Sons

the angle changes to horizontal during TI (Fig. 3.5b). When the moths drop to the ground, dead grass, fallen leaves, and the ground are their main backgrounds, reducing the contrast with their body coloration and making them very difficult to find (Fig. 3.5b).

Honma et al. (2015) experimentally tested whether the forewing patterns of the wood tiger moth have a camouflage effect called disruptive coloration, where relatively high-contrasting patterns are arranged in contact with body contours, which break up the outline of a prey and hinder them from being detected or recognized by predators (Cuthill et al. 2005; Stevens and Merilaita 2009). They crafted artificial prey models printed with forewing patterns of the moth and conducted a large-scale laboratory experiment where they had great tits, *Parus major,* find the prey items

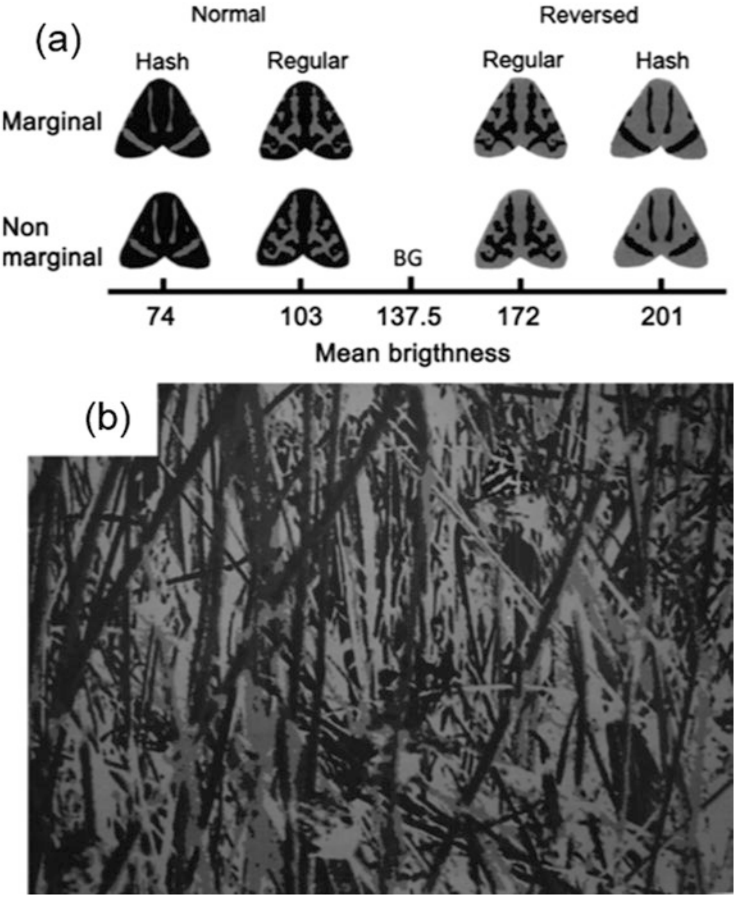

Fig. 3.6 The artificial prey items imitating two extreme types (“Hash” and disruptive “Regular”) of the forewing pattern of the wood tiger moth, *Parasemia plantagenis* (**a**). A prey item placed on the artificial background crafted using the image of natural background (arrow in **b**). This figure is reprinted from Honma et al. (2015) with permission by John Wiley & Sons

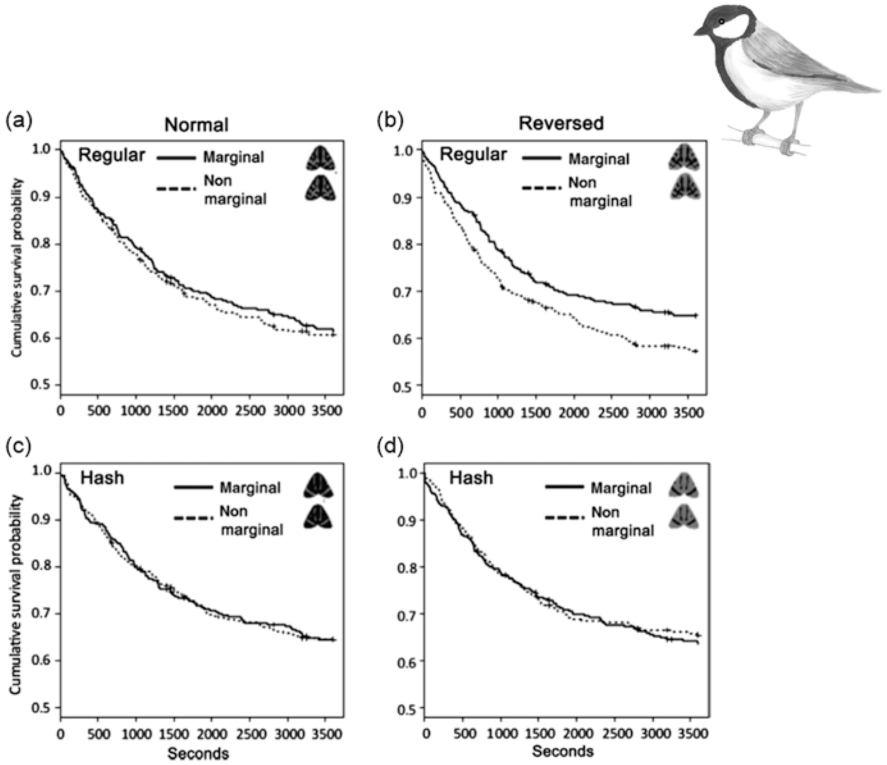

Fig. 3.7 The results of a large-scale indoor experiment where the artificial prey items imitating the forewing pattern of the wood tiger moth, *Parasemia plantagenis* were presented to great tits, *Parus major*. When treated to be non-marginal, the prey item with the "disruptive" pattern that had a higher contrast to the background was attacked significantly more compared to its marginal counterpart (**b**). Such an effect of marginal pattern on survival rate was not observed when the color of the prey item was less contrasting to the background (**a, c**) and the pattern was less disruptive, "Hash" (**c, d**). This figure is reprinted from Honma et al. (2015) with permission by John Wiley & Sons

(Fig. 3.6). The results showed that the prey items where the marking was manipulated to not touch the edge (a non-marginal pattern) were attacked significantly more than those with a marginal pattern (Fig. 3.7b). Thus, the defensive function of the forewing pattern of the wood tiger moth was the disruptive coloration. The disruptive function was apparent only when the prey item was brighter than that of the background. Survival rate of the prey item was not significantly different between marginal and non-marginal when the body color was less contrasting to the background (Fig. 3.7a), suggesting that the effect of the disruptive color was more pronounced when the contrast with the background was relatively higher.

The defense function of warning coloration is acquired by learning of the predators to associate the unpalatability of the prey with its conspicuous body color. Therefore, warning coloration is less effective against predators that have not learned the unpalatability of aposematic prey or do not have a problem with the chemical defenses possessed by the prey. The function of TI, as seen in the wood tiger moth, might be to drop to the ground when attacked by these predators and revert the predation step back to the previous one, which is also effective for these predators (i.e., from attacking to detection; Fig. 3.1). Essentially, warning colors, which make the body color stand out from the background, and concealment colors, which make the body color blend into the background, have contrasting functions; however, TI may function to reconcile the two by switching the background instantly.

This strategic compatibility of warning and disruptive coloration might facilitate the evolution of warning coloration. During the early stages of the emergence of conspicuous mutants in a population of cryptic unpalatable prey and their spread within the population, individuals with conspicuous phenotypes are more likely to be found by predators than those of cryptic one. In addition, since predators need to sacrifice a certain number of prey individuals to establish avoidance learning, conspicuous individuals should receive higher per capita mortality because they are in the minority (Ruxton et al. 2004). Therefore, the fitness of the conspicuous phenotype is lower than that of the cryptic phenotype until they reach a certain number of individuals (the so-called fitness valley). If they evolve to warning coloration via disruptive coloration where a relatively high-contrasting body color prevents the prey from being detected by predators rather than evolving directly from cryptic coloration (e.g., background matching) to conspicuous warning coloration, then the fitness valley would be much shallower.

3.3.3 Proactive Dropping and TI as a "Side Effect"

Insects perched on a substrate such as foliage often drop and engage in TI behavior when they are approached. Such instances are particularly common in beetles. Dropping from a substrate is very effective as a means of quickly escaping from an approaching predator and TI on the ground after dropping is effective in making predator detection difficult (e.g., ladybirds drop with their dark ventral surface facing upwards when they land, making them very difficult to detect). However, insects have a neural mechanism called tarsal reflex. When the tarsi of the flying insect touch an object, the insect stops flapping its wings, but starts flapping again when the tarsi are separated from the object. This mechanism prevents the insects with flying ability from a natural drop. Therefore, this type of TI might have originally evolved to inhibit the tarsal reflex of the insect and allow it to drop naturally. Once triggered, TI cannot be deactivated for some time; therefore, insects that drop to the ground remain stuck in an immobile state for a time. Features that increase the concealment effect after dropping, such as those seen in the wood tiger moth, may have evolved with this pre-adaptation in place.

Avian predators, which are one of the major predators of insects, first frighten insects into taking off and then attack them immediately afterwards (e.g., Davies 1977), which may be due to avoiding the risk of striking the substrate themselves. For an insect that is trying to escape in flight, immediately after take-off is the slowest and most vulnerable moment. In my childhood, I once "helped" a beetle that was struggling on its back on the ground and threw it up in the air to let it fly; however, at the moment it began to spread and flap its wings, a bird flew out of the trees, caught it, and flew away with it in its mouth. Because escape by flight is dangerous at the initial stage of flight, it may be advantageous for insects to assume TI to inhibit the tarsal reflex and drop to the ground. Even if the predator does not lose sight of the prey even after dropping, it would be inefficient for the predator to follow it down to the ground (Humphreys and Ruxton 2018b) and the predator would be at risk of being attacked itself.

However, for small insects, dropping from a substrate may not always be the best tactic because it is associated with several fitness costs and it may increase the risk of being attacked by other predators (see the review by Humphreys and Ruxton (2018b)). Therefore, one would expect TI to evolve more likely in species that obtain more benefit from escaping by dropping than by flying, i.e., species with poor flying ability. Empirically, entomologists have recognized that beetles with poor flying ability are more likely to engage in TI, but this has not been tested to date. In future studies, this hypothesis could be tested using the phylogenetic comparative method (Harvey and Pagel 1991). Ohno and Miyatake (2007) have demonstrated a negative genetic correlation between TI susceptibility and flight ability in the azuki bean weevil.

3.3.4 Other Studies on TI Functions

Other types of functions of TI in beetles have been reported elsewhere. Miyatake et al. (2004) tested the function of TI in the red flour beetle using Adanson's jumping spider as a model predator. After attacking the walking beetle, the jumping spider released it once. If the beetle engaged in TI, it would not be attacked again, but if it continued to move, it was attacked and eaten. The defensive mechanism of this TI, which is similar to that of the wood tiger moth, is to revert the predation step back to the previous one. Miyatake et al. (2009) also demonstrated that when a prey is engaging in TI, the attention of the predator was diverted to other individuals moving around it, helping it avoid predation. By exploiting the systems of the red flour beetle and the azuki bean weevil, Miyatake and colleagues developed comprehensive studies of TI through a multi-disciplinary approach (see Chap. 4 for details).

Another example in invertebrates, although somewhat different from the example of predation avoidance, is TI in the fire ant, *Solenopsis invicta* (Cassill et al. 2008). This species responds differently with age in aggression with workers from neighboring colonies, i.e., days-old workers undergo TI, weeks-old workers flee, and months-old workers fight. This is because young workers, who have relatively soft exoskeletons, are more vulnerable during the struggle. In this case, the goal of the fire ant is not to eat workers of other colonies, but to kill as many opponents as possible and its sudden cessation of movement after being attacked may discourage further attacks.

Males in the nursery web spider utilize TI when mating with cannibalistic females (Blide et al. 2006). The male approaches the female with a characteristic courtship display and presents a nuptial gift to the female. When the gift is accepted and consumed by the female, the male transports its sperm, which is frequently interrupted by the female. Each time that copulation is interrupted, the male performs TI and the female begins to consume the gift again. Such sexual interaction is repeated several times during mating.

3.4 Future Perspective

As with other studies on the function of predator avoidance behavior, an ideal study on the function of TI should identify the following points. First, the predator targeted by the TI should be identified. Because it is difficult to immediately determine the function of some TIs simply by observing them, such as the examples of the pygmy grasshopper and stonefly nymph, it is important to determine what characteristics of predators have been the selection pressure for the evolution of the TI. Second, it is essential to observe predatory behavior and avoidance of predation by TI to elucidate the mechanism of predator avoidance. The direct observations of predation events in the field are rare; thus, laboratory experiments should be used. Third, the effectiveness of TI as predator avoidance behavior can be more accurately assessed if one can determine how much predation the target prey is experiencing in the field. However, this must be done in conjunction with the other two points. If the predator avoidance behavior exhibited by a prey is very effective against a particular predator, then one would expect that the opportunity for that prey to be exploited by the predator would be virtually eliminated. However, the opposite is not true. Simply confirming the fact that a predator is not exploiting a particular predator is not enough to ensure that this is the result of effective predator avoidance. This is because it is impossible to determine whether this is due to the defense or because the predator does not originally exploit the prey. Paradoxically, TI of the pygmy grasshopper against frogs, which may be considered a specialized predator avoidance behavior that has evolved because of strong selection pressure, has resulted in the present nearly invisible predator–prey relationship between the two. From an evolutionary perspective, the fact that a prey is currently well eaten by a predator does not necessarily mean that the predator is an important "partner" to that prey.

Direct experiments and observations are essential for clarifying the function of TI. However, they are difficult to conduct due to ethical problems. An alternative approach would be to conduct experiments in which artificially created models of prey are presented to the predator. This method is a very effective way to extract defensive traits of interest and manipulate them (i.e., eliminating defensive traits), thus allowing for clear conclusions to be drawn. This method has been commonly used in studies of camouflage, warning colors, and mimicry, but has not been used very often owing to the difficulty of artificially manipulating movement. Humphreys and Ruxton (2018a) proposed using remote-controlled robotic simulated prey. In addition to this direct approach, another way to indirectly test the function of TI is to use the phylogenetically comparative method. This approach has not been adopted in the study of TI behavior; however, it is possible to conduct hypothesis-testing studies on traits of prey that are more likely to evolve TI. For example, for TI in beetles, an inter-species phylogenetic comparison of flight ability and engagement to TI would indirectly support the hypothesis of an active dropping due to TI.

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Chapter 4
Environmental, Physiological, and Genetic Effects on Tonic Immobility in Beetles

Takahisa Miyatake

Abstract This chapter first touches on the taxonomic groups of insects that have been observed to tonic immobility. Although I have used the word "death feigning" in many of my previous papers, I used "tonic immobility (TI)" here as the same meaning as death feigning. Next, the two modes of insects the author discovered in our experiments with beetles, stationary and activity, are described. Individuals in the active mode do not TI, while individuals in the stationary mode TI. In other words, TI is a behavior with phenotypic plasticity. Eight factors are outlined as to what conditions cause this plasticity. Finally, I outline the results of direct and correlated responses in artificial selection for duration of TI on beetles as model materials. Using the selected strains, I will also present the results of experiments approaching a group of genes that control duration of TI and the stimuli that arouse from TI.

Keywords Death feigning · Phenotypic plasticity · *Tribolium castaneum* · *Callosobruchus chinensis* · Artificial selection · Dopamine

From my memory—
In the 1990s, I encountered "tonic immobility (TI)." It was the sweetpotato weevil, *Cylas formicarius*, who actually turned me this interesting behavior (Fig. 4.1). When this beetle was stimulated, it stuck in a unique posture with rigid body, legs, and antennae (Miyatake 2001a). I was instantly fascinated by the fun of this behavior as I was engaged in the weevil eradication program at first in the South-western Islands in Japan by male annihilation and sterile insect technique (e.g., Miyatake et al. 1995, 1997, 2000; Moriya and Miyatake 2001). When I moved to Okayama University at October 2000, I was unable to breed the weevil, which inhabits only the South-western Islands in Japan, so I changed my experimental materials to the red flour beetle, *Tribolium castaneum*, and the adzuki bean beetle, *Callosobruchus*

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Fig. 4.1 Tonic immobility of *Cylas formicarius*. Left and right photos show dead and feigned death *C. formicarius* weevil, respectively. The tonically immobilized weevil in a state of extreme rigidity (right: pinch it down with tweezers and take this position) with a characteristic posture that can be discriminated from dead specimens (left) by the following three points. First, both antennae are overlapping and point in the same direction in a death-feigned weevil but usually not in a dead weevil. Second, the dorsal part of a tonically immobilized weevil is bent backward, whereas the dorsal part of a dead specimen is hunched. Third, the hindlegs of a death-feigned weevil are straightened backward, whereas the hindlegs of a dead specimen are bent the same as the other legs. See Miyatake (2001a) for details

chinensis, as we shall see later in this chapter. At Okayama University, I was taught by Professor Sakai who told me about the advances in the study of the physiology of TI behavior (Nishino and Sakai 1996, 1999, Nishino 2004). When I learned several books including Edmunds (1974) and Ruxton et al. (2004), I recognized that the study of TI as an evolutionary ecology has been largely unexplored. I was convinced that a large area of research was opening up here. It was the year 2004.

4.1 Introduction

It has long been well known that many insects show TI when they were attacked by predators or they are stimulated (e.g., Fabre 1900; Frost 1959; Edmunds 1974). Significant roles of this behavior are diverged among taxonomic groups, i.e., cryptic, startling, physical defense, selfish prey, and so on. The TI behavior is sometime called as death feigning or thanatosis. It should be noted that I used the word "death feigning" instead of tonic immobility in many of my previous papers. Personally, the two words are used by in these previous papers to show the same phenomenon, and I do not use them differently in its meaning.

Since Fabre (1900) tried to unravel the significance and mechanism of TI behavior, the development of research over the last 20 years has a sense of change (Miyatake et al. 2004, 2009, Ruxton et al. 2018). In many animal species, TI behaviors have been examined for their adaptability (e.g., Ruxton et al. 2004; Miyatake et al. 2004; Cassill et al. 2008; Rogers and Simpson 2014; Humphreys and Ruxton 2018; Skelhorn 2018). This behavior has some varied function on survival of prey including an adaptive defense against predators (Miyatake et al. 2004, 2009; Honma et al. 2006; Ohno and Miyatake 2007), a tactic to avoid sexual harassment (Khelifa 2017), and a strategy to avoid individuals of conspecific species in a social ant species (Cassill et al. 2008), or of predator to attack and approach the bait without moving (McKaye 1981).

Although natural historical researches and physiological and/or neurobiological experiments have been conducted from long ago as described in an earlier chapter, few researches have been conducted for behavioral ecological approaches (e.g., Miyatake et al. 2004; Ruxton et al. 2018; Humphreys and Ruxton 2018).

In this chapter, first of all, I will note taxonomic groups of insects showing TI. Second, effects of environmental and physiological conditions on TI in insects have been reviewed. In the later part of this chapter, I will introduce the results of genetic base to TI behavior by artificial selection experiments of some beetle species.

4.2 General Feature of Tonic Immobility in the Beetles Used

In this chapter, three beetles were used for TI experiments. First is the sweetpotato weevil, *Cylas formicarius*, which is a serious pest species of sweetpotato. When this insect is stimulated externally by forceps and left it fall, it assumes a very characteristic pose (Fig. 4.1). The weevil immobilize tonically in a state of extreme rigidity (Fig. 4.1 right panel) with a characteristic posture that can be discriminated from dead specimens (Fig. 4.1 left panel) by the following three points. First, both antennae are overlapping and point in the same direction in a tonically immobilized weevil but usually not in a dead weevil. Second, the dorsal part of a tonically immobilized weevil is bent backward, whereas the dorsal part of a dead specimen is hunched. Third, the hindlegs of a tonically immobilized weevil are straightened backward, whereas the hindlegs of a dead specimen are bent the same as the other legs. For more information on how to tell these postures, see Miyatake (2001a). Importantly, we do not understand the adaptive implications of this unique posture of TI.

Second is the red flour beetle, *Tribolium castaneum*, which is a stored product pest, and cosmopolitan species. When this beetle is stimulated externally by forceps, it turns on its back and attaches its six legs to its thorax (Fig. 4.2).

Third is the adzuki bean beetle, *Callosobruchus chinensis*, which is a pest against some peas. When this beetle is stimulated externally by forceps, it turns on its back and attaches its six legs to its thorax as same as *T. castaneum*.

Fig. 4.2 Tonic immobility of *Tribolium castaneum*. Upper and lower show waking and immobilized *Tribolium castaneum* beetles, respectively. In this insect, while tonic immobility, it lies on its back and attaches its legs to its body

4.3 Taxonomic Distribution

TI has been reported in the following ten orders of all 30 insect taxonomic group. That is, in Odonata, Plecoptera, Orthoptera, Phasmida, Neuroptera, Mantodea, Hemiptera, Coleoptera, Lepidoptera, and Hymenoptera. Which stage, larva or adult, TI is shown in Table 4.1, along with the cited references. Therefore, if anyone finds TI in the insects including of remaining 20 orders, the person can note it as a paper

Table 4.1 Taxa (order) of insects in which tonic immobility was observed

Order	Statge	References
Odonata	Larvae	Gyssels and Stoks (2005)
	Asults	Khelifa (2017)
Plecoptera	Larvae	Moore and Williams (1990)
Orthoptera	Adults	Nishino and Sakai (1996), Honma et al. (2006)
Phasmida	Adults	Godden (1972), Farkas (2016)
Neuroptera	Larave	Sendova-Franks et al. (2020)
Mantodea	Adults	Edmunds (1972), Lawrence (1992)
Hemiptera	Adults	Holmes (1996), Villet (1999), Kang et al. (2016)
Coleoptera	Laravae	Miyatake et al. (2008a, b), Matsumura et al. (2017)
	Adults	Duporte (1916), Chemsak and Linsley (1970)
	Adults	Allen (1990), Oliver (1996), Miyatake (2001a, b)
	Adults	Miyatake et al. (2004), Hozumi and Miyatake (2005)
	Adults	Kuriwada et al. (2009), Spencer and Richards (2013)
	Adults	Ritter et al. (2016), Konishi et al. (2020)
Lepidoptera	Larave	Tojo et al. (1985)
	Adults	Dudley (1989), Larsen (1991)
Hymenoptera	Adults	Hölldobler and Wilson (1990), van Veen et al. (1999)
	Adults	King and Leaich (2006), Cassill et al. (2008)

for a novel finding. It is important to ask academic questions about which species within each taxonomic group show TI and which do not. Also it is important to ask how many times TI has evolved independently using molecular phylogenies in the future. Another important question is what physiological and ecological characteristics are present in groups that TI and those that do not. One possibility is that insects that do not immobilized tonically tend to fly better, as we will discuss later (Ohno and Miyatake 2007). In any case, it is important to accumulate information on TI in more taxa because many taxa have not been observed for this behavior.

4.4 Phenotypic Plasticity: Two Modes in Insects

Previous experiments using mainly beetle species showed that insects may universally have two modes, “active” and “inactive.” Therefore, we can consider a hypothesis. That is, in active mode, escape behavior including running and flying are effective against enemies, while immobility including TI (see Chap. 1) is effective against enemies in “inactive” mode.

This hypothesis (originally stated in Miyatake 2001a) was constructed by the observations of insects with different physiological status, including (1) pre-stimulus behaviors, i.e., walking. Resting or feeding, (2) circadian rhythm, (3) mating, (4) starvation, (5) temperature, (6) body size, (7) age, and (8) seasons. These factors indicate that TI has a phenotypic plasticity as described below. So, I will introduce these factors to relation with some insect examples.

Before I get into each of the arguments, I need to be careful about the specific terms. Mainly, “proportion of TI” or “the rate of TI” are shown as the percentage of individuals in the population that immobilized tonically, and the stimulus required for TI is shown as “the number of stimuli required for TI”, and “the duration of TI” shows the time between entering immobility and arousal from the TI.

4.4.1 Pre-Stimulus Behaviors

A weevil’s behavior prior to being startled, i.e., pre-stimulus behavior, influenced on the proportion and duration of TI in *Cylas formicarius* (Miyatake 2001a). In the nocturnal weevil, resting adults immobile more and had longer duration of immobility after stimulation compared than walking and feeding ones. Therefore, an idea that insects have two modes, active and inactive, may deeply concern with releasing of TI. That is, inactive or resting insects feign death well, while active or moving insects do not feign death. TI of *Cylas formicarius* (Miyatake 2001a) supports this idea. Perhaps active weevils have got the terrain ahead mapped out and are more likely to know where shelter is and, therefore, continue running, whereas a resting weevil may be better off TI than wasting time working this out (Miyatake 2001a).

4.4.2 Circadian Rhythm

Many insect behaviors are controlled by biological clock (Saunders 2002). TI is no exception. Appearance of this behavior depends on the time in a day. A weevil's behavior prior to being startled, i.e., pre-stimulus behavior, influences on the proportion and duration of TI in *Cylas formicarius* (Miyatake 2001a). In the sweetpotato weevil, *Cylas formicarius*, the frequency of weevils immobilized tonically were low in daytime, while it was high in nighttime (Miyatake 2001a). Even in the individuals who showed TI in night, they showed shorter duration of immobility in night than day time in *Cylas formicarius* (Miyatake 2001a). This indicates that we should be aware of the times of day at which TI experiments are conducted when the materials have circadian rhythm.

Why is it harder for this weevil to immobilize in night time especially in males? Two reasons were shown as follows; (1) The predation pressures are differed between day and night times, and (2) The nocturnal weevil must search for mates during the nighttime. Therefore, TI behavior at night would pay a potential cost of reduced reproductive fitness. In a seed beetle, *Zabrotes subfasciatus*, TI occurs more in day than night time (Cardoso and Mendonça 2019).

The relationship between circadian rhythms and TI is an issue to be investigated in the future, since TI is deeply related to movement (Miyatake 2001a). The relationship between circadian clocks and TI needs to be studied in the future, even using beetles that are known to be reliably diurnal.

4.4.3 Mating

In *Cylas formicarius*, copulation decreases the duration of TI in males and females (Kuriwada et al. 2009). Also insemination increased duration of TI in this species (Kuriwada et al. 2009). Effect of mating history on TI has been examined. Copulated males did not change the duration of TI in *C. formicarius* (Kuriwada et al. 2009).

However, inseminated females reduced significantly their duration of TI (Kuriwada et al. 2009). Also, mated females had longer duration of TI than that of virgin females in *C. formicarius* (Kuriwada et al. 2011). Pre-copulatory sexual cannibalism by females affects male anti-predator behavior (Bilde et al. 2006). The relationship between mating experience and TI is also limited to certain types of research, so future research is desired.

Also, since only duration of immobility has been studied in relation to mating, the frequency of immobility needs to be investigated in the future.

4.4.4 Starvation

Acheampong and Mitchell (1997) found that unstarved Colorado potato beetles, *Leptinotarsa decemlineata,* took a longer time to recover from TI compared to 24 h starved beetles. However, the authors describes it as an quiescence rather than a TI in this paper, so immobility here is considered to be equivalent to freeze (see Chap. 1).

Also in *C. formicarius*, starved beetles had higher frequency of TI and took shorter duration of immobility than unstarved beetles in both sexes (Miyatake 2001b). In almost of *C. formicarius* starved individuals cannot immobilize tonically and they had shorter duration of TI than those staffed full (Miyatake 2001b). Also in the weevil, *Eucryptorrhynchus brandti*, the TI intensity was negatively correlated with the duration of starvation (Li et al. 2019). It should be noted that studies have shown that the intensity of TI includes both the frequency and duration of TI.

4.4.5 Ambient Temperature

The effect of temperature on TI intensity was examined in two Coleopteran species, *Callosobruchus maculatus* and *C. chinensis*, in which both species show the negative relationships between frequency of immobilized beetles and between duration of TI and temperature (Miyatake et al. 2008b). This indicates the colder the temperature, the longer the TI duration (Miyatake et al. 2008b).

On the other hand, TI occurs more in high than low temperature in another seed beetle, *Zabrotes subfasciatus* (Cardoso and Mendonça 2019). Negative phenotypic correlation between ambient temperature and duration of TI was also observed in water stick-insects (Holmes 1996), that is a water scorpion bug, *Ranatra* sp., has a shorter duration of TI at high temperatures.

The relationship between temperature and TI may also be different for different types of organisms, and needs to be further investigated in the future.

4.4.6 Body Size

Although in *T. cristinae*, smaller individuals are more likely to feign death than larger individuals (Farkas 2016), body size effect is explained by the age of wild-caught individuals. In the adzuki bean beetles, beetles with larger body size showed longer duration of TI than beetles with smaller body size (Hozumi and MIyatake 2005). However, at the present, there are too few examples of research. Beetles do not change in body size as they become adults. However, age and personality have also been found to be related to TI. A discussion of the relationship between these traits and TI must await the results of future studies.

4.4.7 Age

Younger ants adopt TI more than older ants (Cassill et al. 2008). Kuriwada et al. (2009) found the duration of TI decreases with age in females but not in males in *C. formicarius* (Kuriwada et al. 2011). However, no effects of age on the duration of TI were found in the Colorado potato beetle, *Leptinotarsa decemlineata* (Acheampong and Mitchell 1997). Also significant interaction between age and mating history was also observed in *C. formicarius* (Kuriwada et al. 2011). While in a stick insect, *Timema cristinae*, younger individuals adopt TI more than older individuals (Farkas 2016). The meaning of age is likely to be different for insects of different taxa and in different locations. Therefore, relationships between starvation, age at reproduction and mating history should be studied in detail for TI behavior in future.

4.4.8 Season

In *Lycorma delicatula*, tactics depend on season; bugs were more likely to jump away in the early season, whereas TI was more frequent in the late season (Kang et al. 2016). This is considered by different predators by seasons. The relationship between the seasons and TI has hardly been explored.

4.4.9 Conclusion

The physiological or environmental factors, i.e., pre-stimulus behavior, circadian rhythm, mating, starvation, ambient temperature, body size, age, and season, associated with TI in insects have been described above. However, there are very few examples of studies that have examined the relationship between these factors and TI, and they are biased towards certain insect taxa. Furthermore, these factors would be interactively related to TI in future.

4.5 Artificial Selection for Duration of Tonic Immobility and the Correlated Responses

4.5.1 Model Study 1: *Tribolium* Species

4.5.1.1 Direct Responses

Direct response refers to the response to selection found in the trait itself that made the selection. Diverged artificial selections for the duration of TI have been applied to the following three *Tribolium* species (Fig. 4.2; *T. castaneum*; Miyatake et al.

2004, 2008a, b, 2009; Nakayama and Miyatake 2010a, *T. confusum*; Nakayama et al. 2010, *T. freemani*; Konishi et al. 2020). For all the species, strains diverged in duration of TI have been successfully established (Short strains and Long strains) after more than several generations of the selections, namely direct responses were shown in each species. Realized heritabilities (the values of response to selection divided by selection differential) of TI duration have been estimated. For *T. castaneum*, these were from 0.055 to 0.336 for the shorter direction of TI, while these were from 0.107 to 0.133 for the longer duration for the first ten generations (Miyatake et al. 2004). For *T. confusum*, these were from 0.114 to 0.251 for the shorter direction of TI, while these were from 0.112 to 0.182 for the longer duration for the first ten generations (Nakayama et al. 2010). For *T. freemani*, these were from −0.094 to 0.162 for the shorter direction of TI, while these were from −0.094 to 0.189 for the longer duration for the first nine generations (Konishi et al. 2020).

The results of long-term selection experiment for TI duration and the effect of relaxed and reverse selection in strains artificially selected for duration in *T. castaneum* were also reported in Mastumura and Miyatake (2018). To add an annotation, in this section, I use “relaxed selection” to mean the following: stopping the selection for a certain period, i.e., some generations. I also use “reverse selection” as the selection in an experimental situation for a trait opposite to the one selected earlier. The results of Mastumura and Miyatake (2018) showed that each strain clearly responded to relaxation of selection and reverse selection, suggesting that (1) additive genetic variation (the total effect on a trait stemming from one or more gene loci. Each locus contributes to the trait in a measurable way) still existed in both strains after long-term selection, and (2) selection for shorter and longer duration of TI was costly. These results suggest that duration of TI is controlled by many loci, and longer or shorter duration of TI is costly in a laboratory without predators (Mastumura and Miyatake 2018). Correlated responses to the selections in the three *Tribolium* species have investigated as described below (also see Table 4.2).

4.5.1.2 Correlated Response I: Cost of Tonic Immobility

It has been proven that TI behavior in *T. castaneum* is effective against predatory jumping spiders (Miyatake et al. 2004, 2009). This means the behavior has a benefit in avoiding enemies, while it has a cost for mate searching, namely there is a genetic trade-off between abilities to avoid attack and to mate (Nakayama et al. 2010). This relationship related to locomotor activity as described below (Fig. 4.3).

4.5.1.3 Correlated Response II: Activity

Negative genetic correlations have been found between the level of locomotor activity and TI duration in *T. castaneum* (Miyatake et al. 2008a, b), *T. confusum* (Nakayama et al. 2010), and *T. freemani* (Konishi et al. 2020). That is, beetles

Table 4.2 Direct and correlated responses to selections for short (S) or long (L) durations of tonic immobility in beetles

Response	Traits	*T. castaneum*	*T. confusum*	*T. freemani*	*C. chinensis*
Direct	Duration of tonic immobility	S < L	S < L	S < L	S < L
Correlated	Locomotor activity	S > L	S > L	S > L	S > L
Correlated	Avoiding ability from predators	S < L	–	S < L	–
Correlated	Male mating ability	S > L	–	–	–
Correlated	Flight ability	–	–	–	S > L
Correlated	Stress tolerance	S > L	–	–	–
Correlated	Dopamine expression	S > L	S > L	–	–

Fig. 4.3 Adults of feigning death of *T. castaneum* before a female Adanson jumping spider. For relation between tonic immobility and predation rates by the jumping spider, see Miyatake et al. (2004) for details

derived from short strains showed higher locomotor activity, while beetles derived from long strains showed lower locomotor activity in the three species.

4.5.1.4 Correlated Response III: Dopamine and Biogenic Amines

Brain expression of dopamine was measured in adults of long and short TI duration strains in *T. castaneum* (Miyatake et al. 2008a) and *T. confusum* (Nakayama et al. 2012). Higher expression of dopamine was found in short than long strains in *T. castaneum* (Miyatake et al. 2008a) and *T. confusum* (Nakayama et al. 2012).

The effects of dopamine, octopamine, tyramine, and serotonin on TI were also investigated in *T. castaneum* (Nishi et al. 2010). These amines were injected into the abdomens of beetles artificially selected for long or short TI behavior. In beetles of the long strains, the durations of TI were shortened by injection of dopamine, octopamine, and tyramine, and the effects of these amines were dose-dependent. On the other hand, serotonin injection did not affect the duration of TI. In the short-strain beetles that rarely immobilized tonically, no significant effects of the amines were found on the duration of TI. Brain expression levels of octopamine, tyramine, and serotonin did not differ between long- and short-strain beetles, in contrast to the higher dopamine levels in short strains previously reported. Caffeine decreased the duration of TI in both oral absorption and injection experiments. It is known that caffeine activates dopamine. Therefore, the present results suggest that the duration of TI is affected by dopamine via the dopamine receptor in *T. castaneum* (Nishi et al. 2010).

4.5.1.5 Transcriptomic Comparison

The gene expression profiles of short and long strains were compared between short and long strains in *T. castaneum* thoroughly by RNA sequencing (Uchiyama et al. 2019). Differentially expressed genes (DEGs) identified were 518 between the strains including dopamine related genes.

As expected from physiological studies described above, genes associated with the metabolic pathways of tyrosine, a precursor of dopamine, were differentially expressed between the short and the long strains; these enzyme-encoding genes were expressed at higher levels in the long strain than in the short strain. Quantitative real-time PCR analysis showed that the relative expression levels of *TCHPD* (encoding 4-hydroxyphenylpyruvate dioxygenase, *HPD*) and *Tcnat* (encoding N-acetyltransferase, *NAT*) were significantly higher in the L strain than in the S strain, suggesting the influence of these enzymes on the supply of dopamine and duration of TI (Uchiyama et al. 2019). It is thought that there are many genes that govern the duration of TI in this way. Therefore, knockdown or genome editing concerning TI behavior are topics that should be addressed in the future.

4.5.1.6 Arousal from Tonic Immobility

Some researches have been done on stimuli that simulate TI behavior as described above. For example, beside *T. castaneum,* Acheampong and Mitchell (1997) caused TI behavior in Colorado potato beetles, *Leptinotarsa decemlineata*, by dropping weights on the leaves where they were resting or by acoustic vibration, and quantified the strength of vibrations causing immobility in the beetle. However, no research has investigated the stimulation that causes the arousal from TI in animals, except a study reporting that a grasshopper trapped in the mouth of a frog was aroused after being spit out by the frog (Honma et al. 2006). If the predator uses olfactory cues for detecting preys, the prey has to switch its tactic from TI to running to escape due to a strong vibration of substrate, because the greater strength of vibration stimuli might alert an insect to the arrival of larger animals compared to smaller predators. Therefore, the strength of vibration causing arousal from the state of TI (equal to termination of TI) was examined in strains artificially selected for longer duration of long TI strains (L strains) in *T. castaneum* (Miyatake et al. 2019). Miyatake et al. (2019) also clarified that the arousal sensitivity to vibration was well separated in the F2 individuals, i.e., offspring derived from reciprocal crossings of F1 populations. A positive relationship was observed between the duration of TI behavior and the vibration amplitude, suggesting that immobilized beetles are difficult to arouse from a deep depth of immobility, while individuals being in light depth of immobility are easily aroused by even small vibrations. The results indicate a genetic basis for sensitivity to arousal from TI behavior. Here, I defined the depth of tonic immobility as the duration of TI. In other words, the deeper an individual's depth of TI is, the less likely an individual is to rouse.

4.5.2 Model Study 2: Callosobruchus chinensis

Artificial selection for TI duration have been conducted in the adzuki bean beetle, *Callosobruchus chinensis*, and the strains for short and long duration of TI were successfully established (Ohno and Miyatake 2007). Ohno and Miyatake (2007) also made strains selected for flight ability of *C. chinensis*. These artificial selection experiments showed that negative genetic correlation between TI intensity and flying ability was found in *C. chinensis*, i.e. lower flying ability is genetically connected to escaping by dropping from a perch and then tonically immobilized, whereas higher flying ability does not correspond to TI behavior. The strains selected for shorter (longer) duration of TI had higher (lower) flying ability, while the strains selected for lower (higher) flying ability showed longer (shorter) duration of TI. When the two traits were compared in 21 populations of *C. chinensis* derived from different geographical regions (including the populations which we collected from fields and we were provided from persons who have been reared in a laboratory for generations: see Ohno and Miyatake 2007 for detail), a significant negative correlation was found between TI intensity and flying ability. Based on these results, the choice between alternative escaping behaviors in animals is discussed from two points of view: phenotypic plasticity, an individual with two tactics; and pleiotropic genetic correlation, different individuals with opposite strategies.

After the establishments of strains, the life-history traits of individuals derived from two regimes artificially selected for the duration of TI were compared (Nakayama and Miyatake 2010b). Long strains exhibited greater longevity, higher rates of emergence, laid bigger eggs and greater reproductive effort, and also had a tendency of faster development. Fecundity was not significantly different between Long and Short strains.

Locomotor activity levels and mating success were also compared between beetles derived from two regimes artificially selected for the duration of TI behavior in *C. chinensis* (Nakayama and Miyatake 2009). Short strain beetles had higher activity levels than L strains for both sexes, i.e., there is a negative genetic correlation between TI and activity. In addition, we found that short strains had higher mating success than long strains, presumably due to higher activity, in males but not in females.

4.6 Future Perspective

In the research we have done so far using *Tribolium* beetles, jumping spiders are used for the natural enemies. However, there may be other kinds of natural enemies in a place where this insect lives. Therefore, a general role of TI behavior in predator–prey relationships have to examine in futures. Also, the recent researches on TI behavior are coleopteran-biased. Therefore, we need to study the physiology, genetics, and ecological aspects of TI behavior in wider insect taxonomic groups in the future. For molecular analysis, research on target genes with functions is required using knockdown of functional genes or genome editing. For research on stimulus that wakes up from TI, we do not know how similar the stimulus intensity is to the real predator threat. Research on this also needs to be advanced.

The physiological, behavioral, and evolutionary ecology of TI has advanced rapidly in the last 15 years (Ruxton et al. 2018). However, the taxonomic group of insects studied is still largely biased and it is important to investigate the adaptive significance of TI in the field. It can be said that researches on TI has entered the ecological science arena in the last 20 years. Still, many issues remain to be addressed in the ecology of TI, including further studies on community ecology and phylogenetic history. I am very happy that this chapter will lead to the development of future research.

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Chapter 5
Ultrasound-Induced Freezing Response in Moths

Ryo Nakano

Abstract Coevolutionary adaptation leads to modifications of sensory physiology and behavioral responses of predators and prey. From the ecological point of view, insect hearing and acoustic behavior are an attractive study area for researchers in entomology, animal behavior, and neuroethology. Recent technical advances in sound-recording equipment and molecular techniques have contributed to understanding the evolution of acoustic communication, including sexual dialogue, intraspecific competition, and the interspecific arms race between predators (e.g., bats) and prey (e.g., moths). Singing male moths exploit ultrasound-induced freezing responses of potential female mates or unwelcome rival males to enhance mating success. Freezing responses to ultrasound by moths are originally an antipredator reaction to echolocating insectivorous bats. The aim of Chap. 5 is to provide a sensory-behavioral explanation of the freezing response of insects during predator–prey interaction.

Keywords Bat-predator · Calling song · Courtship song · Freezing · Male–male competition · Moth-prey · Sensory exploitation

5.1 Introduction

Nocturnal insects often encounter predatory bats. Most insectivorous bats detect prey insects and obstacles by echolocation using intense ultrasonic cries. As a countermeasure, some insects (e.g., crickets, katydids, lacewings, moths, and tiger beetles) have evolved ultrasound-sensitive ears and defensive behavioral reactions (Miller and Surlykke 2001; Yack 2004). In general, flying insects turn away from

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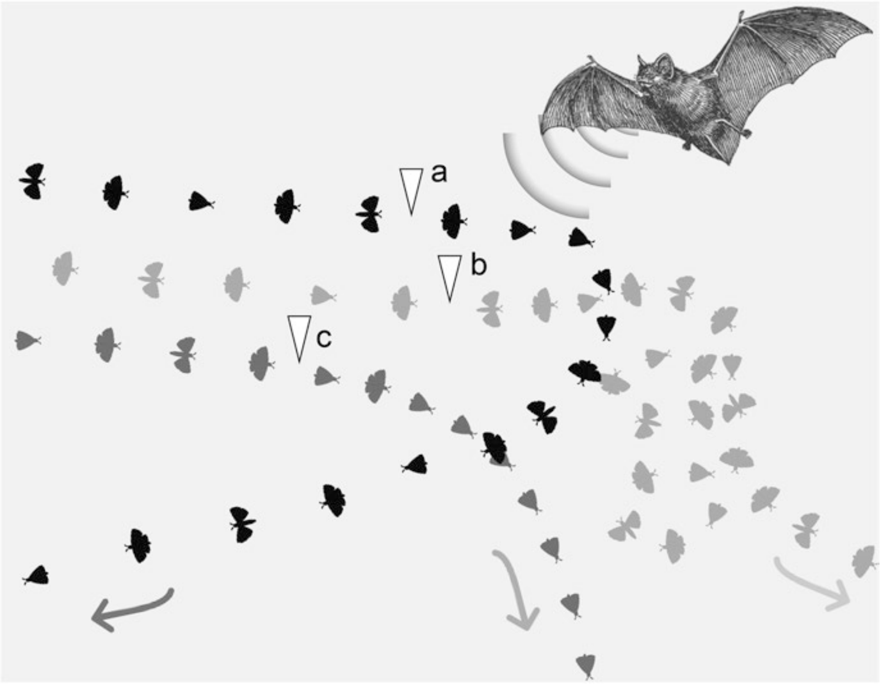

Fig. 5.1 Evasive maneuvers of eared flying insects in response to echolocation calls of a predatory bat. (**a**) turn-away flight, (**b**) zigzagging/spiral/looping flight (power dive), and (**c**) dive to the ground (passive dive). White arrowheads depict the onset of the ultrasonic pulses that insects can detect

the relatively infrequent and faint (but detectable) ultrasonic pulses of a distant echolocating bat (Fig. 5.1a). In contrast, frequent, loud ultrasonic pulses of a nearby bat in hunting mode induce last-ditch responses characterized by protean and erratic zigzagging, spiral, looping flight paths and diving to the ground by the targeted insect (Yager 2012; but see Nakano and Mason 2018) (Fig. 5.1b, c). The dive of prey insects is considered an adaptive defensive behavior to avoid bat predation because a bat chasing an insect in the air suddenly loses the echo reflected from the prey and thus has difficulty in orientating toward the diving insect. After diving and stopping wing movement, i.e., flight cessation, some insects such as moths do not show an immobility response and thus immediately resume flying or walking (Roeder 1962; Nakano et al. 2015a). Therefore, the passive dive could be considered an antibat freezing without tonic immobility. For “tonic immobility,” which is different from “freezing,” see Chaps. 1, 3, and 11; note that some erebid moths show tonic immobility (death feigning) by tactile and vibration stimuli (Chap. 3; Honma et al. 2015). Since the majority of aerially hawking insectivorous bats recognize a fluttering small object as prey, freezing is an effective defense against an approaching predator. Resting insects prior to the approach of a bat also utilize a motionless response (freezing) after detecting the bat’s echolocation calls and wait until the bat goes away.

Thus, roughly 85% of species of the night-active macro-lepidopterans develop a pair of tympanal organs which can respond to broadband ultrasonic wave including echolocation calls of predatory bats (Minet and Surlykke 2003). Moth ears show unique simple structure basically consisting of tympanic membrane, air-sac as a resonator behind the tympanic membrane, and only one to four auditory cells, A_1 to A_4-cell, and a non-auditory B-cell (for detail, see Yack 2004). Although the function of B-cell is still uncertain in sound detection, A-cells with similar frequency-tuning but different thresholds fire in response to mechanical vibration of the tympanic membrane stimulated by ultrasounds. Because a single hearing nerve holds all the A-cells and attaches to single point of the tympanic membrane, moth hearing is not characterized by the tonotopy which is seen in audition of other insects and animals, indicating that acoustic moths do not differentiate the tone of sounds, i.e., frequency (Nakano et al. 2015a). Eared moths, however, acquired both variable escape maneuvers triggered by echolocation sounds of bats and intraspecific acoustic communication in mating behavior and male–male competition. This chapter reviews the relationship between antibat freezing strategy and ecological aspects of acoustic signals of moths. In brief, because moths had developed ultrasound detectors after the divergence of the present lepidopteran superfamily, function and producing-mechanism of sound signals are often diverse in the same subfamily even. Some acoustic moths generate loud advertisement sounds serving as a calling song attracting potential mates and territory song repelling rivals while others generate loud or soft sounds serving as a courtship song attracting mates or freezing both mates and rivals.

5.2 Hidden Ultrasonic Communication

For lepidopteran insects, ultrasonic sexual communication was first reported in pyralid moths, the lesser wax moth, *Achroia grisella* (Pyraloidea, Pyralidae, Galleriinae), and the greater wax moth, *Galleria mellonella* (Pyraloidea, Pyralidae, Galleriinae), in the 1980s (Spangler et al. 1984; Spangler 1985). Sound production in tiger moths (Noctuoidea, Erebidae, Arctiinae; former Arctiidae) was reported as early as 1848 (Haldeman 1848), and a century later the sound was shown to be ultrasonic clicks (Blest et al. 1963). However, the authors described the function of the ultrasonic clicks as a defensive reaction against predacious bats, not as intraspecific communication signals. Up to the early twenty-first century, there are still only a few known examples of ultrasonic intraspecific communication. Thus, sexual acoustic communication has been thought to be quite rare in moths. However, this may be incorrect, based on a failure to notice mating sounds such as courtship songs in many moth species that use low-intensity and high-frequency sounds.

Loud acoustic signals, especially calling songs consisting of low-frequency sounds (<ca. 20 kHz), are advantageous to a distant receiver for sexual communication over long distances because of their low attenuation in the air. Hence, many insects, such as cicadas and crickets, use male calling songs to attract female

receivers that are far from the male signalers. However, conspicuous signals subject the signalers to eavesdropping by unintended receivers, such as predators, parasitoids, and conspecific rivals (e.g., satellite males) (Zuk and Kolluru 1998; Gerhardt and Huber 2002; Hedwig and Robert 2014; Legett et al. 2019). Predatory gleaning bats and eared parasitoid flies locate sound-producing insects using passive hearing (Belwood and Morris 1987; Zuk et al. 2006; Alem et al. 2011; Siemers et al. 2012). In contrast, the quiet courtship songs of male corn borer moths *Ostrinia* spp. (Crambidae) suggest that whispering “soft” ultrasonic courtship songs may be widespread among moths because they are less susceptible to eavesdropping (Nakano et al. 2008). A survey of 26 species of singing moths (Noctuidae, Erebidae, Pyralidae, Crambidae, and Geometridae) found that males of 11 species (42%) produced low-intensity (<80 dB peSPL at 10 cm) ultrasounds close to females with peak frequencies of 30–120 kHz (Nakano et al. 2009; Nakano and Nagamine 2019). Soft courtship songs were not found in all the species but were more common than previously thought. As shown in Fig. 5.2, the temporal and spectral features and sound pressure levels of the male courtship sounds are quite diverse, probably reflecting the different body parts involved in the two main types of sound production, stridulation by file-and-scraper (Fig. 5.2a, b) and clicking by tymbal buckling (Fig. 5.2c–e; presumably these males emit ultrasounds from tymbal organs). For the sound-producing organs of moths, see the previous review papers which described more details (e.g., Conner 1999; Greenfield 2014; Nakano et al. 2015a).

Among the noctuoid species producing courtship songs, metathoracic ventral tymbals are commonly found in male *Spodoptera* spp. (Nakano et al. 2010a; Nakano and Nagamine 2019) and metathoracic lateral tymbals are seen in tiger moths and lichen moths (Conner and Corcoran 2012; Nakano et al. 2013), but not in *Herminia* spp. and others (Nakano et al. 2009, 2015a). These findings suggest independent evolution of the sound-producing organs and ultrasonic signals. This corroborates the evolutionary scenario for the development of sexual communication sounds in moths: relatively recently, some moths have evolved male courtship songs after speciation of the ancestral species that had already developed functional hearing organs of which structures are shared in the same superfamily (Kawahara et al. 2019). Recent data combined with phylogenetic information indicate that whispering courtship song may be quite common in moths because it has the advantage of avoiding eavesdropping (Nakano and Nagamine 2019).

5.3 Calling Ultrasound

Ultrasound can also be a useful signal in moths to advertise the presence of the signaler. When they detect a nearby echolocating bat, tiger moths (Erebidae) and beggar moths (Geometridae) reply with ultrasonic clicks as antipredator defense (Conner and Corcoran 2012; Corcoran and Hristov 2014). Besides aposematic and jamming sounds above, some male moths generate loud sounds for intraspecific communication like common singing insects, cicadas, katydids, and crickets.

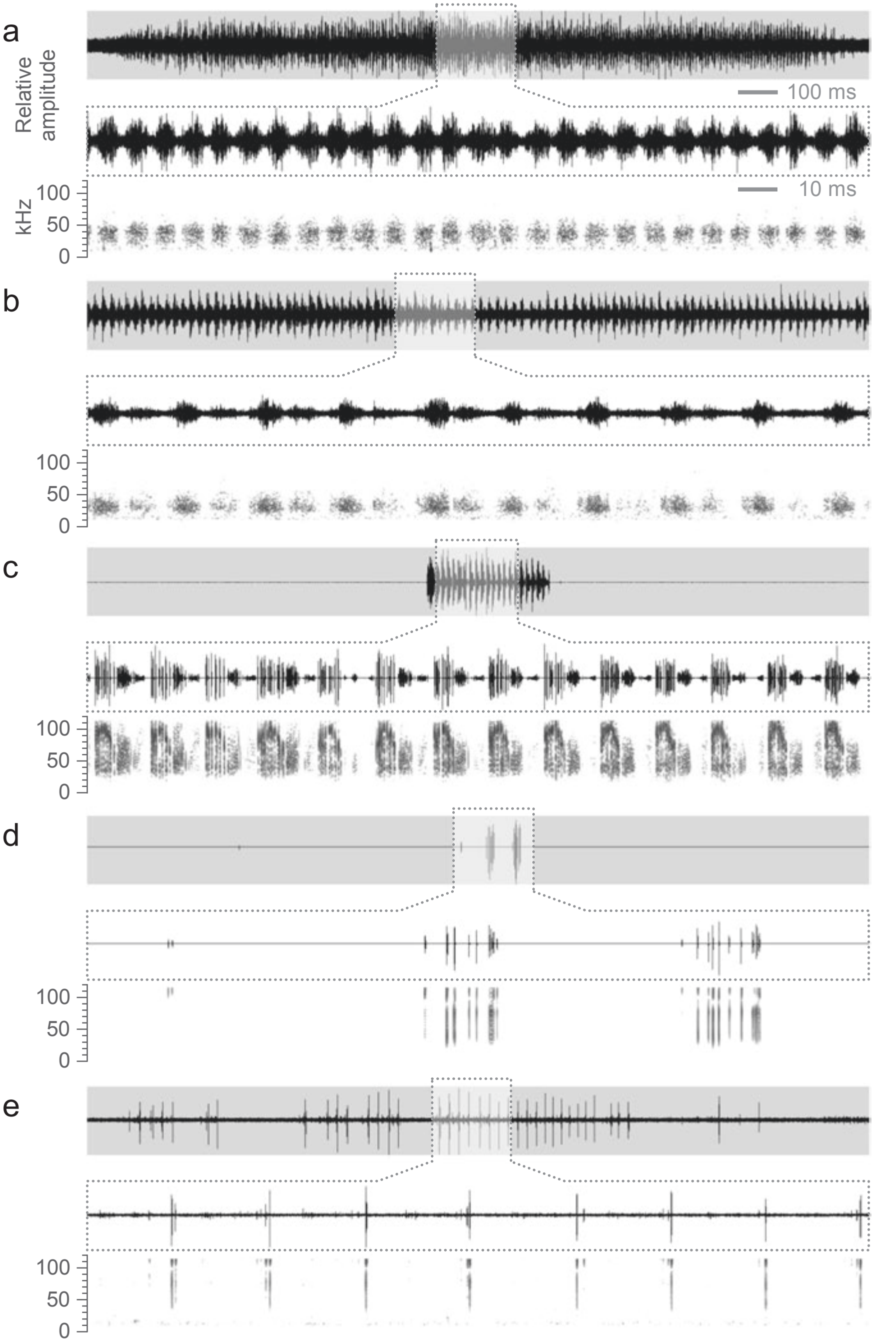

Fig. 5.2 Diversity of temporal and spectral characteristics of male sounds. Examples of male courtship songs in crambid moths: (**a**) *Ostrinia palustralis*, (**b**) *Ostrinia zealis*, (**c**) *Desmia funeralis*, (**d**) *Palpita nigropunctalis*, and (**e**) *Glyphodes pyloalis*. Oscillograms in 2 s (top) and 200 ms (middle; magnified area surrounded with box in top panel) and spectrogram (bottom) are shown. Adapted from Nakano and Nagamine (2019)

5.3.1 Mating System with Calling Songs

5.3.1.1 Noctuoidea

Males of diurnal Australian whistling moths, *Hecatesia* spp. (Noctuidae, Agaristinae), produce percussive sounds by striking knobs on the forewings ("alar castanets") together above the back (Bailey 1978). These sounds, which are partially ultrasounds emitted by males in flight, function as territorial signals against conspecific male rivals, and attract female mates (Alcock et al. 1989; Alcock and Bailey 1995). Males of *Rileyiana* (former *Phlogophora* and *Thecophora*) *fovea* (Noctuidae, Cuculliinae) rub a bubble (fovea) on the hindwing with the jagged hindleg (Surlykke and Gogala 1986). Perching males of *Amyna natalis* (Noctuidae, Eustrotiinae) also produce loud ultrasonic clicks from tymbal-like structures on the forewing (Heller and Achmann 1993). The functions of the male ultrasounds in the two nocturnal noctuids above, *R. fovea* and *A. natalis*, have not yet been determined, but they seem to function for attraction of female moths.

Males of the nocturnal Nolidae (Chloephorinae) moths *Bena bicolorana* and *Pseudoips prasinana* produce loud ultrasonic clicks with ventral tymbal organs located on the second abdominal sternite. The positive correlation between microtymbal structure and the number of clicks in these two species is similar to that in tiger moths: *B. bicolorana*, which has corrugated tymbals with striae on the medial part, produces a series of clicks for each buckling of the tymbal, whereas *P. prasinana*, which has smooth tymbals, produces a single click for each buckling of the tymbal (Skals and Surlykke 1999; Dowdy and Conner 2019). It is suggested that male ultrasonic clicks in the two nolid species are used as calling songs to attract female moths although no direct observations or experiments have been conducted.

In Erebidae, including the former Arctiidae (tiger moths and lichen moths), only a few species are known to use ultrasonic clicks produced from metathoracic tymbals for orientation before copulation (Conner and Corcoran 2012). Females of the polka-dot wasp moth, *Syntomeida epilais* (Erebidae, Arctiinae), release a sex pheromone at night to attract male moths, which is the typical mating system of moths. But remarkably, males start to sing a calling song after they begin to approach the females (Sanderford and Conner 1995). The female moth replies with ultrasonic clicks with a temporal structure different from that of the male's clicks, and the male reaches the female for mating. Mating sequence involving the "duet" is rather uncommon in moths.

5.3.1.2 Pyraloidea

In Pyraloidea (Pyralidae and Crambidae), there are several examples of loud ultrasound signals for calling females (Greenfield 2014). The lesser wax moth, *Achroia grisella* (Pyralidae, Galleriinae), is one of the most intensively studied moths for investigation of sexual selection and evolutionary processes in male calling song and female mate acceptance (e.g., Spangler et al. 1984; Greenfield and Weber 2000; Rodríguez and Greenfield 2004; Greenfield and Hohendorf 2009). Wing-fanning male moths emit a sex pheromone and ultrasonic clicks to attract female moths.

In Crambidae, males of *Symmoracma minoralis* (Pyraustinae) (Heller and Krahe 1994) and *Syntonarcha iriastis* (Odontiinae) (Gwynne and Edwards 1986) produce loud ultrasounds, which are most likely to work as calling songs. The sound-producing organs are located on the last abdominal segment and the genitalia, respectively, but the structure differs between the species: *Symmoracma* has a symmetrical tymbal and *Syntonarcha* has an asymmetrical file-scraper.

5.3.2 Evolution of Calling Songs

Noctuoidea moths possess homologous tympanal ears on both sides of the metathorax, which suggests that a common ancestor evolved ears once (Kawahara et al. 2019). In contrast, the diversity of sound-producing organs even within the same phylogenetic clade indicates that sound production has evolved independently several times (Conner 2014; Greenfield 2014). Sound-producing organs in moths that produce calling songs may be located in a variety of body parts, and the mechanisms may vary accordingly.

The temporal structure of calling songs of moths, including the duration of pulses (or series of clicks), interpulse interval, and duty cycle, is basically species-specific. The frequency components, however, commonly range from about 20 to 60 kHz and are a little higher in the smaller species such as pyralid moths; male *A. grisella* emits 70–130 kHz ultrasonic clicks. The dominant frequency of the calling sounds roughly matches the sensitive frequency range of the auditory cells, indicating that the frequency range of the calling song in moths is within the frequency range of the echolocation calls emitted by sympatric insectivorous bats (Schnitzler and Kalko 2001; Schnitzler et al. 2003). The incomplete tuning of the best frequency in hearing to the maximum energy in the calling song frequencies suggests that moths evolved acoustic communication by a sensory exploitation process after modification of ultrasound-sensitive hearing for bat detection (Nakano et al. 2013). The peak sound pressures of these advertisement sounds are usually greater than 90 dB peSPL at 10 cm. Based on hearing thresholds, attenuation in the air, and emitted sound pressure levels, the maximal communication distance is estimated at about 10–20 m. The loud sounds are therefore probably effective at medium distances, which are less than the effective distances of >200 m for volatile sex pheromones (Schlyter 1992) but are considerably greater than the distances involved in courtship.

5.4 Courtship Ultrasound

The majority of moths have not been thought to be acoustic insects with regard to mating behavior. Female moths release species-specific sex pheromones to attract conspecific male moths prior to copulation. After approaching the female releasing sex pheromone, the male attempts copulation. Thus, sex pheromones, which are effective in attracting a mate from a long distance, play an essential role in pair formation (Ishikawa 2020). This courtship sequence, which is described in textbooks, does not involve sounds; however, an increasing number of studies reports the role of ultrasonic songs in the courtship of male moths (Nakano et al. 2015a; Nakano and Nagamine 2019). Courtship ultrasounds produced by a male attracted to a female are found rather ubiquitously in moths, especially in Pyraloidea. The male moth sings a courtship song shortly before copulation attempts after orientation toward the female.

5.4.1 *Loud Courtship Songs*

Loud courtship songs are found in Pyraloidea. In the bee moth, *Aphomia sociella* (Pyralidae, Galleriinae), males attract conspecific females by sex pheromones (“male odor” or “male scent”) and subsequently emit loud courtship songs in close proximity to the female to stimulate her to accept a mate (Kindl et al. 2011). The sound pressure level of the song reaches 96 dB SPL at 10 cm, which is about 1600-fold louder than the song of the Asian corn borer, *Ostrinia furnacalis* (Crambidae, Pyraustinae) (Nakano et al. 2008). Males of *A. sociella* have a mating territory and produce a rival song against intruding males, which is similar to the system in the Australian whistling moths, *Hecatesia* spp. (Noctuidae). In *Galleria mellonella*, which belongs to the same subfamily as *A. sociella,* males also emit ultrasonic rival songs against other males. The function of the rival songs in *Galleria* is still uncertain; females adopt a mate acceptance posture in response to rival songs as well as to courtship songs, indicating that females do not discriminate between the songs for rival males and female mates.

Male yellow peach moth, *Conogethes punctiferalis* (Crambidae, Spilomelinae), also emit loud courtship songs when they are close to a female. After approaching a female that is releasing sex pheromone, males hover around her and generate a series of brief pulses and then one long pulse (83 dB SPL at 10 cm) (Fig. 5.3a) (Nakano et al. 2012a, b). Only the long pulse, not the short pulses, causes the female to raise her wings upright and accept copulation (Fig. 5.3b). This specific wing-raising response is essential for copulation, as it triggers the male’s landing and attempting genital coupling. Wing-raising is evoked by any long ultrasonic pulse, i.e., with duration >200 ms (Nakano et al. 2012a, 2014) (Fig. 5.3c). The series of short pulses with 28-ms pulse duration and 26-ms interpulse interval emitted before the long pulse does not directly affect the female’s mate acceptance. However, the

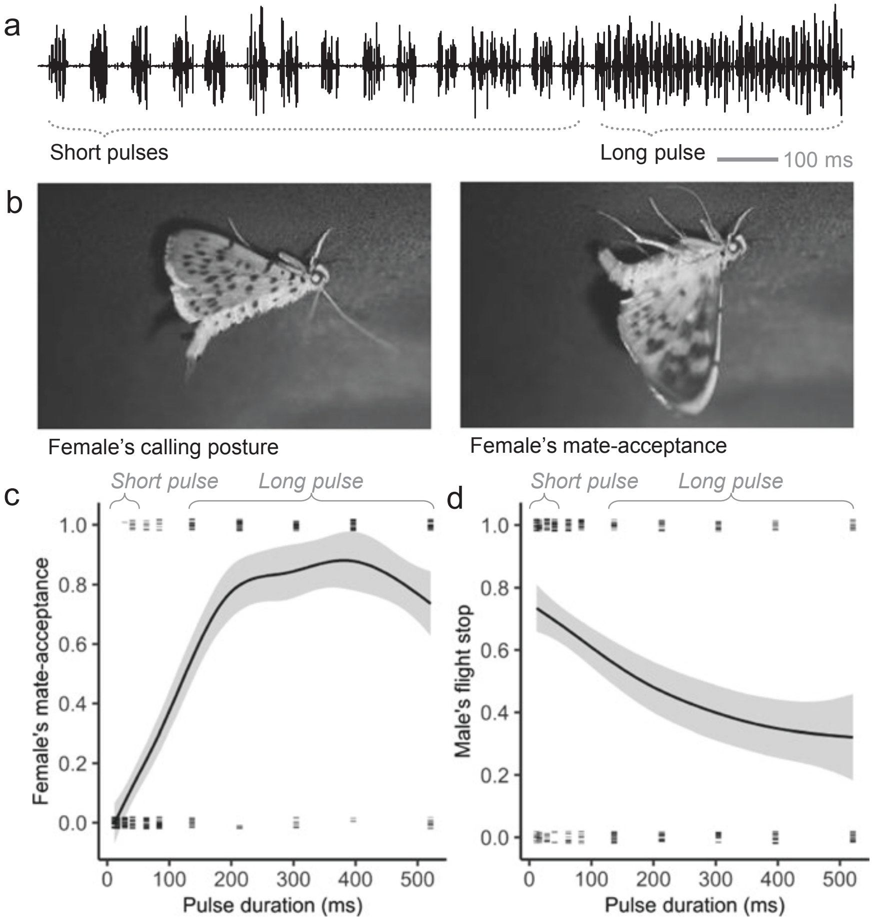

Fig. 5.3 Loud courtship song of the yellow peach moth, *Conogethes punctiferalis*. When close to a female, the male hovering around her generates a series of short pulses and one long pulse (**a**). Calling females adopt the mate acceptance posture (**b**) only in response to long pulses (**c**), whereas short pulses evoke cessation of the rival male's flight. Figure 5.3b is adapted with permission from Nakano et al. (2014)

temporal pattern is similar to that of echolocation calls in the approach phase of horseshoe bats, *Rhinolophus* spp., which frequently hunt moths. These calls elicit flight cessation in moths (Nakano et al. 2014, 2015b). Therefore, a possible function of the short pulses is to suppress the approach flight of rival males (Fig. 5.3d). Thus, male courtship songs of *C. punctiferalis* have a dual function to fend off rivals with the short pulses and make the female accept mating with the long pulse. The evidence that male moths produce loud ultrasounds before courtship to avoid interference from rival males in both *A. sociella* and *C. punctiferalis* suggests that the loudness of the male courtship song has primarily evolved for male–male competition. The high sound pressure level of the courtship song for communicating with the females might be a by-product of the use of the same sound-producing mechanism for rival songs and courtship songs.

5.4.2 Soft Courtship Songs

Males of the Asian corn borer, *Ostrinia furnacalis*, produce ultrasonic courtship songs of extremely low-intensity (46 dB SPL at 1 cm = 26 dB at 10 cm) in close proximity (≈1 cm) to a receptive female (Figs. 5.4 and 5.5a) (Nakano et al. 2006, 2008). The female's hearing threshold, 38 dB SPL, at their best frequency (46 kHz) allows females to detect the male song only when a male is singing within 3 cm of the female (Nakano et al. 2008). Males produce these sounds by stridulation; they rub specialized scales on their forewings against scales on the thorax (Fig. 5.4b) (Nakano et al. 2008). Relatively simple sound-producing scales on the body may have evolved more easily than other more elaborate cuticular sound-producing apparatuses such as tymbal organs, which require sophisticated modifications of the integument. The male songs suppress the escape behavior of the stationary female as she releases sex pheromones, thereby increasing the male's attempts and success in copulation with the motionless female (Nakano et al. 2010b).

Production of soft sounds has also been observed in other *Ostrinia* congeners (European corn borer *Ostrinia nubilalis*, adzuki bean borer *Ostrinia scapulalis*, etc.) (Fig. 5.2a, b). The sound pressure levels of male courtship songs are similar

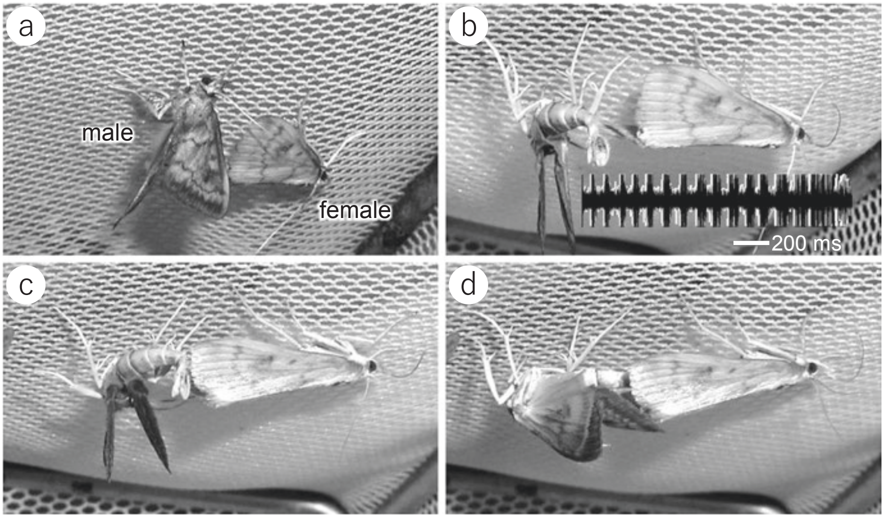

Fig. 5.4 Courtship sequence in the Asian corn borer, *Ostrinia furnacalis*. After approaching the female (**a**), the male erects both wings, rubs them against his thoracic scales (**b**) to produce soft courtship songs (inset oscillogram in **b**) shortly before attempting copulation (**c**), and copulates with the freezing female (**d**). Adapted with permission from Nakano et al. (2006)

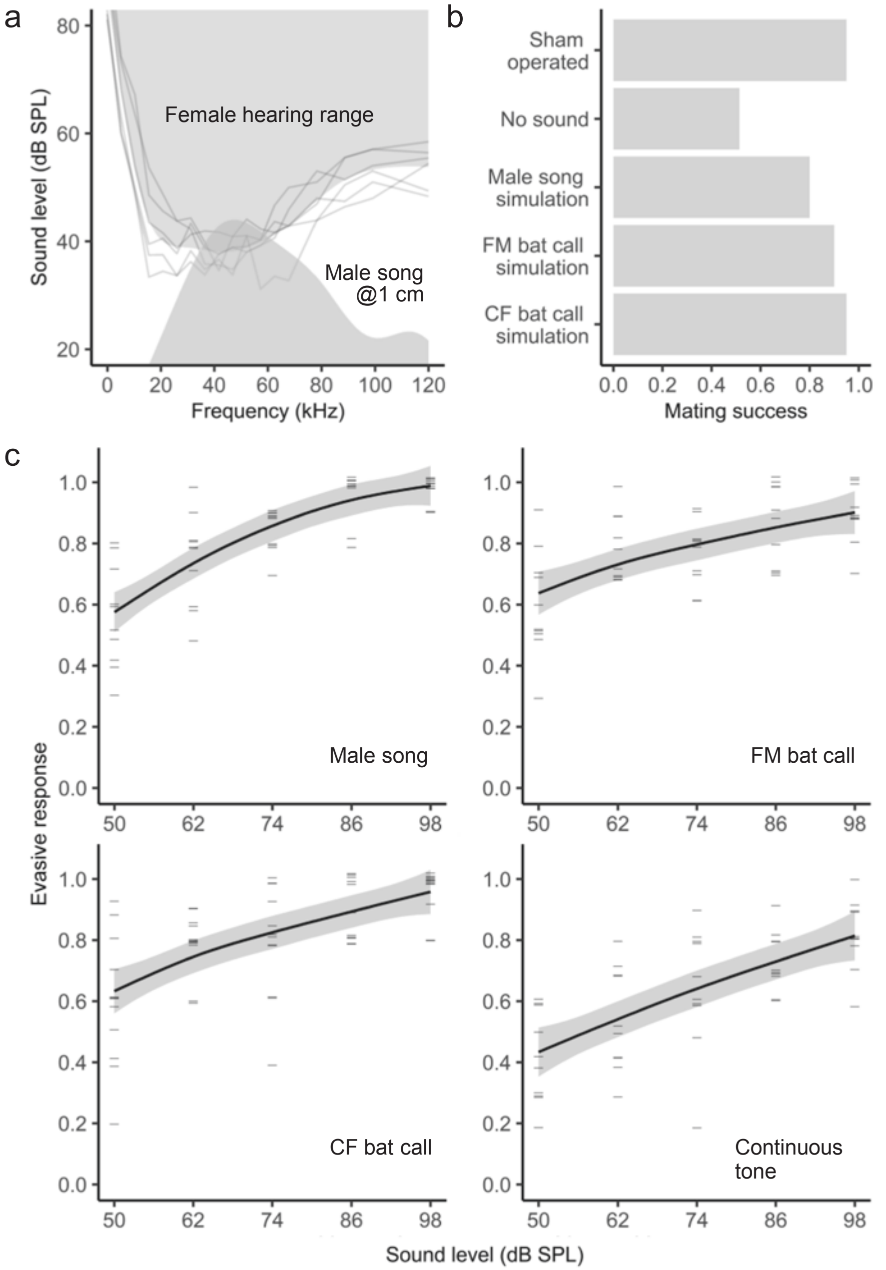

Fig. 5.5 Secondary use of freezing response in acoustic communication of *Ostrinia furnacalis*. (**a**) Spectrum of male ultrasound at a distance of 1 cm (at the female's location) corresponds to the most sensitive hearing range of the female moth. (**b**) Females accept muted males with simultaneous playback of male courtship song as well as simulated bat calls [frequency-modulated (FM) pulses and constant-frequency (CF) pulses]. (**c**) Rates of evasive responses to male song, two types of bat calls, and continuous tone in tethered flying moths. Adapted from Nakano et al. (2008, 2013, 2015a)

(23–26 dB SPL at 10 cm) and the hearing thresholds are also comparable (38–44 dB SPL at the best frequency) among *Ostrinia* spp. (Takanashi et al. 2010). Male courtship songs, thus, function only at very short distances in these species as well as *O. furnacalis* (Fig. 5.5a). However, the spectral features and temporal patterns differ, reflecting the phylogenetic relationships of *Ostrinia* species. The songs of male *O. nubilalis* and *O. scapulalis* consist of pairs of pulses (similar to Fig. 5.2a, b), whereas males of *O. furnacalis* emit pulse-group songs (Fig. 5.4b) with a higher frequency (peak frequency, 48 kHz; bandwidth 3 dB below the peak, 38–60 kHz) than in *O. nubilalis* (39 kHz; 31–45 kHz) and *O. scapulalis* (38 kHz; 29–45 kHz) (Nakano et al. 2006, 2008; Takanashi et al. 2010).

Although sounds are used for sexual communication in *Ostrinia* moths, the physiological threshold and hearing range have been conserved as a result of predatory pressures imposed by insectivorous bats (Takanashi et al. 2010). Thus, for these "soft-spoken" Crambidae, the most likely scenario is that evolution of ultrasonic hearing for bat detection came first, and later ancestral males of *Ostrinia* species acquired the ability to produce courtship songs that can be heard only by females in close proximity. The functions of male courtship songs are highlighted in the next section.

5.5 Secondary Use of Freezing Response

Moth acoustic communication is believed to have evolved through the receivers' preexisting ability to hear ultrasonic echolocation calls emitted by hunting bats ("sensory exploitation" or "receiver bias"; Endler and Basolo 1998; Ryan 1998; Greenfield 2014; Nakano et al. 2015a). Thus, prominent sound production for mating is restricted to eared moth species. The use of mating sounds via exploitation of a sensory bias, however, involves more than just detecting the sounds.

5.5.1 True Love Songs

Modification of opposing behavioral responses between aversion maneuvers against predatory bat calls and positive orientation toward and acceptance of a mate's signal is also essential for the evolution of sexual communication in general. For example, in the lesser wax moth, *A. grisella*, female moths show negative phonotaxis to echolocation calls of bats but are attracted to male ultrasonic calling songs (Rodríguez and Greenfield 2004; Greenfield and Weber 2000; Greenfield and Hohendorf 2009). In the lichen moth, *Manulea* (former *Eilema*) *japonica* (Erebidae, Arctiinae), and the yellow peach moth, *C. punctiferalis*, females discriminate between bat echolocation calls and male courtship songs on the basis of the temporal structure of sound signals and adopt the mate acceptance posture to male courtship songs of their own species (Nakano et al. 2013, 2014). Nevertheless, except for moths like the two species above, *M. japonica* and *C. punctiferalis*, the requirement of behavioral reversal (negative to bat calls and positive to male songs) might not be critical in the courtship context. Courtship song functions at extremely short range, which renders positive orientation unnecessary. Hence, addition of courtship song to the typical moth mating system has probably made courtship singing more common than loud calling song in moths. Adaptive elaboration of moth acoustic communication through sensory exploitation results in tuning of the characteristics of the sender's sounds to the detection abilities of the receiver, e.g., detectable sound intensity and high-frequency sounds of bat echolocation calls. Subsequently, both the sender's signal and the receiver's response have coevolved by way of sexual selection (Greenfield 2014). Thus, a prerequisite for establishing sensory exploitation in moths is to demonstrate that the receiver's preference for the mate's communication signal evolved after the development of evasive action in response to bat ultrasounds. This means that it is extremely hard to corroborate the sensory exploitation experimentally by using species that have evolved a receiver's preference for the sender's sound signal. For Arctiinae moths of which males generate courtship songs and both sexes generate antibat aposematic/jamming signals, female's preference for male courtship songs is likely to have evolved after evolution of the aposematic and jamming sounds against echolocating bats.

5.5.2 *Deceptive Love Songs*

Males of *Ostrinia furnacalis* and the common cutworm, *Spodoptera litura* (Noctuoidea, Noctuidae, Noctuinae), emit soft courtship songs with low sound pressure levels (see Sect. 5.4.2). The females do not distinguish male songs from bat echolocation calls. When not only playback of male courtship songs (46 dB SPL for *O. furnacalis* and 70 dB SPL for *S. litura* at the position of the female's ear), but also simulation of bat echolocation calls (74 dB SPL for *O. furnacalis* and 100 dB SPL for *S. litura*) was presented to the receptive female moth, aphonic males with ablated sound-producing organs were not rejected by females in the courtship context and could complete fertile copulation (Fig. 5.5b) (Nakano et al. 2010a, 2013). The behavioral responses of females to bat calls and conspecific male songs are similar. They "freeze" when they hear either sound, which is one of the maneuvers that aids resting moths to avoid capture by the insectivorous bat's biosonar. By secondarily exploiting the female's freezing response to ultrasounds, the singing male can persistently attempt genital coupling and thus increase his copulation success because the female is made motionless. Regardless of sex, females and males of *O. furnacalis* in flight also freeze, i.e., dive to the ground, or take evasive maneuvers in response to simulations of echolocation calls of the big brown bat, *Eptesicus fuscus* (Vespertilionoidea, Vespertilionidae, Vespertilioninae), and the greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophoidea, Rhinolophidae, Rhinolophinae), which are representative insectivorous bat species. In addition to bat call simulations, playbacks of male courtship song caused bat avoidance behavior in flying moths if the playbacks were presented at >70 dB SPL at the location of the moth (Fig. 5.5c). Because males of *O. furnacalis* and *S. litura* do not generate loud courtship songs, flying moths do not exhibit drastic reactions, such as protean and unpredictable flight responses, to their actual courtship songs. It is therefore inferred that the female interprets a male's soft courtship song as an echolocation call emitted by a distant bat and remains stationary in order not to be detected by the bat. The identical reactions of females to bat echolocation calls and male courtship songs support the hypothesis that eared moths evolved acoustic sexual communication based on a preexisting sensory bias.

5.5.3 Evolutionary Origin of Courtship Songs

After acquiring hearing sensitive to ultrasound and defense strategies as a countermeasure to echolocation in predatory bats emitting ultrasonic pulses, there were probably some males that incidentally generated weak noise, including sounds in the ultrasonic range, in courtship displays, such as wing-fanning for diffusing the male sex pheromone to the intended female or collecting the female sex pheromone (Obara 1979; Spangler 1988; Kindl et al. 2011). This assumption implies that females were able to detect the by-product ultrasounds, which caused a freezing response and thus led to increased copulation success. This process accelerated the use of bat-like male songs in courtship because of the initial lack of females' preference and/or recognition of an appropriate conspecific mate on the basis of male song characteristics, with a mainly species-specific temporal structure. In contrast to the deceptive courtship song, if the acoustic features of males' original mating sounds were extremely far from those of bat calls, females would not show antibat reactions to these sounds. This is why moths do not always take evasive action to all the detectable sounds (Nakano and Mason 2018). A species that had been able to distinguish male courtship sounds from bat echolocation calls and to increase mating success could evolve female mate preference and recognition and develop "true" love songs via coevolutionary sexual selection (Endler and Basolo 1998; Ryan 1998; Greenfield 2014).

There are an increasing number of case studies of moths in which the males produce ultrasounds during courtship (Conner 1999; Nakano et al. 2009; Greenfield 2014; Nakano and Nagamine 2019). The functions of most of these courtship ultrasounds are still unclear. Experiments are warranted using behavioral tests with moths in which the hearing organs or sound-producing organs have been ablated and using playbacks or simulations of courtship ultrasounds. Thus, it is unknown whether males' ultrasounds are used for sexual communication with females, for resource competition with rival males, or both. Even if male courtship song serves as a mating signal, the mode of action should be determined by comparing behavioral and auditory responses to bat echolocation calls and male courtship songs. The function of moth courtship songs is divided into two types on the basis of the receiver's reaction. The "deceptive" function is found in the crambid *O. furnacalis*

(Nakano et al. 2013) and the noctuid *S. litura* (Nakano et al. 2010a), and the "true" attractive function is found in the pyralid *G. mellonella* (Spangler 1985) and the erebid *M. japonica* (Nakano et al. 2013). Only in the case of "true" mating signals, receptive females discriminate between bat echolocation calls and male courtship songs and thus perform a freezing response to bat calls but not to male songs.

It is difficult to conduct persistent observations of courtship behavior of animals with uncertain times of day for mating activity. Nocturnal moths usually produce soft and brief courtship ultrasounds that are not audible to the human ear (Nakano and Nagamine 2019). Our sensory bias toward low-frequency (<20 kHz), loud, and long duration sounds has contributed to the failure to detect the actual number of insects communicating with soft courtship songs (Balenger 2015; Reichard and Anderson 2015). Future studies are anticipated to reveal the use of soft sounds for private communication in more moths as well as other animals. Subsequently, these mating sounds will be confirmed to be deceptive or true signals. These studies will be essential for deepening our understanding of the evolutionary scenario of moth acoustic communication. To demonstrate that females have acquired the current mate preference to male sounds from original negative responses to predator signals may contribute to our knowledge of the evolution of novel communication modalities.

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Chapter 6
Vibration-Induced Immobility in Coleopteran Insects

Takuma Takanashi and Wataru Kojima

Abstract Many insects demonstrate immobility, such as death feigning (tonic immobility) and freeze responses in the context of interactions within and between species. Immobility is induced by environmental information, including substrate vibrations generated by predators or conspecifics. In this chapter, we review immobility induced by vibrations in the order Coleoptera. Tonic immobility and freeze responses are observed in 18 coleopteran families, particularly in characteristic postures during tonic immobility with extended or flexed legs. Chordotonal sensory organs located in the legs play important roles for detecting vibrations and triggering tonic immobility. Vibration-induced immobility has various functions, e.g., defense from predators, and interactions within the species. As individual case studies, behavioral mechanisms for immobility related to vibrations have been described in the orders Cerambycidae, Nitidulidae, and Scarabaeidae. Adults of a longicorn beetle *Monochamus alternatus* (Cerambycidae) detect vibrations on their host plant via leg chordotonal organs. They show freeze or startle responses to vibrations, presumably for conspecific and predator recognition. Larvae of a group living beetle *Trypoxylus dichotomus* (Scarabaeidae) show freeze responses to vibrations produced by both conspecific pupae and mole predators in the soil. Vibrational interactions and freeze responses among larvae and predators in Scarabaeidae also shed light on the evolution of deceptive communication in *T. dichotomus*.

Keywords Vibration · Tonic immobility · Freezing · Chordotonal organ · Predator · Cerambycidae · Scarabaeidae

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6.1 Introduction

6.1.1 Vibration-Induced Immobility

Vibrations transmitted through substrates are one of the essential channels that insects use to detect environmental information (Greenfield 2002; Hill 2008). Insects exhibit various behaviors in response to mechanical stimuli such as vibrations (Greenfield 2002; Hill 2008; Takanashi et al. 2019). In many insects, immobility is induced by mechanical stimuli. Immobility includes two reactions: tonic immobility and freezing (Chap. 1). Tonic immobility (TI) is also called as death feigning or thanatosis. Humphreys and Ruxton (2018) define TI as motionless posture by a prey individual triggered by physical contact or very close proximity of a predator. Fabre (1900) reported for the first time that tenebrionid beetles demonstrate TI with a range of several tens of minutes. In coleopterans and other insects, the typical posture during TI consists of either legs extended irregularly or flexed (Fig. 6.1). On the other hand, freezing is a kind of startle response, which is a short-latency, abrupt movement induced by mechanical or other stimuli (Friedel 1999). For instance, a male cricket demonstrates a “silencing reaction” during sound production in response to vibrations (Dambach 1989). In coleopterans and other insects, cessation of walking and other movement in response to stimuli is classified as freezing (Kojima et al. 2012b; Tsubaki et al. 2014; Takanashi et al. 2016). In Chap. 1, freezing is used only in the context of predator–prey interactions from an ecological point of view. In this chapter, however, freezing is also described in the context of social communication. To discriminate the two, “freeze response” is used in this chapter in the context of predator–prey interactions and social communication.

6.1.2 Vibration-Induced Immobility and Communication

Subsequent to discoveries of vibrational sexual communications in hemipteran insects (Ichikawa and Ishii 1974), vibrations have also been found to play essential roles in the context of predator–prey interactions and other social communications among insects (Greenfield 2002; Hill 2008; Takanashi et al. 2019). Indeed, vibrations induce TI and freeze response in beetles (Acheampong and Mitchell 1997; Kojima et al. 2012b; Tsubaki et al. 2014; Kiyotake et al. 2014; Takanashi et al. 2016; Kishi and Takanashi 2019a), fruit flies (Fabre et al. 2012; Mazzoni et al. 2013), and honeybees (Michelsen et al. 1986; Kirchner 1993; Nieh 1993). For instance, *Drosophila melanogaster* uses both vibration and sound signals for sexual communication. Male vibration signals generated by shivering induce a freeze response in females to accept copulation (Fabre et al. 2012; Mazzoni et al. 2013). As another example, worker honeybees of *Apis mellifera* produce a vibrational “stop signal” during the waggle dance to freeze other workers and leave the nest (Michelsen et al. 1986; Kirchner 1993; Nieh 1993).

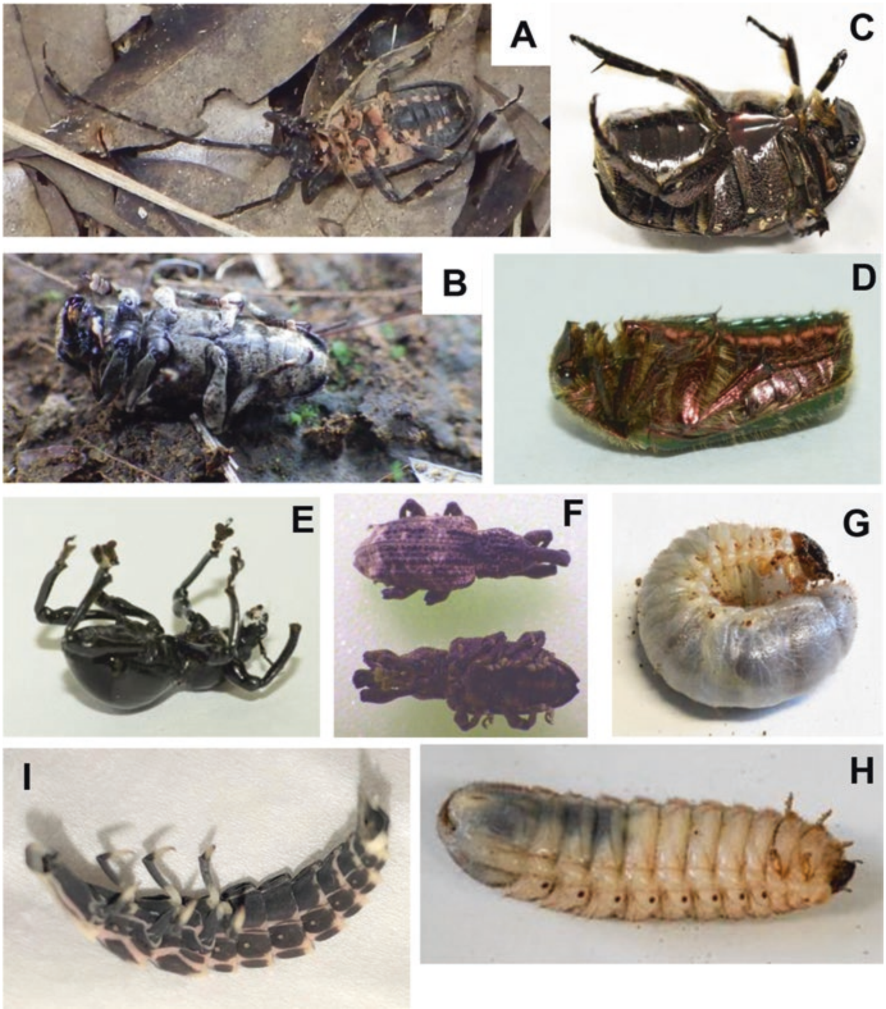

Fig. 6.1 Different postures displayed in tonic immobility in Coleoptera. Adults of (**a**, **b**) Cerambycidae, (**c**, **d**) Scarabaeidae, and (**e**, **f**) Curculionidae are shown. Adults of (**a**) *Moechotypa diphysis*, (**c**) *Protaetia brevitarsis brevitarsis*, and (**e**) *Pachyrhynchus infernalis* exhibit extended legs. Adults of (**b**) *Mesosa (Aplocnemia) longipennis*, (**d**) *Cetonia pilifera*, and (**f**) *Dyscerus perforates* exhibit flexed legs. Larvae of (**g**) *Trypoxylus dichotomus* (Scarabaeidae), (**h**) *Dicronorhina derbyana* (Scarabaeidae), and (**i**) *Pyrocoelia atripennis* (Lampyridae) are shown. (**a**) was adapted from a photo published by Takanashi and Nishino (2021)

6.2 TI and Freeze Responses in Coleoptera

6.2.1 Survey of TI and Freeze Responses

Coleoptera is the largest insect order, and includes numerous species exhibiting TI and freeze responses (Table 6.1, Fig. 6.1). Based on the findings of Bleich (1928) and others, we surveyed 49 genera from 18 families exhibiting TI with different postures and freeze responses to vibrations (Table 6.1), alternatively called thanatosis or death feigning in the literature (e.g., Bleich 1928; Crowson 1981). Acheampong and Mitchell (1997) also classified the freeze response to vibrations as quiescence. TI occurs in adults and larvae of 18 families, whereas freeze responses occur in only five of those families. Adults exhibit TI postures with two different leg positions, either legs extended irregularly or legs flexed regularly (Bleich 1928; Crowson 1981). Even within same families, both of these postures are adopted. Six families are known to exhibit extended postures (Brentidae, Silphidae, Carabidae, Histeridae, Geotrupidae, and Anobiidae), five families exhibit flexed postures (Nitidulidae, Elateridae, Coccinellidae, Byrrhidae, and Dermestidae), and six other families exhibit extended and flexed postures dependent on individual species (Cerambycidae, Tenebrionidae, Scarabaeidae, Chrysomelidae, Curculionidae, and Lucanidae).

6.2.2 TI and Other Behavior in Coleopteran Adults

In the family Cerambycidae (Table 6.1), *Moechotypa diphysis* exhibits a TI posture with femoro-tibial joints in extended positions at various angles and extended antennae after dropping from a host tree (Takanashi and Nishino 2021; Fig. 6.1a). On the other hand, *Mesosa (Aplocnemia) longipennis* demonstrates a flexed posture after dropping from a tree (Fig. 6.1b). In the Scarabaeidae and Curculionidae families, extended postures are observed in *Protaetia brevitarsis* and *Pachyrhynchus infernalis* (Fig. 6.1c, e), whereas flexed postures are observed in *Cetonia pilifera* and *Dyscerus perforatus* (Fig. 6.1d, f). In Curculionidae, adults of *Euscepes postfasciatus* and *Erodiscus proximus* make stridulatory sounds during TI with flexed postures (Tatsuta and Kumano 2015; Gaiger and Vanin 2006) (Table 6.1). *Erodiscus proximus* produces sounds from the elytro-femoral stridulatory organ for 15 s during TI lasting 60 s or more (Gaiger and Vanin 2006). This sound, in conjunction with TI, might play roles in defense from predators. In the family Chrysomelidae, Caprinera (1976) observed different proportions of TI, stridulation, and other behaviors between *Crioceris asparagi* and *C. duodecimpunctata*. *Crioceris asparagi* frequently exhibits dropping and/or TI with flexed postures and stridulation, whereas *C. duodecimpunctata* take flight and stridulate easily with infrequent TI. These observations suggested that both TI and sound production can serve as multiple defenses against predators. Also, chemical defense by the exocrine gland secretion,

Table 6.1 Tonic immobility (TI) and freeze responses in Coleoptera. TI in adults with legs extended or flexed, TI in larvae, and freeze responses in adults and larvae in response to vibrations are shown

Family	Species	Tonic immobility	Freeze responses	References
Cerambycidae	*Moechotypa diphysis*	Adult extended	Adult	Takanashi and Nishino (2021)
	Mesosa (Aplocnemia) longipennis	Adult flexed	–	Takanashi pers.com
	Hippopsis lemniscata	Adult flexed	–	Bleich (1928)
	Monochamus alternatus	None	Adult	Takanashi et al. (2016)
	Paraglenea fortunei	None	Adult	Tsubaki et al. (2014)
	Eburia ulkei	Adult extended	–	Chemsak and Linsley (1970)
Scarabaeidae	*Trypoxylus dichotomus*	Larvae	Larvae	Kojima et al. (2012a, b)
	Protaetia spp. (*P. orientalis, P. cuprea, P. brevitarsis brevitarsis*)	Adult extended, larvae	Larvae	Kojima et al. (2012a), pers.com, Bleich (1928)
	Dicronorhina derbyana	Larvae	Larvae	Kojima et al. (2012a)
	Cetonia pilifera	Adult flexed, larvae	–	Kojima pers.com
	Taeniodera nigricollis	Adult flexed	–	Kojima pers.com
	Anomala albopilosa	–	Larvae	Kojima et al. (2012a)
	Phyllopertha horticola	Adult extended	–	Bleich (1928)
	Apogonia sp.	Adult flexed	–	Kojima pers.com
	Holotrichia kiotonensis	Adult extended	–	Kojima pers.com
	Onthophagus sp.	Adult flexed	–	Kojima pers.com
Tenebrionidae	*Tribolium castaneum*	Adult flexed[a], larvae	–	Kiyotake et al. (2014)
	Blaps mortisaga	Adult extended	–	Bleich (1928)
	Zophobas atratus	Adult extended	–	Nishino pers.com
Nitidulidae	*Phenolia (Lasiodites) picta*	Adult flexed[a], larvae	–	Kishi and Takanashi (2019a, b)
Curculionidae	*Euscepes postfasciatus*	Adult flexed[b]	–	Tatsuta and Kumano (2015)
	Trigonopterus vandekampi	Adult flexed	–	de Kamp et al. (2014)
	Erodiscus proximus	Adult flexed[b]	–	Gaiger and Vanin (2006)

(continued)

Table 6.1 (continued)

Family	Species	Tonic immobility	Freeze responses	References
	Dyscerus perforatus	Adult flexed	–	Ichikawa et al. (1987)
	Lixus sp.	Adult extended		Kojima pers.com
	Pachyrrhynchus infernalis	Adult extended		Kojima pers.com
Chrysomelidae	*Callosobruchus maculatus*	Adult flexed	–	Ohno and Miyatake (2007)
	Crioceris spp. (*C. asparagi* and *C. duodecimpunctata*)	Adult flexed[b]	–	Caprinera (1976)
	Leptinotarsa decemlineata	–	Adult	Acheampong and Mitchell (1997), Metspalu et al. (2002)
	Phaedon brassicae	Adult flexed, larvae	–	Matsubara and Sugiura (2018)
	Zabrotes subfasciatus	Adult flexed	–	Cardoso and Mendonça (2019)
	Galeruca tanaceti	Adult extended	–	Bleich (1928)
Brentidae	*Cylas formicarius*	Adult extended	–	Miyatake (2001), Kuriwada et al. (2009)
Elateridae	*Agriotes* spp. (*A. lineatus, A. obscurus, A. sordidus,* and *A. ustulatus*)	Larvae	–	Ritter et al. (2016)
	Selatosomus aeneus	Adult flexed	–	Bleich (1928)
Lucanidae	*Lucanus cervus*	Adult extended	–	Bleich (1928)
	Dorcus rectus	Adult flexed	–	Kojima pers.com
Coccinellidae	*Epilachna varivestis*	Adult flexed	–	Happ and Eisner (1961)
	Coccinella septempunctata	Adult flexed	–	Bleich (1928)
Silphidae	*Silpha obscura*	Adult extended, larvae	–	Bleich (1928)
	Phospuga atrata	Adult extended, larvae	–	Bleich (1928)
	Nicrophorus quadripunctatus	Adult extended	–	Kojima pers.com
Lampyridae	*Pyrocoelia* spp. (*P. fumosa, P. atripennis*)	Larvae	–	Ohba (2007), Kojima pers.com
Carabidae	*Broscus cephalotes*	Adult extended	–	Bleich (1928)

(continued)

Table 6.1 (continued)

Family	Species	Tonic immobility	Freeze responses	References
Histeridae	*Hister quadrinotatus*	Adult extended	–	Bleich (1928)
Geotrupidae	*Geotrupes silvaticus*	Adult extended	–	Bleich (1928)
Byrrhidae	*Byrrhus pilula*	Adult flexed	–	Bleich (1928)
Dermestidae	*Dermestes lardarius*	Adult flexed, larvae	–	Bleich (1928)
Anobiidae	*Anobium striatum*	Adult extended	–	Bleich (1928)

[a] Vibration-induced TI.
[b] TI with stridulation.

so-called reflex bleeding, during TI has been observed in Chrysomelidae, Coccinellidae, and Lampyridae (Happ and Eisner 1961; Caprinera 1976; Ohba 2007).

It has been assumed that the flexed and extended postures could play roles in defense against predators, but there is no direct evidence for defense with regard to the postures in Coleoptera. In the pygmy grasshopper *Criotettix japonicus*, extended posture with the hind legs, pronotum, and lateral spines is crucial to avoid being swallowed by frog predators (Honma et al. 2006). Thus, coleopteran extended postures may function as a defense against large predators. A flexed posture could make insects more difficult for bird and insect predators to hold or pick up, compared to postures with extended legs. Apart from the posture, Miyatake and his colleagues elegantly revealed that the length of TI in *T. castaneum* adults (Tenebrionidae) played an important role in defense against jumping spider predators *Hasarius adansoni* (Miyatake et al. 2004, Chap. 8). As these spiders attack and eat only active beetles, immobile beetles are able to avoid predation. Induction and arousal of TI in *T. castaneum* are triggered by 33–60 Hz vibrations (Table 6.1) (Kiyotake et al. 2014; Miyatake et al. 2019), which are supposed to be generated by approaching predators. Future studies are necessary to address the adaptive significance of TI with different postures across the order Coleoptera.

6.2.3 TI and Freeze Responses in Coleopteran Larvae

To our knowledge, larvae in eight families (Tenebrionidae, Scarabaeidae, Nitidulidae, Chrysomelidae, Elateridae, Silphidae, Lampyridae, and Dermestidae) are more likely to exhibit postures with extended legs during TI (Table 6.1; Fig. 6.1g–i). For instance, larvae of *Pyrocoelia atripennis* (Lampyridae) display lengthy TI, with extended legs and a ventrally curved abdomen in response to physical contact (Ohba 2007). Scarabaeid larvae show unique postures during TI; for example, larvae of *Protaetia orientalis* and *Cetonia pilifera* roll up their body, as with some woodlice and millipedes, while those of *Dicronorhina derbyana* and *Pseudotorynorrhina japonica* stretch out their body (Fig. 6.1h). The posture of some Dynastinae (e.g., *Trypoxylus dichotomus*) is U-shaped (Fig. 6.1g), intermediate between the "roll up" type and "stretch" postures. Despite this diversity, the insects' body is always hardened during TI (Kojima, personal communication). Although deterring effects against predators have not been determined, the hardened integument may be less vulnerable to attack from predators. We will describe in more detail in further sections the freeze response to vibrations in Scarabaeidae, Cerambycidae, and Nitidulidae.

6.3 Vibration Sense Organs

6.3.1 Chordotonal Organs

Chordotonal organs, which are located in the legs and other body parts of insects (Nishino and Sakai 1997; Field and Matheson 1998), are internal mechanoreceptors that can detect vibrations via the legs and positions of leg joints. There are two different major chordotonal organs found in all six legs: the femoral chordotonal organs in the femur, and subgenual organs in the tibia (Field and Matheson 1998). Characteristically, coleopteran insects possess only the femoral chordotonal organ (Schneider 1950; Nishino et al. 2016; Takanashi et al. 2016). There are other minor chordotonal organs, including a tibio-tarsal chordotonal organ and a tarso-pretarsal chordotonal organ, each with small numbers of sensory neurons (Takanashi et al. 2016). The femoral chordotonal organs of the cerambycid species *Monochamus alternatus* consist of the scoloparium, including sensory neurons, and a chord-like cuticular apodeme, which is connected to sensory neurons and the joint pivot of the tibia (Takanashi et al. 2016) (Fig. 6.2a). Sensory neurons possess stretch-sensitive mechanosensory channels localized in the sensory cilia of the dendrite (Field and Matheson 1998), which is inserted into the scolopale cap (Field and Matheson 1998; Takanashi et al. 2016). Each pair of sensory neurons may be bifunctional, able to detect vibrations as well as mediate proprioceptive feedback (Takanashi et al. 2016; Takanashi and Nishino 2021), such as resistance reflex in the leg muscles (Field and Burrows 1982; Sauer and Stein 1999).

In *M. alternatus*, there are 37 scolopale rods in the prothoracic FCO, and therefore there are an estimated 74 sensory neurons (Takanashi et al. 2016; Takanashi and Nishino 2021) (Fig. 6.2b, c). In *M. diphysis*, the estimated number of sensory neurons is 82 (Takanashi and Nishino 2021), indicating that both cerambycid species have similar structures. The number of sensory neurons is mostly identical among pro-, meso-, and metathoracic FCOs (Takanashi et al. 2016). Both species also have a single scoloparium; however, a tenebrionid species *Zophobas atratus* has two ventral and dorsal scoloparia with and an apodeme (Nishino et al. 2016) (Fig. 6.2d, e). The number of scolopale rods in ventral and dorsal scoloparia of the

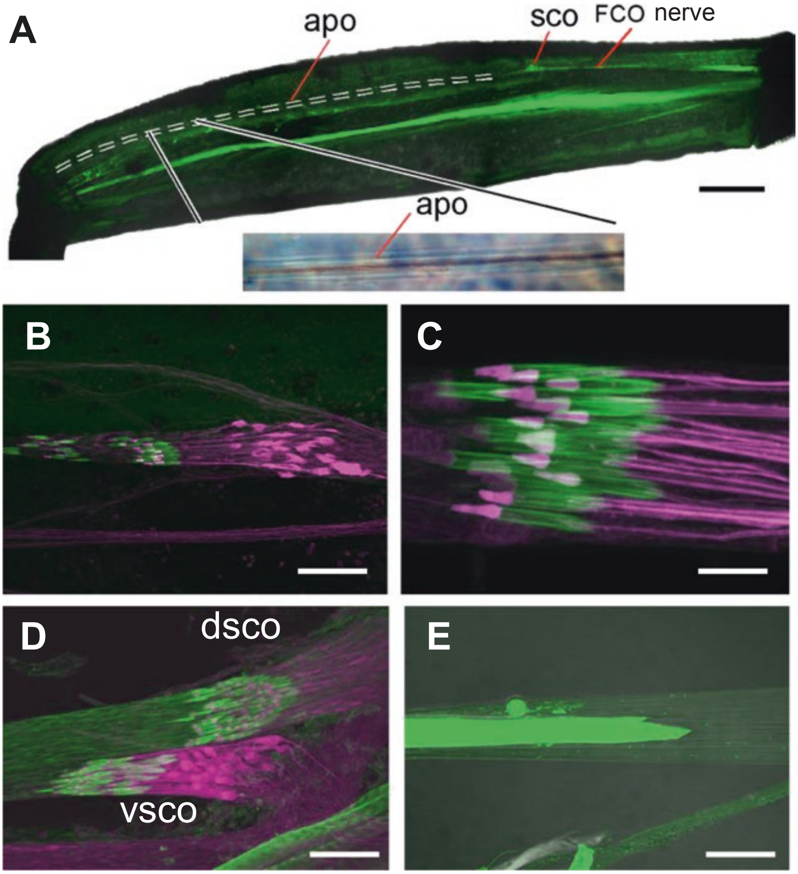

Fig. 6.2 Femoral chordotonal organs in *Monochamus alternatus* and *Zophobas atratus*. (**a**) Prothoracic femoral chordotonal organs (FCOs) of *M. alternatus*. The scoloparium (sco), including sensory neurons, is connected to a cuticular apodeme (apo). (**b**–**e**) Sensory neurons (magenta) and scolopale rods (green) in (**b**, **c**) the prothoracic FCO of *M. alternatus* and (**d**) mesothoracic FCO of *Z. atratus* are shown by differential fluorescent labeling. (**d**) The mesothoracic FCO is divided into the ventral scoloparium (vsco) and dorsal scoloparium (dsco). (**e**) A cuticular apodeme (green) of *Z. atratus* is shown. Adapted from photos published by Takanashi and Nishino (2021) and Nishino et al. (2016). Scale bars = 500 μm in **a**; 50 μm in **b**, **d**, **e**; 10 μm in **c**

mesothoracic FCO (Nishino et al. 2016) is approximately 150; therefore, there are an estimated 300 sensory neurons (Fig. 6.2d). Because there are distinct differences of subdivision in scoloparium and the number of sensory neurons in FCOs between cerambycid species and tenebrionid species, FCOs are assumed to be diversified functionally between these species.

Central projections of FCO axons are revealed by bilateral labeling of FCO nerves in the legs, which enter the medio-ventral and the lateral association centers in *M. alternatus* (Takanashi and Nishino 2021). The medio-ventral association center, specialized for vibratory/auditory processing, is conserved among different insect orders (Boyan 1993; Pflüger et al. 1981; Newland 1991). The lateral association center, located laterally from the medio-ventral association center, is the motor association neuropil (Pflüger et al. 1981, 1988; Mücke and Lakes-Harlan 1995).

6.3.2 Mechanosensilla and Chordotonal Organs

Insects possess other mechanoreceptors, such as external campaniform sensilla and hair (trichoid) sensilla, which are touch receptors for detecting low-frequency vibrations or strain on cuticular surfaces (Keil 1997; Field and Matheson 1998), although the vibrational sensitivities of the sensilla are lower than those of chordotonal organs (Kühne 1982; Ai et al. 2010). In pupae of *Z. atratus*, many campaniform and hair sensilla distributed in various body parts play roles in the detection of forces by physical contact, in order to induce the defensive behavior of pupal rotation (Kurauchi et al. 2011).

Bleich (1928) reported that the main receptors involved in TI seemed to be located in the thorax, particularly in the meso- and metathorax in several coleopteran species. In the cricket *Gllylus bimaculatus*, prothoracic femoral chordotonal organs were involved in triggering both the flexion reflex and TI, whereas hair sensilla on the prothorax and campaniform sensilla in the legs were involved in the elicitation of the flexion reflex alone (Nishino and Sakai 1996; Nishino et al. 1999). Considering the positions and functions of the femoral chordotonal organs and the mechanosensilla in coleopterans (Nishino and Sakai 1996), the femoral chordotonal organs are assumed to be mainly involved with TI. Additionally, mechanosensilla on the thorax and legs are also assumed to be involved with coleopteran TI.

6.4 Freeze Responses and TI in Cerambycidae

Adults and larvae of longicorn beetles (Cerambycidae) feed on tree bark or other plant tissues, thereby damaging the plants (Wang 2017). Various behaviors, such as freeze responses and walking, are induced by vibrations in adults of *M. alternatus*, *M. diphysis,* and *P. fortunei* (Takanashi et al. 2016, 2019; Takanashi and Nishino 2021; Tsubaki et al. 2014). These three species are distributed mainly in Asia, including Japan (Kobayashi et al. 1984; Ohbayashi and Niisato 2007; Kosaka 2011). *Monochamus alternatus* is the vector of a plant disease caused by the pine wilt nematode *Bursaphelenchus xylophilus* (Kobayashi et al. 1984; Yazaki et al. 2018), *Moechotypa diphysis* is an invasive pest of Shiitake mushroom (*Lentinula edodes*) bed logs from oak trees (Ohbayashi and Niisato 2007; Kosaka 2011), and *Paraglenea fortunei* feed on ramie and other plants (Ohbayashi and Niisato 2007; Tsubaki et al. 2014).

Adults of *M. alternatus* show various behavioral responses to vibrations. Walking adults show freeze responses to vibrations of 100 Hz and 1 kHz. Takanashi et al. (2016) confirmed that FCOs detect vibrations because of behavioral experiments described below. After the scoloparia attached to the apodemes were removed, adults with altered FCOs in all six legs did not freeze in response to 100 Hz or 1 kHz vibrations while walking. In contrast, freeze responses were frequently induced in intact and sham-operated beetles. In this way, the FCO involvement in the freeze response has been identified in a coleopteran species.

In addition to *M. alternatus*, freeze responses to vibrations are also observed in *P. fortunei* and *M. diphysis* (Table 6.1). Startle responses are found in all three species, as well as in the house longhorn beetle *Hylotrupes bajulus* (Breidbach 1986; Tsubaki et al. 2014; Takanashi et al. 2016). A startle response—that is, small movement of the legs and antennae—is induced by vibrations when resting in quiescence, distinct from immobility. Behavioral thresholds of these responses have been determined in *P. fortunei* and *M. alternatus* (Tsubaki et al. 2014; Takanashi et al. 2016). Briefly, behavioral responses in quiescence were observed on a flat steel plate attached to a vibration exciter. The amplitude of the stimulus was gradually increased, and the threshold was defined as the least acceleration necessary to evoke a response at frequencies of 20–1 kHz. In freeze responses, *P. fortunei* showed high sensitivity to frequencies of 20–500 Hz, with response thresholds of 2–10 m/s^2 (Fig. 6.3a). Similarly, *M. alternatus* and *P. fortunei* showed high sensitivity to these same frequencies of 20–500 Hz (Fig. 6.3a, b). The thresholds of the startle response are 2–20 m/s^2, which is higher than that of the freeze response in *P. fortunei*. In contrast, the desert locust *Schistocerca gregaria* exhibits startle responses with a threshold of 0.23 m/s^2 (zero-to-peak) at 60 Hz, which is lower than the threshold of 1.0 m/s^2 in the freeze response (Friedel 1999).

Cerambycid adults can show behavioral responses to vibrations generated by approaching conspecifics or predators. In *P. fortunei*, vibrations from approaching individuals on host plant leaves were recorded (Tsubaki et al. 2014). Vibrations generated by a walking individual were 2 m/s^2 at <30 Hz, and those generated by a landing individual were 9 m/s^2 at <30 Hz. These values were high enough to induce freeze responses in *P. fortunei*, indicating that this species can recognize approaching conspecifics according to characteristic vibrational frequencies and accelerations, presumably without sexual recognition (Tsubaki et al. 2014).

In addition to conspecifics, cerambycid adults can recognize approaching predators with higher vibrational accelerations (Tsubaki et al. 2014; Takanashi et al. 2019). Subsequent to startle responses, adults show behavior triggered by vibrations or other sensory information. Indeed, *M. alternatus* begins walking in response to vibrations at 100 Hz, which allows the adult to avoid predators or orientate to conspecifics (Takanashi et al. 2016, 2019).

Moechotypa diphysis is more sensitive to vibrations than *M. alternatus* and *P. fortunei*, as the thresholds of startle responses were shown to be 1 m/s^2 at 100 Hz in *M. diphysis* (Takanashi and Nishino 2021). In the field, *M. diphysis* and *M. (A.) longipennis* likely drop from a host tree, the so-called drop-off reflex (Crowson 1981), and subsequently exhibit TI on the ground (Takanashi and Nishino 2021)

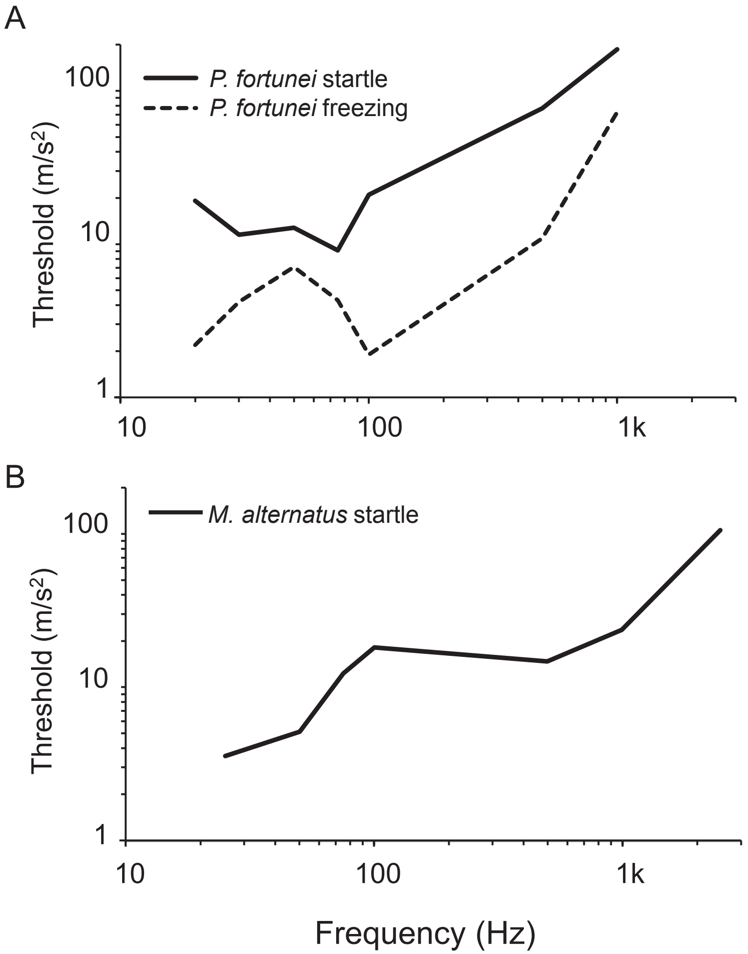

Fig. 6.3 Thresholds of behavioral responses to vibrations in (**a**) *Paraglenea fortunei* and (**b**) *Monochamus alternatus*. Startle responses (solid lines) in both species and a freeze response in *P. fortunei* (dashed line) are indicated. Modified from Takanashi et al. (2019)

(Fig. 6.1). In contrast, *M. alternatus* and *P. fortunei* do not show TI (Takanashi personal communications). *Monochamus alternatus* rarely drops from the host tree, and *P. fortunei* is likely to drop from the host and/or take flight following disturbance (Takanashi personal communications). Thus, high sensitivities of startle responses to vibrations may explain why cerambycid species exhibiting TI drop easily from the host, like *M. diphysis* and *M. (A.) longipennis*. Dropping, in conjunction with TI, is widespread among many insects, including beetles (Crowson 1981; Humphreys and Ruxton 2019; Caprinera 1976; Miyatake et al. 2004; Ohno and Miyatake 2007; Honma et al. 2015; Matsubara and Sugiura 2018; Kishi and

Takanashi 2019a); however, the mechanism of dropping by virtue of TI has not yet been clarified in coleopteran insects.

6.5 TI in Nitidulidae

Sap beetles of *Phenolia* (*Lasiodites*) (Nitidulidae) consist of many species native across the Eastern Hemisphere (Kirejtshuk and Kvamme 2002), while *P.* (*L.*) *picta* is distributed in Asia and Australia (Kishi and Takanashi 2019b). Both adults and larvae feed on fallen rotten fruits, such as the Japanese apricot *Prunus mume* (Kishi and Takanashi 2019b).

Vibrations have been shown to induce TI, startle responses, or other behavioral responses in the adults and larvae of the sap beetle *P.* (*L.*) *picta* (Kishi and Takanashi 2019a, b). In adults at standstill, TI and startle responses are induced by 60 and 120 Hz vibrations with different accelerations from 1 to 16 m/s^2 (Fig. 6.4) (Kishi and Takanashi 2019b). These beetles display TI in a position with fixed legs, normally lasting for several seconds (Kishi and Takanashi 2019b). At 60 and 120 Hz, the startle responses are induced by accelerations of 1–8 m/s^2, whereas TI is induced by accelerations of 2–16 m/s^2 (Fig. 6.4). This indicates that the thresholds of startle responses are lower than those of TI, with a range of 1–2 m/s^2. Furthermore, different intervals from 1 to 9 s of pulsed vibrations with 1-s durations at 120 Hz and 8 m/s^2 affect TI and startle responses during walking, compared with control stimuli of continuous vibrations or without vibrations (Kishi and Takanashi 2019b). Long intervals of 9 s induced the highest proportion of TI and startle responses per pulsed vibration (50%) among the different intervals tested. A negative relationship was observed between the numbers of TI and startle responses and walking durations.

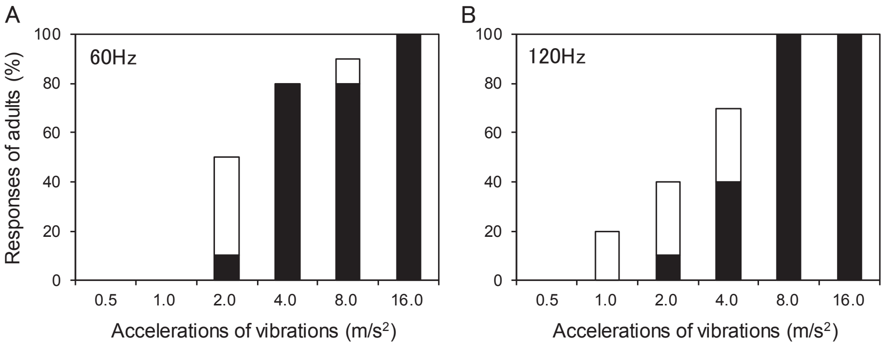

Fig. 6.4 Percentages of adults of *Phenolia* (*Lasiodite*) *picta* that responded to different accelerations of pulsed vibrations at (**a**) 60 Hz and (**b**) 120 Hz. Open and closed sections of vertical bar represent the startle response and tonic immobility, respectively. Reproduced from Kishi and Takanashi (2019b)

Short intervals of vibrations decreased the induction of TI and startle responses, presumably due to habituation (Kishi and Takanashi 2019b).

In larvae of *P.* (*L.*) *picta*, TI presents with a ventrally curling abdomen within several seconds following stimulation by physical contact or dropping to the ground (Kishi and Takanashi 2019a). An escape behavior of quick crawling is also observed after stimulation. This escape behavior was observed when the larvae were presented with various vibrations through the fruit of a sliced banana (Kishi and Takanashi 2019a). At 120 Hz and ≥8 m/s^2, pulsed vibrations of 1-s duration at intervals from 1 to 29 s induced this behavior in 40–50% of larvae within 30 min. Vibration-induced TI was not investigated, as it could not be observed within the tested fruit (Kishi and Takanashi 2019a). Vibrations caused by predators can induce a range of defensive behaviors, including TI, startle responses, and escape responses in both the adults and larvae of *P.* (*L.*) *picta*.

6.6 Freeze Responses in Scarabaeidae

6.6.1 Pupal Vibratory Signals and Larval Freeze Responses

Freezing of insects typically functions as a method of resistance against predators. However, freeze responses also occur in conspecific interactions, such as in larvae of *T. dichotomus* (Coleoptera, Scarabaeidae, Dynastinae). *Trypoxylus dichotomus* is native to east Asia, including Japan. This beetle has a relatively large body; the maximum mass of the larval form can reach 35 g. Larvae live in accumulated dead leaves, and are often found in composts for agricultural use, where they feed on decaying organic matter (Kojima et al. 2014). More than one hundred larvae are sometimes found within a few square meters of humus. This clumped distribution is formed via the following two mechanisms: first, female adults lay eggs at intervals of a few centimeters, and the larvae therefore hatch in close proximity to each other; second, larvae are attracted to carbon dioxide (CO_2) emitted by neighboring larvae (Kojima 2015a). Although the preference for CO_2 is probably an adaptation to locate fermented food rather than to form colonies, this habit results in a clumped distribution. Larvae build their own pupal cells in early summer by compacting a mixture of fecal pellets and humus. The pupal cells are oval (7–8 cm along the major axis, 3–4 cm along the minor axis) and built vertically underground at the same site where the larvae matured. The timing of building pupal cells is not completely synchronized, with 5–10 days of lag between the fastest and slowest larvae within microhabitats (Kojima 2015b). Larvae do not cannibalize pupae, but they do actively move in the humus (ca. 30 cm/h). Considering the high larval density within microhabitats, frequent encounters of larvae with other pupal cells are likely common. Pupal cells are fragile, and therefore at high risk of being accidentally damaged by neighboring larvae.

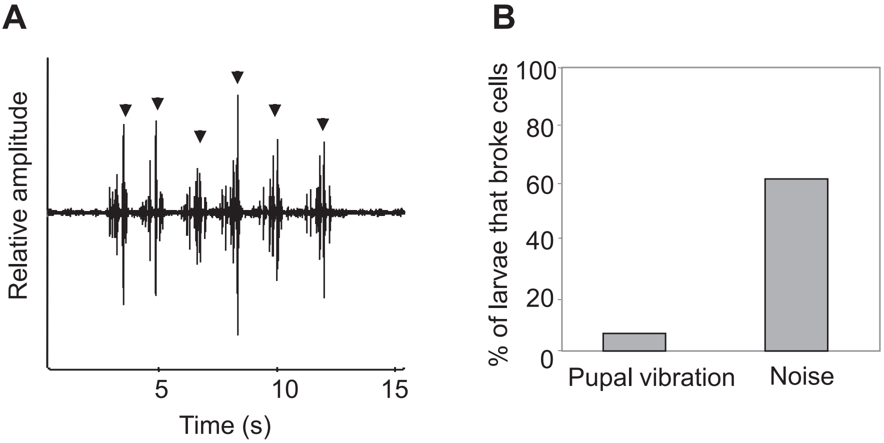

Fig. 6.5 Vibratory interactions between pupae and larvae in *Trypoxylus dichotomus*. (**a**) An oscillogram of vibration produced by a pupa. Six pulses generated through pupal rotating behavior are indicated by arrows. (**b**) Percentage of larvae that broke an artificial pupal cell when pupal vibrations or background noise were played near the artificial pupal cell. Reproduced from Kojima et al. (2012a)

Kojima et al. (2012a) found that pupae and prepupae of *T. dichotomus* rotated their abdomen 3–7 times in succession when conspecific larvae approached their pupal cells (<5 cm). This rotating behavior is likely induced by vibrations emitted "unintentionally" by the burrowing larvae. When pupae (or prepupae) rotate their abdomen, vibration is produced, consisting of several pulses with 1.3 s inter-pulse interval and a low-frequency range, below 500 Hz (Fig. 6.5a). According to synchronous recordings of this vibration and the rotating movement of pupae via high-speed video, the pulses are produced when pupae beat the dorsal side of their prothorax against the inner wall of the pupal cells. The interval between each two successive pulses is equivalent to the time required for pupae to complete a rotation inside their pupal cells (Fig. 6.5a).

To test deterring effects of the pupal vibrations to burrowing conspecific larvae, pupal vibrations were artificially induced near vacant artificial pupal cells (Kojima et al. 2012a). The percentage of damaged pupal cells was remarkably decreased when pupal vibrations were played, compared to when a negative control background noise was played instead (6% vs 60%) (Fig. 6.5b). Monitoring of vibrations associated with larval movement revealed that nearby larvae stopped moving immediately after pupal vibratory signals were played (Kojima et al. 2012b). This suggests that these larvae showed a freeze response, which was prolonged for 3 to 22 min. Thus, pupae produce vibrations to protect themselves from larval disturbance by arresting the movement of conspecific larvae. However, whether the body of the larvae in the freeze response is hardened, as occurs in larvae in TI (Fig. 6.1g), is unknown, since the larval response to pupal signals was characterized by vibrations caused by larval movement in soil, prohibiting visual observation.

6.6.2 Evolution of Freeze Responses

Why do *T. dichotomus* larvae respond to conspecific pupal vibrations? Considering that freeze responses generally play a role in predation avoidance in insects (Takanashi et al. 2019; Chap. 1), the larval response to pupal signals may have originated from an anti-predator adaptation. The predominant predators of *T. dichotomus* larvae in Japan are moles *Mogera* spp. Moles emit vibrations during burrowing in the soil or foraging. Kojima et al. (2012c) provided *T. dichotomus* larvae with mole vibrations, and found that the larvae exhibited freeze responses similar to those following conspecific pupal vibrations. The response to mole vibrations might enhance larvae survival given that various fossorial mammals locate prey using vibrations associated with their activity (Narins et al. 1997; Hill 2009). Kojima et al. (2012c) also found that mole vibrations and *T. dichotomus* pupal vibrations were similar in that both predominantly contain low-frequency components (mostly below 500 Hz) and successions of pulses at intervals of a few seconds. These results imply that pupal vibrations function to mimic vibrations from predators including moles, eliciting larval freeze responses.

To assess the evolutionary process of pupae-larvae communication in *T. dichotomus*, Kojima et al. (2012b) provided the vibrations of *T. dichotomus* to larvae of other scarab species in different subfamilies of Scarabaeidae, including *Protaetia orientalis* (Cetoniinae), *Dicronorhina derbyana* (Cetoniinae), and *Anomala albopilosa* (Rutelinae), whose pupae do not produce vibrations. These three species were found to exhibit similar freeze responses as *T. dichotomus* larvae. The presence of a freeze response instinct in these three subfamilies (Dynastinae, Rutelinae, and Cetoniinae) suggests that the freeze responses to certain types of vibration are not unique to *T. dichotomus*, but have been conserved across scarabaeid beetles, presumably due to the fitness advantage in predator avoidance (Fig. 6.6). Pupae of

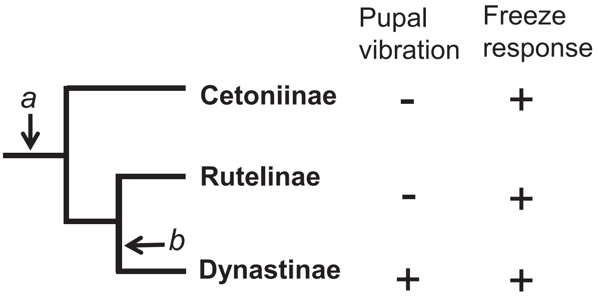

Fig. 6.6 Phylogenetic relationships among Cetoniinae, Rutelinae, and Dynastinae. As larval freeze responses to vibrations have been observed in all three subfamilies, it was likely present in the common ancestor indicated by **a**. Pupal vibration was found in only Dynastinae, suggesting that pupal vibration has been acquired in the ancestor of Dynastinae indicated by **b**. Reproduced from Kojima et al. (2012b)

T. dichotomus likely exploit this anti-predatory response for the protection of their own pupal cells.

If pupae of *T. dichotomus* manipulate larval behavior with deceptive signaling, the larvae may incur a fitness cost through restrictions in their own behavior. This may lead to the counter-selection of larvae that can identify and disregard these deceptive signals (Ruxton and Schaefer 2011); however, larvae do not distinguish pupal signals from signals associated with predators. Additional playback experiments using synthetic vibrations indicate that *T. dichotomus* larvae show stereotypic freeze responses upon perceiving low-frequency vibrations with various temporal patterns (Kojima 2013). The counter-adaptation of larvae may be hindered because the patterns of predator vibrations vary with environmental factors, and larvae risk being attacked by predators in cases of misidentification. In addition, kin selection may favor a larval response to pupal signals. If larvae can avoid breaking the pupal cells of siblings, the cost imposed by pupae is partly offset; however, relatedness of larvae within microhabitats is unknown. The deceptive communication in *T. dichotomus* sheds a new light on the functions of freeze responses in insects.

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Chapter 7
Tonic Immobility in a Cricket: Behavioral Characteristics, Neural Substrate, and Functional Significance

Hiroshi Nishino and Masaki Sakai

Abstract The field cricket (*Gryllus bimaculatus*), a model organism for neurobiological studies, shows tonic immobility (TI) upon restraint of its legs and maintains the restrained posture for several minutes. All bodily movements including abdominal ventilations are inhibited during TI. Catalepsy, in which the animal maintains a new posture forcibly given by an experimenter, is observed in all appendages. Due to the cataleptic nature, the cricket can assume any posture. Ablations of sense organs revealed that exteroceptors such as short trichoid sensilla on the pronotum and campaniform sensilla on the legs were necessary for the flexed-leg posture, and chordotonal organs that detect the tibial movement/position were critical for the induction of TI. Cooling of the brain led to immediate termination of TI, suggesting that immobility of the entire body is maintained by continuous activity of descending neurons originating from the brain. In the natural habitat, TI occurs during escape in which the cricket creates self-imposed restraint by crawling into a small opening made by pebbles or plants, supporting the view that TI is functional for dazzling predators that primarily use vision for prey detection.

Keywords Cricket · *Gryllus bimaculatus* · Tonic immobility · Catalepsy · Chordotonal organ · Command-like neuron · Functional significance

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7.1 Introduction

Many insects show an immobility reaction when they are attacked or restrained by predators. This reaction has been called death-feigning, thanatosis, or animal hypnosis. We call it tonic immobility (TI) here according to the recommendation in Chap. 1.

Representative insects showing TI are insects in the order Coleoptera, Hemiptera, and Phasmatodea. Studies on TI in these insects and some spiders were all published in the early 1900s (Fabre 1910; Robertson 1904; Rabaud 1919; Holmes 1906; Mangold 1920; Bleich 1928; Steiniger 1936). In those studies, characteristics and mechanisms of TI were investigated by using various stimuli and ablation of the nervous system. Since then, there has been no systematic study on TI in insects except for some studies in stick insects. Therefore, TI in insects is summarized on the basis of the above reports.

TI was induced in beetles in the order Coleopteran (Fabre 1910; Mangold 1920; Bleich 1928) by rolling them on the palm of the hand or dropping them onto a table. The beetle assumed an immobile posture on its back for several minutes to 1 h. TI was not easily interrupted by disturbing stimuli but was terminated by some kinds of mechanical stimulation to particular parts of the body such as contact to the mouth or legs (Fabre 1910). There were considerable differences in features of TI among species.

On the other hand, water stick insects in the order Hemiptera (Holmes 1906) and stick insects in the order Phasmatodea (Steiniger 1936; Godden 1972; Bässler 1983) are mostly standstill in a cryptic manner. During the daytime, the former waits for prey underwater, while the latter hides from predators in trees. Although such immobility is not induced by a predator's attack but occurs spontaneously or due to environmental stimuli such as sunlight, it has been traditionally classified as thanatosis (=TI) and their cryptic mimicry is therefore included in TI. TI is also induced by mechanical stimulation. In water stick insects, TI occurred when the slender body was held or stroked with fingers or dropped on a table. TI continued for several minutes to several hours and was terminated by a light stimulus. On the other hand, in stick insects, TI is induced by light with a latency of 5–15 min and continues as long as the light is on. TI, induced by mechanical stimulation, is relatively short and the insect becomes habituated after several trails. As in beetles, TI is not easily interrupted by mechanical stimulation but is terminated by contact to the abdominal tip (Bässler 1983).

As for the mechanism of TI, insects in the three groups mentioned above share two physiological properties: one is that they showed an increase in muscle tonus and inhibition of righting reflex during TI so that they were unable to maintain their normal postures, and the other is that TI was abolished when the head ganglia (especially the brain) were removed from the body. This was strengthened by the fact that the posture in the posterior half of the body became loosened when the connectives were transected between the prothorax and the mesothorax (Bässler 1983). These findings suggested that TI is not a passive state but a state actively controlled by the head ganglia.

Apart from insects, some spiders showed TI in response to a vibratory stimulus such as shaking the web or tapping the box containing them (Robertson 1904). The occurrence and manner of TI were different among three species in line with their different behavioral properties. Such differences were due to the different controls by the brain.

As mentioned above, characteristics and mechanisms of TI in insects were studied almost a century ago. However, electrophysiological techniques have been used only in stick insects (Godden 1972; Godden 1974; Bässler 1983; Driesang and Büschges 1993). The mechanism of light-induced TI was studied at a neural level, and the results provided an important clue for understanding the mechanism of TI in stick insects (Chap. 9). However, the mechanism of TI induced by mechanical stimulation remains unknown.

Are there any other insects that are suitable for neuroethological study of TI? It is known that TI occurred in orthopteran insects such as locusts (Rabaud 1919; Faisal and Matheson 2001) and grasshoppers (Miriyala et al. 2013) and in crickets in the family Gryllidae such as the species *Nemobius sylvestris* and *Liogryllus campestris* (Rabaud 1919). We found that the field cricket *Gryllus bimaculatus* showed distinct TI by mechanical stimulation. This species has a suitable size of the nervous system for electrophysiology and has been one of the model animals for neuroethology (Huber et al. 1989). Thus, we conducted experiments on *Gryllus bimaculatus* to elucidate the neural mechanism of induction and maintenance of TI. In this chapter, we first describe the behavioral characteristics of TI in the cricket and then we show the neural basis of TI and propose its functional significance.

7.2 Time Course of TI

TI in the cricket is induced by simple methods in laboratory conditions. TI with all legs fully flexed is induced by gently pressing both sides of the pronotum and prothoracic legs for 3–5 s with the thumb and forefinger, upon which the cricket enters TI with all legs flexed (Fig. 7.1a, Nishino Lab 1 2018a). This immobility is defined as flexed-leg TI (Nishino et al. 1999). The appearance of flexed-leg TI in the cricket resembles in some species of coleopteran insects (Bleich 1928; van de Kamp et al. 2015). Occasionally, the provoked crickets resisted the restraints instead of assuming leg flexion but somehow entered a state of immobility with all knee joints opened while struggling, which is referred to as “weak TI” as described later.

A single bout of TI in the cricket comprises three phases: (1) induction phase, (2) maintenance phase, and (3) termination phase (Fig. 7.1b; Nishino and Sakai 1996; Nishino et al. 1999). Bodily movements monitored with a photo-coupler placed beside the abdomen (P, Fig. 7.1a) during flexed-leg TI indicated that the induction phase always entails a brief struggle upon restraint (Fig. 7.1b). The struggle stops immediately after all knee joints reach fully flexed positions (Fig. 7.1b; Nishino Lab 1 2018a). The onset of the maintenance phase is often indicated by cessation of

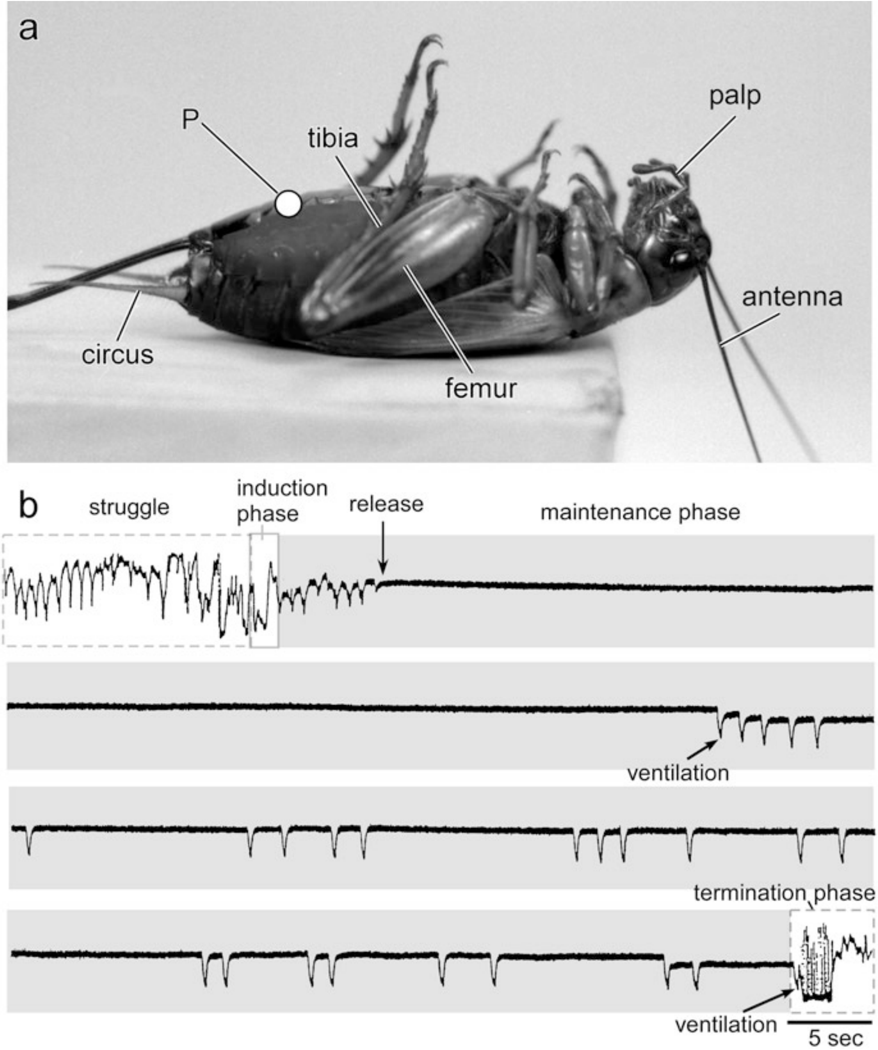

Fig. 7.1 Flexed-leg TI induced experimentally and its time course. (**a**) A cricket in TI showing a rigid posture with all knee joints fully flexed and the antennae and palpi extended in parallel. (**b**) A typical example of the time course of TI in which abdominal movements were monitored by a pair of photodiodes (P). During the induction phase, a brief struggle occurred under a restrained condition. During the maintenance phase, ventilatory movements stopped upon release of gentle pressure applied by fingers and recommenced 1 min after the release, and then ventilations became frequent. During the termination phase, a righting behavior occurred abruptly

ventilation movements in the abdomen, resulting in complete immobility of the entire body (Fig. 7.1b; Nishino Lab 1 2018a). Both antennae are straight and aligned in parallel with the rostral surface of the head (Fig. 7.1a). The palpi and legs are flexed (Fig. 7.1a). The hindlegs are pressed to the abdomen with the femurs directed downward (Fig. 7.1a). The head is raised from the substrate, the abdomen is contracted, and the cerci are crossed. All of these features are indications that the entire body is tonically tensed. Postural adjustment including a righting reflex is inhibited during TI. Usually, abdominal ventilations occur more frequently as time passes (Fig. 7.1b), suggesting that the motor inhibition is progressively weakened toward the end of TI. Regardless of sex, the mean duration of the maintenance phase was 259 s (Table 7.1).

Recovery from the TI, which is expressed as a righting behavior for flexed-leg TI, occurs abruptly, often concurrent with a single ventilation movement in the abdomen (Fig. 7.1b). Immediately after righting behavior, the cricket showed environment searching behavior by vigorous palpation and antennation (Nishino Lab 1 2019a). When the cricket was disturbed, it tended to run very fast, suggesting the presence of a post-inhibitory rebound in the central nervous system.

7.3 Strong TI and Weak TI

The posture of flexed-leg TI varied from individual to individual and from trial to trial in the same individual (Nishino and Sakai 1996). We classified TI into two types (strong TI and weak TI) based on its appearance. In strong TI, all of the appendages including the legs and palpi were strongly flexed (Fig. 7.2a). The hindlegs were tightly pressed to the abdomen. Both antennae were straight and aligned parallel with the rostral surface of the head, which was raised from the substrate. When the body and appendages were touched with fingers, they were stiff and resistive. On the other hand, in the weak TI, the legs and palpi were flexed intermediately, the abdomen was less contracted, the head was raised only slightly, and the antennae were in different positions (Fig. 7.2b).

The durations of TI were measured. The duration of strong TI was significantly longer (264 ± 150 s) than that (92 ± 61 s) of weak TI. Both durations were longer than that (66 ± 55 s) in a quiescent state as a control that spontaneously occurred between searching movements (Nishino and Sakai 1996). The ventilation rate was

Table 7.1 Comparison of parameters in different immobile states induced by different methods

Immobile state	Leg flexion (degree)	Respiration rate (min)	Duration (s)	Auditory response (%)
Flexed-leg TI	2 ± 3	1 ± 2	259 ± 62	9.9
Extended-leg TI	61 ± 27[a]	2 ± 5	241 ± 63	10.0
TI in a natural habitat	/	3 ± 6	216 ± 96	/
Quiescence				
Extended-leg	66 ± 37[a]	3 ± 8	16 ± 32[a]	20.5[a]
Flexed-leg	0 ± 1[a]	9 ± 4[a]	368 ± 241	70.0[a]

[a] Presence of statistical significance with respect to the TI test group (Student's T-test). Thirty trials were conducted for each group of 10 crickets. /, not measured

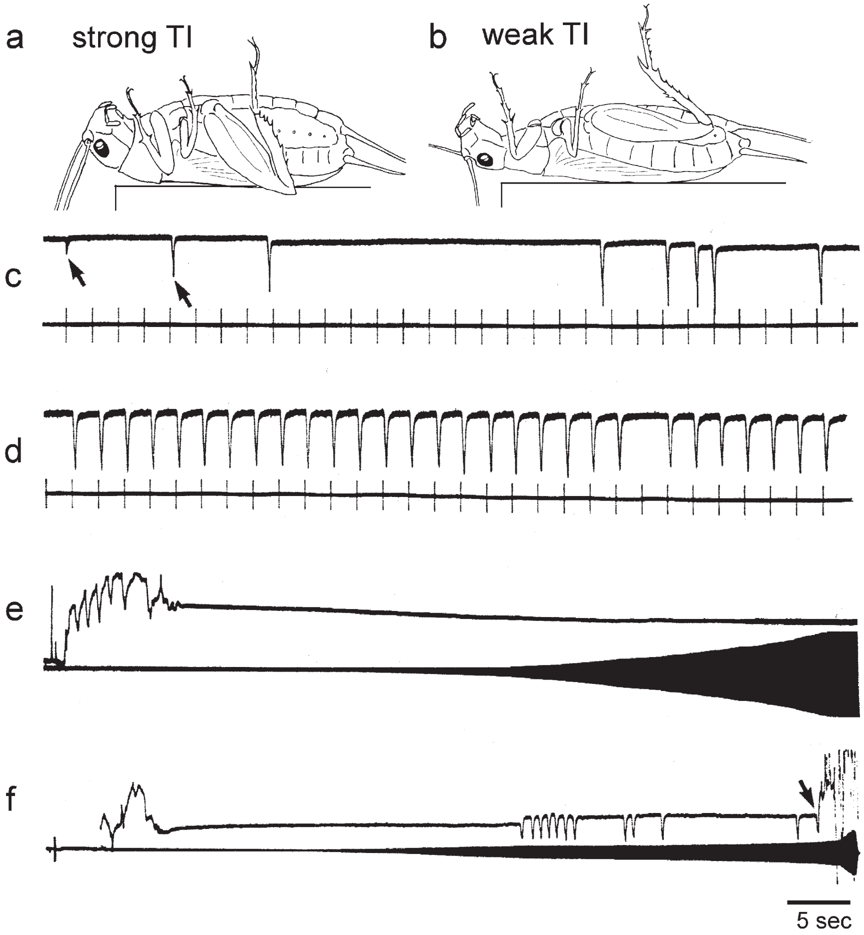

Fig. 7.2 Sensory responsiveness in strong TI and weak TI. (**a**) and (**b**) show strong TI and weak TI, respectively. In (**a**), the cricket is stiffened with all appendages rigidly flexed, while in (**b**), it shows weaker flexion in all body parts. In C ~ F, the upper trace shows abdominal ventilatory movements and the lower trace shows sound stimulation. (**c**) and (**d**) show abdominal ventilatory reactions to a series of sound pulses at 95 dB. The reaction is sporadic during strong TI and the amplitudes of the first two responses were smaller of the beginning of a bout (arrows, **c**), whereas ventilatory reactions occur at almost every stimulation during weak TI (**d**). In strong TI (**e**), the cricket remained immobile without ventilation when the sound intensity was gradually increased up to 103 dB at maximum. In weak TI (**f**), a ventilatory response first appeared at approximately 90 dB and then righting response took place at 98 dB (arrow)

lower in strong TI (1 ± 2 min^{-1}) than in weak TI (5 ± 4 min^{-1}). On the other hand, heart pulsation rate was higher in strong TI (105 ± 7 min^{-1}) than in weak TI (75 ± 8 min^{-1}) although heart pulsation rate in weak TI was still significantly higher than that (59 ± 8 min^{-1}) in the quiescent state (Nishino and Sakai 1996). Whether the increment of heart pulsation rate during TI reflects the struggle before the start of TI or represents the energy consumption for maintaining a low rate of ventilations remains to be clarified. It is noteworthy that crickets exhibiting strong TI ran away more quickly than did those exhibiting a weak posture when disturbed.

7.4 Responsiveness to Sensory Stimuli During TI

In order to examine sensory responsiveness, various stimuli were applied to the cricket during the maintenance phase of TI. For auditory stimulation, a series of sound pulses elicited abdominal ventilations only sporadically in crickets exhibiting strong TI with initial responses (arrows) being weaker than the following responses (Fig. 7.2c). Some ventilations were not stimulus-coupled (Fig. 7.2c). In contrast, crickets in weak TI reacted with abdominal ventilations at nearly one-to-one correspondence to the sound pulse stimulation (Fig. 7.2d). With continuous sound stimulation, crickets in strong TI remained immobile in response to the stimulation even at the maximum intensity of 103 dB (Fig. 7.2e). In contrast, in weak TI, a bout of ventilations appeared at around 90 dB and righting behavior took place at 98 dB (arrow, Fig. 7.2f).

For mechanical stimulation, gentle contact to the body often elicited a ventilation movement but did not affect the maintenance of TI. No response occurred during gentle stroking of the antenna or cercus in strong TI. In the case of the resting state, the cricket instantly responded to contact stimulation to a cercus; that is, the cricket brushed off the stimulus with its hindleg. In the weak posture, stroking an antenna occasionally elicited oscillatory movements of antenna and stroking a cercus induced small leg movement or righting response. On the other hand, TI was terminated by prodding the cricket with a needle regardless of the type of TI (Nishino Lab 1 2020).

Visual stimuli such as intense cold-light illuminating compound eyes were not effective in either strong or weak TI.

The results revealed that responsiveness to sensory stimulation is generally decreased during TI compared with that during the resting state. The sensory responsiveness in strong TI is lower than that in weak TI, as shown in different depths of TI of stick insects (Bässler 1983).

7.5 Emergence of Catalepsy

An index characterizing TI in the cricket was the emergence of “catalepsy” (Fig. 7.3). Catalepsy is defined as “maintaining a new posture forcibly given by an experimenter” (Godden 1974; Bässler 1983; Driesang and Büschges 1993: see Chaps. 2, 8 and 9). During TI, catalepsy is observed in any appendages including the legs, antennae, and palpi. Catalepsy is phenomenally explained by resistance reflex (stretch reflex in vertebrates, Pflüger and Sillar 2013) and its rapid decay in skeletal muscles (Bässler 1983). For example, resistance reflex in the flexor tibiae

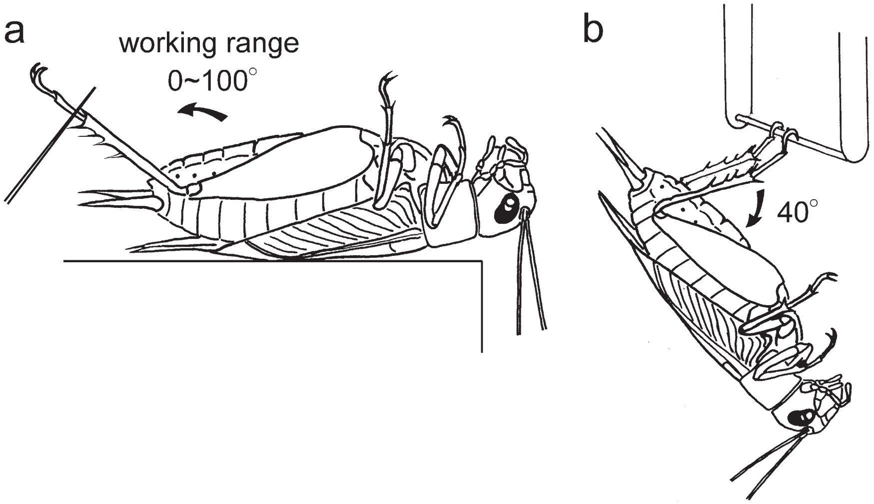

Fig. 7.3 Induction of catalepsy. (**a**) Catalepsy during lying. It was induced by displacing the metathoracic tibia to an arbitrary position. Catalepsy in an intact cricket occurs at any knee joint angle from 0° to ca. 100°. (**b**) Catalepsy during hanging. The cricket continued to be immobile with knee joints opened (ca. 40°) by the body weight

muscle occurs even when the tibia is slightly extended, and the reflex is therefore important for an animal to maintain the initial posture against mechanical perturbations (Field and Burrows 1982). Two types of catalepsy tests in crickets were feasible, though care was needed not to disturb the cricket during TI.

Catalepsy During Lying The metathoracic tibia was forcibly extended to an arbitrary position from full flexion (= 0°) to an extended position with a needle (Fig. 7.3a), held for ca. 3 s, then released to test the degree of imposed catalepsy (Nishino Lab 1 2019b). The experimenter initially felt resistance during displacement of the tibia because of the resistance reflex in the flexor tibiae muscle, but the reflex soon ceased while holding the tibia at the extended position. In the intact cricket, this test caused the F-T joint to be maintained at any angle up to ca. 100° (Fig. 7.3a). When the tibia was extended beyond this limit (maximum extension = 160°), it returned quickly to an angle of around 100°. The range of 0–100° is exactly the working range of the flexor tibiae muscle but not that of the extensor tibiae muscle, suggesting that only flexor muscles contribute to the emergence of catalepsy (Nishino et al. 1999).

Catalepsy During Hanging The cricket was hung by the tarsal claws of the metathoracic legs on a horizontal wire. The body weight caused the knee joints to open from 0° to 40–50°, which was maintained until recovery (Fig. 7.3b; Nishino Lab 1 2019c). This treatment allowed measurement of the strength of the resistance reflex in the flexor tibiae muscle (particularly in slow motor units) during the tonic extension stimulus. In this case, continual resistance reflex occurred to counter the continuous extension by the weight.

Another important feature that underlies the catalepsy is muscle rigidity. To maintain a given posture, skeletal muscles need to retain not only plasticity but also sufficient rigidity so that the knee joint is under complete immobilization. Emerging catalepsy explains why various animals including crickets maintain varied and sometimes even peculiar postures passively taken during TI.

7.6 Survey of Sense Organs Involved in Induction of TI

To facilitate an understanding of the neural substrate underlying the induction and maintenance of TI, we here introduce results of dysfunctioning of sensory elements. Insects are equipped with two types of mechanoreceptors in the body and appendages: exteroceptors and internal proprioceptors. Typically, the exteroceptors have short to long cuticular protrusions housing one mechanosensory neuron (Keil 1997). These receptors are functionally equivalent to nociceptors embedded in the skin of vertebrates (Abbracchio and Reggiani 2013). The primary internal proprioceptors are chordotonal organs distributed extensively in appendages and body segments that measure the position and movement of their own segments and vibrations via a chord-like cuticular apodeme (Field and Matheson 1998). There are various sizes and forms of chordotonal organs (Field and Matheson 1998), as exemplified by subgenual organs and tympanal organs specialized for detecting substrate vibration and airborne sound, respectively (Yack 2004; Nishino et al. 2019).

The central question for deciphering triggering mechanisms of TI is which sense organ detects physical restraint of the legs, inasmuch as TI in the cricket is commonly induced by restraint of the legs. In fact, amputation of the legs prevented crickets from entering TI; more legs ablated lead to more difficulty in entering TI (Nishino and Sakai 1996), indicating that the total amount of sensory input from legs is related to inductivity of TI. Amputation of three hemilateral legs resulted in reduced inductivity of TI, similar to results when all six legs were amputated, suggesting that bilateral restraint of legs is important for induction of TI (Nishino and Sakai 1996).

Ablation of various exteroceptors on the body surface revealed that sense organs sensitive to deformation of the exoskeleton, such as campaniform sensilla on prothoracic legs (Fig. 7.4a) and short trichoid sensilla numerously distributed on the pronotum (Fig. 7.4b, c, d), participate in the leg flexion reflex (Nishino and Sakai 1996). However, extended-leg TI still occurred in animals with these exteroceptors eliminated, suggesting that exteroceptors mediate flexion reflex but are not critical for triggering TI. In contrast, ablation of the internal proprioceptors, femoral chordotonal organs (FCOs), in all six legs (see Fig. 7.4a for the location) somehow mimicked the results of ablation of the entire leg, preventing the induction of TI.

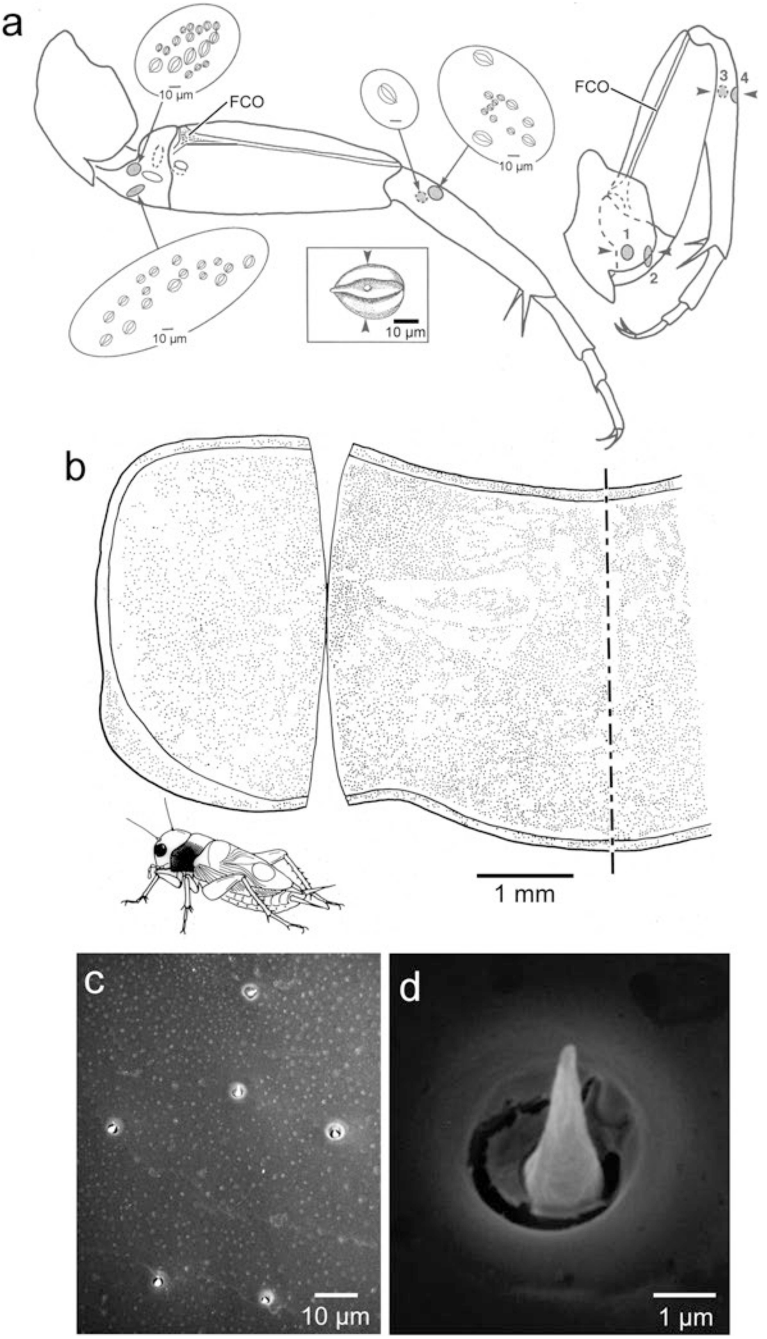

Fig. 7.4 Mechanoreceptors that elicit leg flexion reflex in the prothoracic leg and the pronotum. (**a**) Groups of campaniform sensilla indicated by gray (individual sensilla magnified in circles in

7.7 Contribution of the Central Nervous System to TI

In order to evaluate the contribution of the central nervous system to maintenance of an immobile state, cooling of the brain was performed during TI by placing a small piece of dry ice on the frontal cuticle of the head. Crickets showed ventilatory movements as soon as cooling started. Such an early ventilation response can be taken as an indication of the commencement of neural inactivation because a similar effect was observed immediately after the connective cut between the brain and subesophageal ganglion. The latency of the ventilatory response was 5 ± 3 s (n = 10), which was much shorter than the latency of 150 ± 95 s in the control (non-cooled) animals. The duration of TI was 6 ± 3 s (n = 10), which was also much shorter than the duration of 288 ± 150 s in the control animals. Separate experiments (see Materials and methods in Nishino and Sakai 1996) indicated that the brain temperature was lowered to about 11 °C at 5 s after the start of cooling, while it was 16 °C on the surface of the suboesophageal ganglion after 1 min. Synaptic transmission or spike conduction in neurons of the head ganglia was not severely interrupted until the head temperature was lowered below about 10 °C. These findings indicated that the effect of head cooling on termination of TI is caused by inactivation of the brain and suggested that the brain is crucial for the cricket to maintain TI.

It is known that the head ganglia play an important role in maintenance of TI in insects. In the water stick insect, death-feigning did not continue for more than several seconds after the brain had been removed (Holmes 1906). The removal of the brain abolished twig mimesis in the stick insect because locomotor activity was increased (Godden 1972). Our results in crickets are consistent with those in other insects for the role of the brain in maintenance of TI (see Introduction). It was reported that there is a command interneuron in the hemi-connective originating from head ganglia in the crayfish (named statue fiber) that evokes a sudden cessation of ongoing movements during electrical stimulation (Bowerman and Larimer 1974). A pair of command neurons that evoke cessation of ongoing movements by optogenetic activation have been identified recently in fruit flies (Zacarias et al. 2018). Command-like neurons playing a similar role in movement inhibition may be present in the cricket. In our preliminary study (Nishino and Sakai 1991), it was found that some descending interneurons from the head ganglia were discharging throughout the period of TI, while many others ceased to discharge, supporting the view that the brain in the cricket may contain command-like fibers responsible for induction and maintenence of TI, as in other arthropods.

◄

Fig. 7.4 (continued) the left figure) were more effective for eliciting the flexion reflex than were those indicated by white, presumably because the former groups are mechanically compressed when prothoracic legs are pressed laterally (arrowheads in the right figure). One representative campaniform sensillum is illustrated in the rectangle, indicating the direction of the force that was effective for stimulating the sensillum. The location of the femoral chordotonal organ (FCO) is also indicated in **a**. (**b**) Each dot in B represents a single trichoid sensillum in the left half of the pronotum (inset). (**c**) and (**d**) show low-power and high-power photos of trichoid sensilla and a single sensillum taken by a scanning electron microscope

7.8 TI that Occurs in Natural Settings

TI can be induced in more natural situations in which the ventral side of the thorax and abdomen was in contact with the substrate (Nishino and Sakai 1996). Under experimental conditions, TI occurs in conjunction with fleeing when disturbed or urged by an experimenter: a physical restraint when the cricket attempts to crawl into a small tunnel made on the substrate makes the cricket immobilize (Fig. 7.5a; Nishino Lab 1 2018b). The legs were immobilized at extended positions as restrained between the substrate and the ceiling (61° ± 27°, n = 30), thus designated as extended-leg TI (Fig. 7.4b). The duration of TI, ventilation rate, and responsiveness to sensory stimulation are similar to those in flexed-leg TI, suggesting that both states are basically identical (Table 7.1). When the extended-leg TI was terminated, the cricket raised its body with the palpi and antennae moving and began to walk slowly with searching movements (Nishino Lab 1 2018b). When the imposed leg restraint was insufficient due to a large space on the substrate, the cricket never entered TI and walked out from the tunnel. These results indicated that TI in the cricket always entails a self-generated restraint resulting from the flexion reflex or struggling in a narrow space. An adequate stimulus to elicit TI in crickets is therefore restraint of the legs, and a minimum pressure not to permit resistive leg movements appears to be effective for smoothly guiding the cricket into TI.

In the natural habitat, we observed that TI indeed occurred when the cricket (*G. bimaculatus* in Okinawa Islands of Japan) attempted to hide by crawling into a small opening made by pebbles or roots of plants (Fig. 7.5c). This is supposed to occur by the same mechanism as that in the extended-leg TI. The sudden stopping by the cricket is probably effective for dazzling the eyes of predators (Chap. 1; Channel Wani 2020).

7.9 Differences in TIs and Escape Reactions in Related Species

Variations in inductivity and duration of TI were observed in four species of field crickets. *G. bimaculatus* and the genus *Teleogryllus* species, which are phylogenetically close (Chintauan-Marquier et al. 2016) and have similar body sizes. However, *G. bimaculatus* prefers to inhabit a gravel-rich field, whereas *T. emma* inhabits a grass field. *G. bimaculatus*, which has shorter legs and rarely jumps, rushed into a small opening between pebbles and was immobilized for a while when urged by experimenters (Fig. 7.6a). In contrast, *T. emma* and *T. occipitalis*, both having longer legs relative to their body size, ran fast in combination with jumps when disturbed (Fig. 7.6b). *T. infernalis*, another *Teleogryllus* species tends to live in a gravel-rich field as does *G. bimaculatus*. However, *T. infernalis* stops only briefly upon restraint in a narrow space and tries to crawl deeper to search for a safer place. Since *T. infernalis* is more agile than *G. bimaculatus,* finding safe places might be

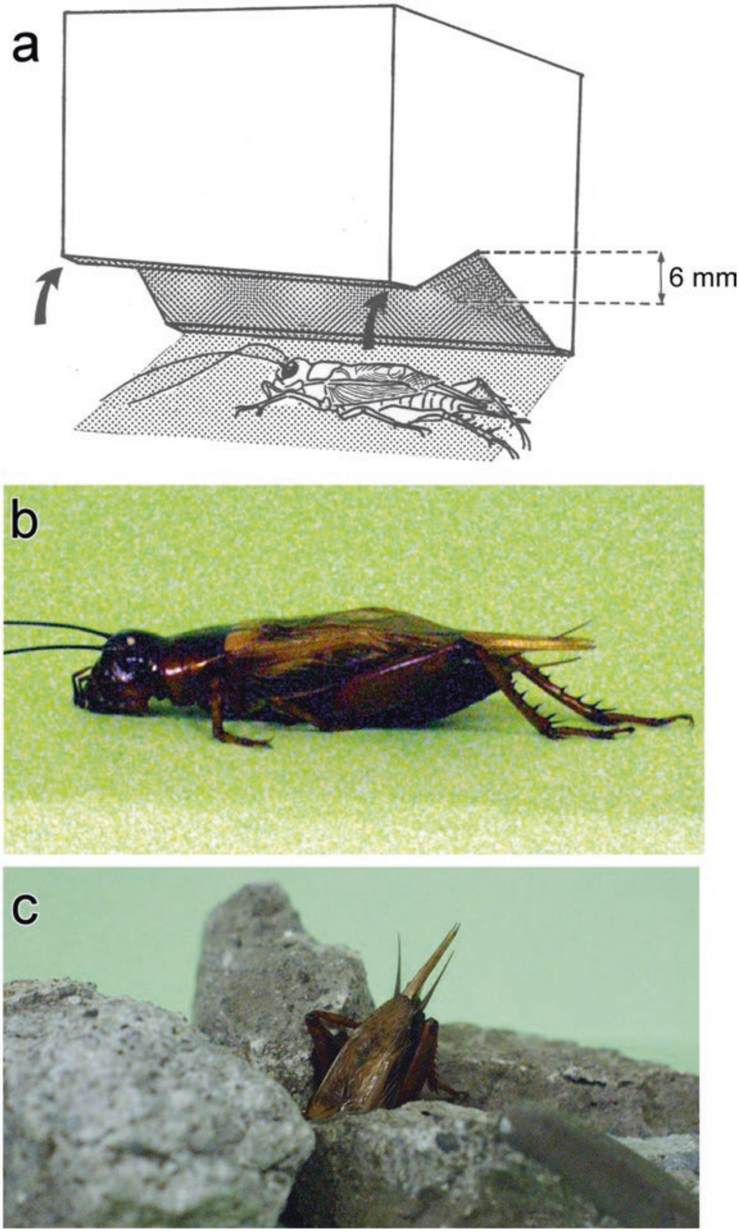

Fig. 7.5 TIs in natural settings. (**a**) TI occurred when the cricket crawled into a small tunnel made by a manipulator base and the substrate. Note that the posture in TI is maintained even after removal of the manipulator base. (**b**) A picture of the cricket taken in **a**. (**c**) TI occurred in a space between pebbles

more adaptive for *T. infernalis* than immobilizing in exposed places. The occurrence rates of TI in the three *Teleogryllus* species were lower and the periods of TI were shorter than those in *G. bimaculatus* (Fig. 7.6c, Yukari Konishi, unpublished data). These results suggest that TI in crickets depends on anti-predator strategies linked to their habitats.

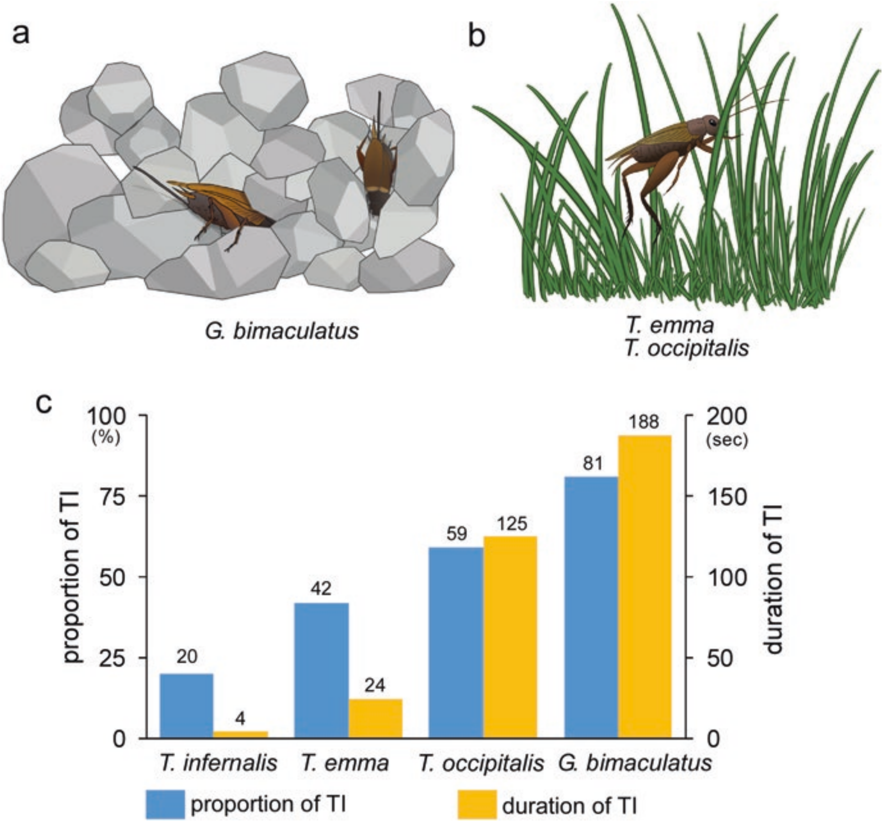

Fig. 7.6 Different escape strategies linked to habitats in four species of field crickets. (**a**) *G. bimaculatus* prefers to inhabit a gravel-rich field and tends to rush into a small opening between stones or between roots of grasses when disturbed and exhibits TI close to the entrance. *T. infernalis* inhabits a gravel-rich field and tends to crawl into small spaces to hide. (**b**) *T. emma* and *T. occipitalis* inhabit grassland and escape by jumping mixed with running. (**c**) Comparisons of the proportions of crickets showing TI and the durations of TI in the four species of crickets. Note that two species with a higher proportion of TI had longer duration of TI

7.10 Summary

TI in the cricket has the following characteristics: (1) TI is induced by physical restraint of the legs, (2) the duration of TI is several seconds to several minutes, (3) during TI, responsiveness to sensory stimulation is decreased, (4) TI is terminated spontaneously or by a mechanical disturbance, (5) catalepsy emerges during TI, (6) TI is used as one of the anti-predator strategies in conjunction with fleeing behavior. Many of these findings for TI in the cricket are similar to those for vertebrates such as birds and mammals (Ratner 1967).

Regarding the mechanisms of TI in the cricket, chordotonal organs which are internal proprioceptors are critically important for inducing TI and the brain is necessary for maintaining immobility of the whole body. In Chap. 8, concrete roles of chordotonal organs in induction and postural control of TI and motor output characterizing TI are described in more detail.

Acknowledgments We thank M. Domae for kindly providing an illustration shown in Fig. 7.6a, b.

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Chapter 8
Tonic Immobility in a Cricket: Neuronal Underpinnings of Global Motor Inhibition

Hiroshi Nishino

Abstract Tonic immobility (TI) in the field cricket *Gryllus bimaculatus* is triggered by physical restraint of the legs, and is locally characterized by resistance upon imposed restraint and its rapid decay that occur regardless of the tibial position for each leg. The neuronal substrate underlying the induction and maintenance of TI was investigated by sensory and motor neuronal recordings in free-moving crickets. Isometric contraction of flexor tibiae muscles that occurred under the condition of leg restraint intensely activated sensory neurons in the femoral chordotonal organ (FCO). The "restraint signals" detected by prothoracic FCOs intersegmentally inhibited active movements of all legs, which was a hallmark of the widespread motor inhibition. In each leg, two neuronal groups in the FCO mediate the tibial resistance reflex and its inhibition in the same leg, respectively. The flexor muscle rigidity unique to TI was maintained by a weak discharge of slow excitatory motor neurons together with suppression of the activity of common inhibitory motor neurons. Taken together with the requirement of the brain for maintaining TI, I propose that a local reflex that mitigates isometric muscle loading is incorporated into a brain-controlled long-lasting immobilization for camouflaging against predators during evolution.

Keywords *Gryllus bimaculatus* · Common inhibitory motor neuron · Isometric contraction · Accessory flexor muscle · Resistance reflex · Freezing reaction

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8.1 Introduction

The field cricket *Gryllus bimaculatus* displays a tonic immobility (TI) when leg movements are physically hindered (Nishino Lab 1 2018). During TI, knee joints in all legs are stiffly immobilized for several minutes. As an index characterizing TI, catalepsy in which the new tibial position passively given is maintained emerges during TI (Bässler 1983). Catalepsy is physiologically characterized by a sudden decay of the resistance reflex to a forced displacement of appendages including legs and antennae. Catalepsy that emerges during TI, therefore, explains why TI occurs at various and sometimes peculiar postures including fully flexed to extended-leg postures.

The procedure for eliciting catalepsy is similar to that for eliciting TI. A forced displacement of tibiae at new positions and holding them for a while (e.g., 3 s) result in induction of TI and, at the same time, catalepsy emerges for each leg. Therefore, similar sensory mechanisms are very likely to underlie induction of both TI and catalepsy. In this context, results of ablation experiments (Chap. 7) suggested that femoral chordotonal organs, major internal proprioceptors that govern the knee joint control, are critical for induction of TI. Studying concrete roles of chordotonal organs would provide a clue for further elucidation of the mechanism by which TI is induced.

On the other hand, the motor output characterizing TI has been enigmatic because TI occurs at any posture due to its cataleptic nature, superficially resembling a normal quiescence state. During TI, knee joints are stiff, not flaccid, in the cricket (Nishino and Sakai 1996) as in wetas (Hoyle and Field 1983) and beetles (Chap. 6). As an immobile state resembling TI, a sudden pause of ongoing movements occurs when an insect during locomotion detects an unexpected stimulation such as substrate vibrations or sound (Hoy 1989; Takanashi et al. 2016). This behavior has been termed the freezing reaction by Friedel (1999).

In contrast, a deep resting state (i.e., sleep-like state) differs from TI in that a decrease of the muscle tonus (antennal and neck inclination) is prominent in resting crickets (Nishino 2004) and honey bees (Kaiser 1988; Sauer et al. 2003). This immobility is frequently interrupted by short bouts of limb or antennal movements (Kaiser 1988; Nishino and Sakai 1996; Sauer et al. 2003).

Given that catalepsy is observed in any appendages (Nishino and Sakai 1996), the neuronal events observed in skeletal muscles of a single leg are expected to occur in other appendages. Therefore, I focused on large muscles, flexor and extensor tibiae in the femur, which elicit knee joint flexion and extension, respectively. The flexor tibiae is one of the principal posture-controlling muscles and is fundamental to the maintenance of tibial flexion during TI. Sensorimotor controls of these muscles have been intensively studied in locusts (Hoyle and Burrows 1973; Burrows 1996) and stick insects (Godden 1972; Bässler 1983). Since crickets are easily provoked in a largely dissected condition or in a continually restrained condition and

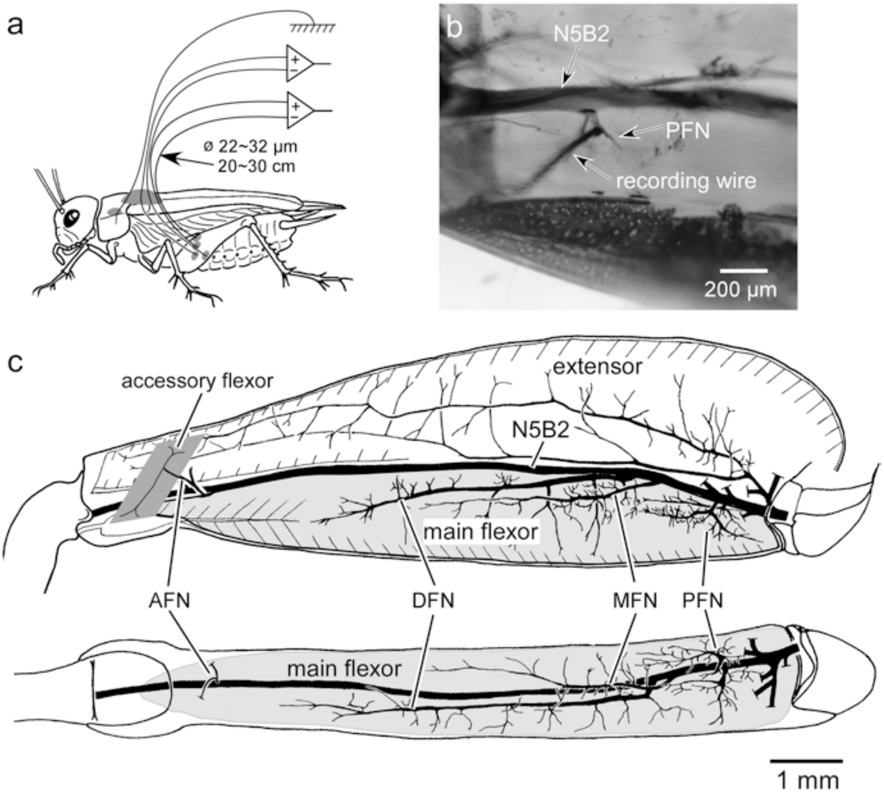

Fig. 8.1 Procedure for recording motor neuronal activity and anatomy of the metathoracic leg. (**a**), (**b**) Neurogram recordings were achieved in a tethered condition; the tip of the recording electrode (copper wire, ø 22–38 μm) was positioned closely to the targeted nerve. The reference electrode was placed on the surface of the muscle innervated by that nerve. (**c**) *Camera lucida* drawing of the motor innervation in the metathoracic femur viewed posteriorly. The flexor tibiae comprise the main flexor that primarily contributes to the leg flexion movement and the accessory flexor that contributes to maintaining the flexed posture. The main flexor is differently innervated by the proximal flexor nerve (PFN), middle flexor nerve (MFN), and distal flexor nerve (DFN). See Fig. 8.9 for detailed neuroanatomy of the accessory flexor

have difficulty in entering into TI, neuronal recordings during TI were achieved under a condition in which free movements of the cricket were not hindered.

By using extracellular recordings from crickets in tethered conditions (Fig. 8.1a, b), I successfully characterized the sensory signaling underlying the induction of TI and related motor neuronal activity. Taken together with the results shown in Chap. 7, I propose the most plausible picture of neural mechanisms of TI from an evolutional viewpoint.

8.2 Neuroanatomy of Flexor and Extensor Tibiae Muscles

The flexor tibiae and extensor tibiae muscles in the cricket occupy the ventral and dorsal regions in the femur, respectively (Fig. 8.1c). The flexor tibiae comprises the main flexor that lies axially in the ventral femur and the accessory flexor that lies diagonally (45°) in the distal femur, both inserting onto a common flexor apodeme (Fig. 8.1c; Heitler 1974; Hustert and Gnatzy 1995).

As in locusts (Hoyle and Burrows 1973; Hoyle 1980), excitatory motor neurons are classified into slow, intermediate, and fast types according to their physiological properties (Table 8.1). The slow-type fires tonically to maintain muscle tonus, though high-frequency firing of slow exciters evokes slow movement (Hoyle and Burrows 1973). The fast type fires phasically and evokes a strong twitch contraction in muscle fibers. The intermediate type fires phasotonically in longer-lasting bursts with a progressively declining frequency during visible movements (Nishino et al. 1999; Nishino 2003). Common inhibitory motor neurons (CIs), which are unique to arthropods, innervate specifically slow muscle fibers of multiple muscles (Burrows 1973; Hale and Burrows 1985) and counteract the function of slow exciters by eliminating the muscle tonus.

The main flexor tibiae in the cricket is compartmentalized into the proximal, middle, and distal regions, each of which is innervated by a separate nerve branch diverging from the main leg nerve N5B2: the proximal flexor nerve (PFN), middle flexor nerve (MFN), and distal flexor nerve (DFN) (Fig. 8.1c). Each nerve contains axons of different subsets of motor neurons (Table 8.2). Recordings from the motor nerve branches revealed that the PFN has approximately 12 exciters (3–4 slow types, 7–8 intermediate types, and 1–2 fast types), the MFN has three exciters (two intermediate and one fast types), and the DFN has four exciters (one intermediate and three fast types). Thus, the main flexor muscle changes progressively from slow to fast innervation distally (Table 8.2).

On the other hand, the accessory flexor comprises two groups of 5–6 muscle bundles inserting into the anterior and posterior edges of the cushion. This muscle is innervated by bifurcated branches of the accessory flexor nerve (AFN) diverging from N5B2 (Fig. 8.1c). Each branch contains three slow excitatory and one

Table 8.1 Roles of distinct types of motor neurons in the cricket *G. bimaculatus*

Motor neuron type		Spike size	Activity	Function
Exciter	Fast	Large	Phasic	Eliciting strong twitch contraction in muscles
	Intermediate	Medium	Phasotonic	Eliciting prolonged contraction, linked to visible movement
	Slow	Small	Tonic (3–50 Hz)	Increasing muscle tonus
Inhibitor		Medium	Tonic (0.5–1 Hz)	Eliminating muscle tonus

The classification of and activity profiles of distinct types of motor neurons are based on results in Nishino (2003)

Table 8.2 Local innervation patterns of flexor and extensor tibiae motor neurons in the cricket *G. bimaculatus*

	Main flexor				
Motor neuron type	PFN	MFN	DFN	Accessory flexor	Extensor
Fast	1 ~ 2	1	3	0	1
Intermediate	7 ~ 8	2	1	1	0
Slow	3 ~ 4	0	0	3	1
Inhibitor	2	0	0	2	1

The results are based on extracellular recording and backfilling of individual nerve branches (Nishino 2003). Since one neuron or two neurons are overlapped between different pairs of flexor nerve branches (see Fig. 8.1c), the total number of neurons that innervate the flexor muscle was estimated to be at least 19 (see Nishino 2003 for details)

intermediate excitatory motor neurons (Table 8.2). Therefore, the accessory flexor is predominantly slow in nature (Nishino 2003).

Whereas the main flexor acts to drive the tibia to the flexed position, the accessory flexor acts to maintain the tibia at the fully flexed position by a small contraction force due to lever action (Hustert and Gnatzy 1995). Crickets in which the metathoracic accessory flexor was ablated were not capable of maintaining the metathoracic legs at flexed positions in "catalepsy during hanging" (see Fig. 7.3b in Chap. 7).

In contrast, innervation of the extensor tibiae is simple as in other orthopteran insects (Godden 1972; Hoyle 1980). This muscle is innervated by one fast type excitatory motor neuron and one slow-type excitatory motor neuron (Table 8.2), the latter of which shows a firing profile similar to that of the intermediate type of flexor motor neurons (Nishino 2003).

In the following sections, the flexor tibiae muscle and the extensor tibiae muscle are abbreviated as the flexor and the extensor, respectively, and excitatory motor neurons and common inhibitory motor neurons are abbreviated as exciters and inhibitors, respectively (Tables 8.1 and 8.2).

8.3 Isometric Muscular Contraction Characterizes the Induction of TI

Simultaneous recordings of flexor and extensor motor neurons under a tethered condition (Fig. 8.1a) were achieved during TI and walking. The extensor and flexor were activated reciprocally during walking though some co-activation was evident (Fig. 8.2a).

Despite distinct appearances of flexed-leg TI and extended-leg TI, the induction phase of both TIs was characterized by similar motor patterns: intense flexor activity recruiting fast exciters (that caused cross-talk in the extensor tibiae) and its rapid decay. For flexed-leg TI, brief but strong flexor activation occurred during the flexion reflex (Fig. 8.2b). However, immediately after the tibia reached the fully

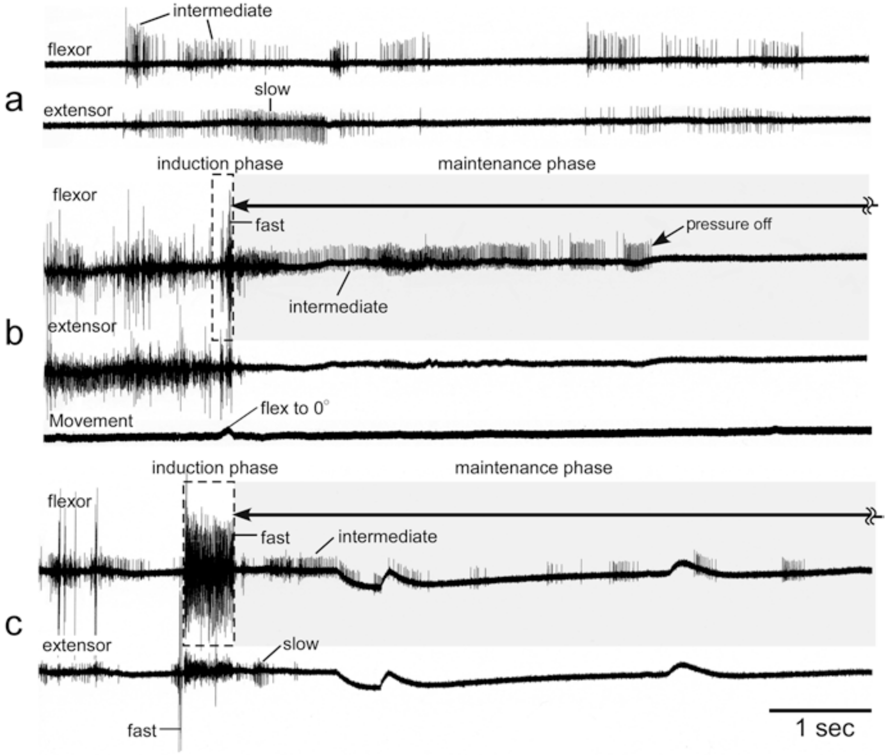

Fig. 8.2 Neurograms of the metathoracic flexor and extensor tibiae during walking, induction phase of flexed-leg TI, and induction phase of extended-leg TI recorded from the same cricket. (**a**) During walking, the flexor tibiae and extensor tibiae are activated generally reciprocally. (**b**), (**c**) The induction phase of the two types of TI is characterized by massive flexor discharge recruiting fast exciters (fast). Some cross-talk is observed in the extensor during the induction phase. A sharp reduction of flexor activity occurs after the knee joint becomes 0° (upper deflection in the movement monitor, b) or at a restrained position (**c**) and the maintenance phase commences. During TI, intermediate flexor exciters (intermediate) and slow extensor exciters (slow) are activated with the occurrence of ventilatory movements. Upper deflections of baselines in c are artifacts

flexed position (0°), the flexor activity suddenly waned and TI commenced (Fig. 8.2b).

Strong flexor activity also occurred in the induction phase of extended-leg TI but tended to be prolonged (Fig. 8.2c) because it took more time before all knee joints were properly fixed in the tight space compared to flexed-leg TI. In any case, the induction phase of TI was characterized by isometric contractions of skeletal muscles that occur under the condition of no change in muscle length (Pflüger and Sillar 2013) when the knee joint is fixed at the fully flexed position or at a restrained position.

During the maintenance phase, the extensor activity ceased except for brief activations of the slow exciter concurrent with ventilatory movements (Fig. 8.2c). In

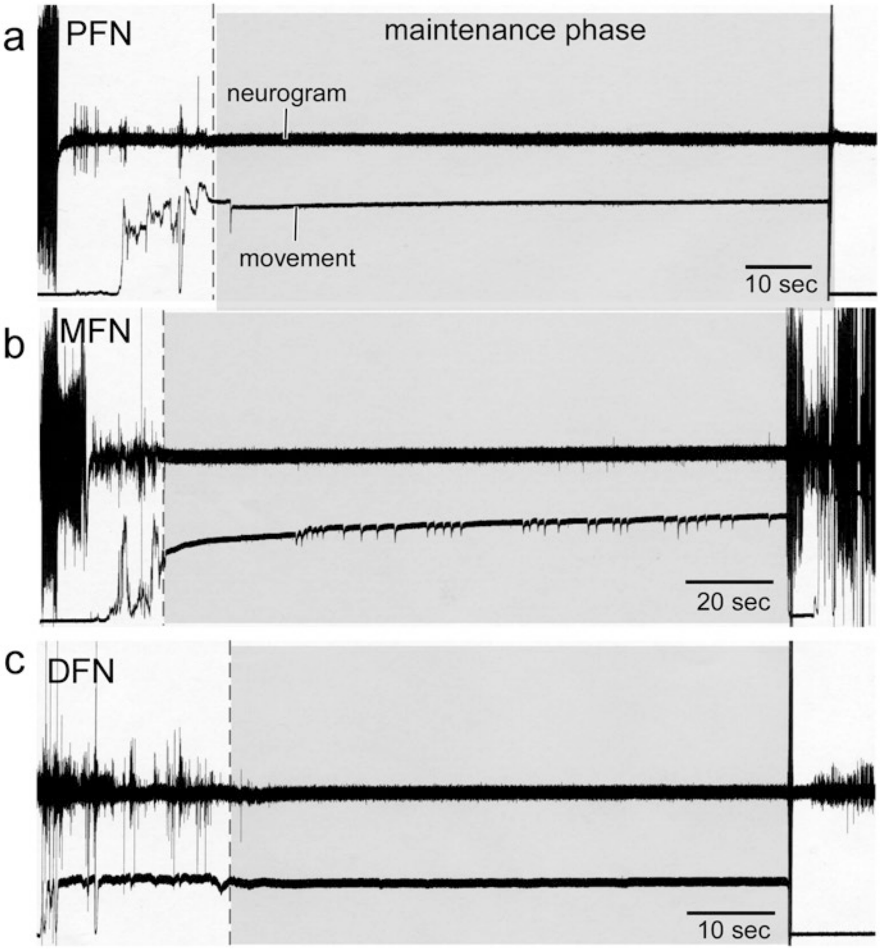

Fig. 8.3 Neurograms recorded from PFN (**a**), MFN (**b**), and DFN (**c**) supplying the main flexor during the course of TI. During TI (shaded by gray), activity of intermediate and fast exciters was almost completely suppressed. The intermediate exciters tended to be activated more frequently when ventilatory movements (deflections, lower trace) occurred during TI. Intense activation of intermediate and fast exciters occurred when righting behavior took place on arousal. Fast exciters are truncated

fact, the extensor does not contribute to maintenance of the TI posture. This was unequivocally supported by the finding that dysfunctioning of the extensor has no effect on the posture during TI (Nishino et al. 1999).

Activities of fast and intermediate exciters in the flexor muscle also ceased during the maintenance phase (Fig. 8.2b, c), being consistent with results of extracellular recordings of the PFN, MFN, or DFN (Fig. 8.3). Toward arousal (equal to termination of TI), intermediate exciters tended to be activated progressively concurrent with ventilatory movements (e.g., Fig. 8.3b). Although activities of slow exciters in the flexor are not evident in these neurograms (Fig. 8.3), their activities persist for maintaining the flexed posture, as described in the Sect. 8.7.

8.4 Neuroanatomy of the Femoral Chordotonal Organ

The femoral CO (FCO), running in parallel to the extensor, is one of the largest proprioceptors in the cricket (red area in Fig. 8.4a). Its location and sensory neuronal organization are similar in all six legs (Nishino and Sakai 1997). There are two distinct clusters of bipolar sensory neurons called “scoloparia” (Field and Matheson 1998). The ventral scoloparium contains small to large sensory neurons, while the dorsal scoloparium contains only small neurons (Fig. 8.4b, c). The two scoloparia are distally connected to two parallel running ligaments that join in a common cuticular apodeme extending from the pivot of the knee joint of the tibia (Fig. 8.4a). Therefore, the tibial flexion stretches the sensory dendrites distally and tibial extension pushes the dendrites proximally. Each sensory neuron has been shown to be functionally specialized for measuring position, velocity, acceleration, and their combinations in locusts (Matheson 1990) and in stick insects (Hofmann et al. 1985; Büschges 1994). Some neurons responded across the full range of knee joint angles, while others had restricted response ranges (Matheson 1992). The dorsal scoloparium neurons (DSNs, Fig. 8.4b, c) have been suggested to tune to substrate vibrations at approximately 300–500 Hz in locusts (Field and Pflüger 1989) and stick insects (Stein and Sauer 1999).

In the cricket, sensory neurons of the ventral scoloparium are further classified into two anatomically separable groups, the ventral group (VG) and dorsal group (DG) (Fig. 8.4b, c; Nishino and Sakai 1997). The VG neurons are orderly arranged and become smaller distally. The DG neurons with similar soma sizes cluster in the proximal region (Fig. 8.4b, c).

The axonal projections of the three neural groups into the central nervous system provide clues for inferring their functions. Heavy metal ($NiCl_2$) infusion into individual neural groups and subsequent silver intensification revealed distinct central projections conserved in FCOs of all legs (Fig. 8.5a, b, c; Nishino and Sakai 1997). The VG neurons projected broadly to lateral neuropils (Fig. 8.5ai,aii) including the motor association center (lateral association center: LAC), where extensor and flexor tibiae motor neurons possess dendrites (Fig. 8.5aii; Pflüger et al. 1988). Their projection fields shifted lateral to medial according to the disto/proximal soma locations, implying their progressive recruitment on range fractionation (Nishino 2000). The DG neurons gave rise to some collaterals in the LAC (Fig. 8.5bii) and provided abundant terminal arborizations in the region just lateral to the medial ventral association center (mVAC) (Fig. 8.5bi), which is known as a neuropil that processes auditory/vibratory signals (Pflüger et al. 1988). The termination fields of the DG neurons largely overlapped with those of the subgenual organ specialized for detecting substrate vibrations (Nishino and Field 2003). DSNs projected exclusively to the dorsal region in the mVAC (Fig. 8.5ci, cii; Nishino 2000).

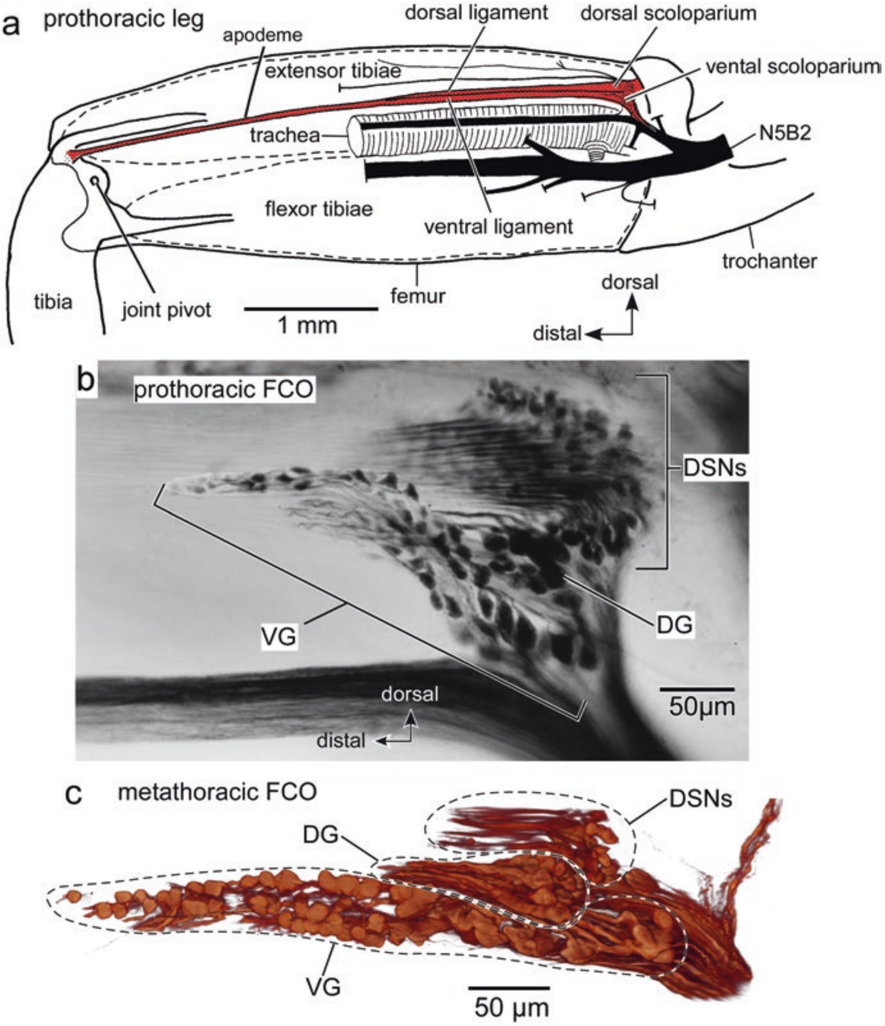

Fig. 8.4 Morphologies of femoral chordotonal organs (FCOs). (**a**) The location and innervation pattern of FCOs (red area) are similar in all legs. The ventral and dorsal scoloparia (clusters of bipolar sensory neurons) are distally connected to a cuticular apodeme via the ventral and dorsal ligaments. The apodeme is distally inserted in the dorsal region of the knee joint pivot. (**b**, **c**) The ventral scoloparium is composed of two neuronal groups: the ventral group (VG) and the dorsal group (DG). Cell bodies (6.5–18.5 µm in diameter) sequentially become smaller distally for the VG, while similar-sized neurons (8.5–19.5 µm in diameter) are concentrated proximally for the DG. The dorsal scoloparium neurons (DSNs) comprise small neurons (5.5 and 13.3 µm in diameter), and the number being much smaller for the metathoracic FCO (**c**) than for the prothoracic FCO (**b**)

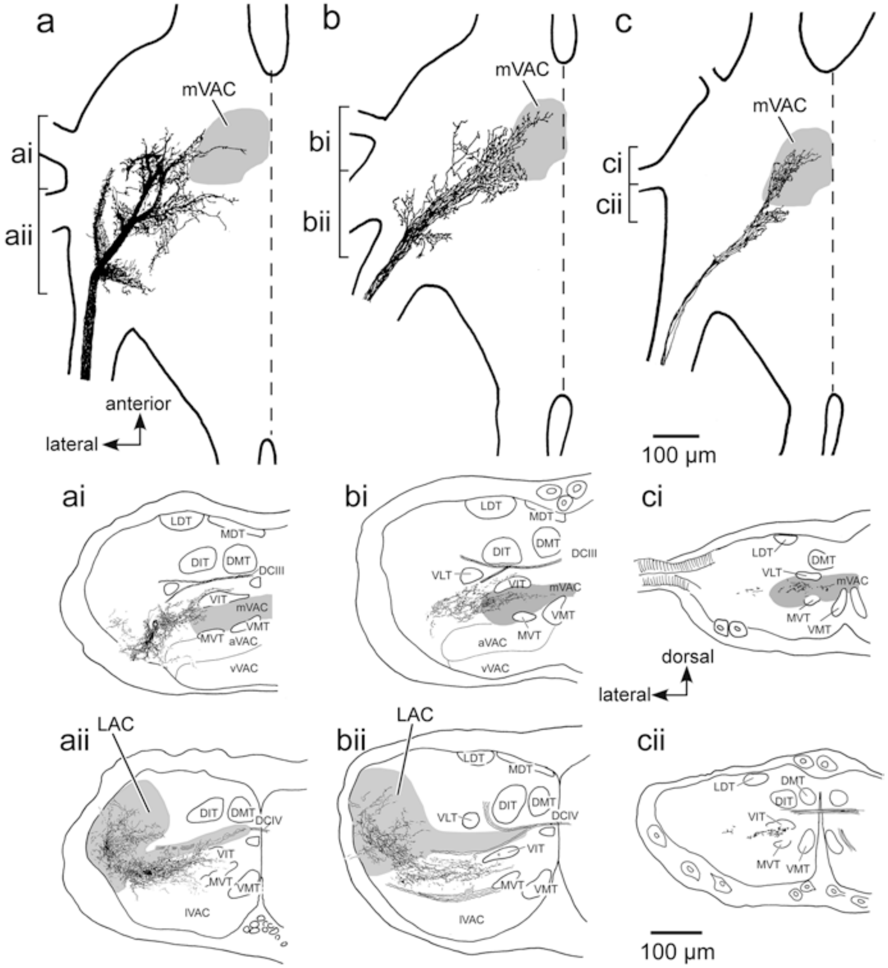

Fig. 8.5 Central projections of the three neural groups of the metathoracic FCO. Whole mount specimens (**a**–**c**) and their transverse sections (**ai**–**cii**) show partly overlapping but distinct projection profiles of the VG (**a**) and DG neurons (**b**) in the ventral scoloparium and DSNs (**c**). The lateral association center (LAC) is the motor association center where sensory afferents make synaptic connections with dendrites of motor neurons. The medial ventral association center (mVAC) is the neuropil specialized for processing auditory/vibratory signals. See Pflüger et al. (1988) for naming of tracts and association centers in **ai-cii**

8.5 Femoral Chordotonal Organ Measures Muscular Isometric Contraction

Now the question arises as to whether FCOs are activated by isometric muscular contraction during the induction phase of TI. Simultaneous extracellular recordings of the flexor tibiae and FCO were achieved from crickets with the tibiae completely immobilized. To avoid inadvertent mechanical stimulation by the electrode itself

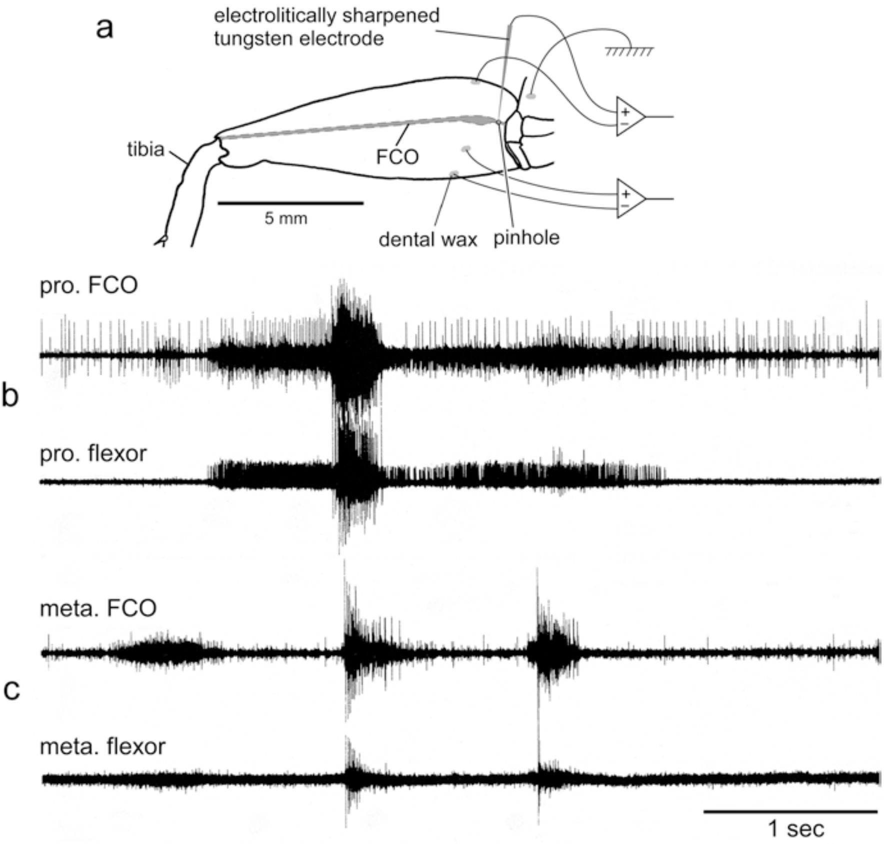

Fig. 8.6 Simultaneous recordings of sensory neurons in the FCO and the flexor tibiae muscle. For low-invasive extracellular recordings, an electrolytically sharpened tungsten electrode was positioned on the surface of the FCO nerve through a small pinhole made in the cuticle (**a**). Sensory neurons in the prothoracic FCO and metathoracic FCO were strongly activated by isometric contraction of the respective flexor tibiae during struggling in the tibial immobilization (**b**, **c**). In both preparations, the tibia was immobilized at 30°

(Yack and Fullard 1993), recordings from FCO afferents were made by placing an electrolytically sharpened tungsten electrode on the FCO nerve through a small pinhole made on the overlaying cuticle (Fig. 8.6a). Isometric contraction of muscles was conducted by touching the head of the cricket with a wooden stick.

The neuronal recordings of FCOs consistently revealed that (1) there were always several units that were tonically active (Fig. 8.6b, c), as is known in the locust *Locusta migratoria* (Matheson 1990), (2) regardless of the tibial position, many units were strongly activated when an isometric contraction of the flexor muscle occurred in the same leg (Fig. 8.6b, c), and (3) some of the activated units included large-amplitude spikes (Fig. 8.6b, c), presumably velocity and acceleration-sensitive units (Matheson 1990).

Observations of muscle movements through a small window under an objective microscope revealed that the flexor, but not the extensor, showed strong isometric contractions when the cricket was provoked under the condition of restraint of the prothoracic tibia (Nishino Lab 1 2020). Inasmuch as the muscle vibrated during the isometric contraction (Nishino Lab 1 2020), the FCO neurons appeared to be activated by low-frequency vibrations transmitted through the apodeme or through the trachea that interconnect the flexor with FCO scoloparia.

8.6 Inhibitory Functions of the FCO in Motor Control

8.6.1 Resistance Reflex and Its Inhibition

Participation of the FCO in the resistance reflex has been established because cutting the FCO apodeme resulted in an immediate abolishment of the resistance reflex in the flexor and extensor in locusts (Burns 1974; Field and Burrows 1982) and stick insects (Godden 1974). To attain further insight into the FCO function in catalepsy, I achieved microablation of individual neuronal groups of the metathoracic FCO leaving tracheal innervation and attachment with surrounding tissues intact (see Nishino et al. 1999 for methodology) and recorded activity of the flexor and extensor tibiae (Fig. 8.7ai-cii). Whether the individual neuronal group had been properly removed was evaluated later by retrograde staining of sensory neurons (Fig. 8.7a, b, c).

Motor neuronal recordings of the proximal flexor nerve in intact crickets (Fig. 8.7a) during the flexed-leg TI (see Fig. 8.1a, b for recording procedures) showed that a forced tibial extension evoked a resistance reflex in the flexor; fast exciters were activated briefly and intermediate exciters were activated phasotonically during the tibial flexion (Fig. 8.7ai). The activation, however, ceased immediately after the displacement, and the tibia was maintained at the newly imposed extended position (Fig. 8.7ai), meaning that catalepsy occurred normally.

The removal of attachment of VG neurons with the ventral ligament (Fig. 8.7b) resulted in a loss of muscle tonus (Fig. 8.7bi). On induction, the tibia reached full flexion transiently but soon extended to 50° due to the weakening of activity of slow exciters, which is not seen in the recording (Fig. 8.7bi). The forced extension evoked a weak activation of intermediate exciters related to the stimulation of exteroceptors and the tibia was maintained in the new position without rigidity, meaning that the resistance reflex had disappeared (Fig. 8.7bi).

The removal of DG neurons (Fig. 8.7c) led to the opposite effect. Once TI was conducted, the flexor activity was unusually potentiated and some intermediate exciters that participate in tibial flexion continued to fire even after the knee joint had reached the fully flexed position (Fig. 8.7ci). When the tibia was displaced to an extended position, it returned to full flexion, indicating that the flexor resistance reflex is abnormally strengthened and prolonged. For this reason, the catalepsy was abolished. An additional cut of the FCO apodeme in the same specimen resulted in

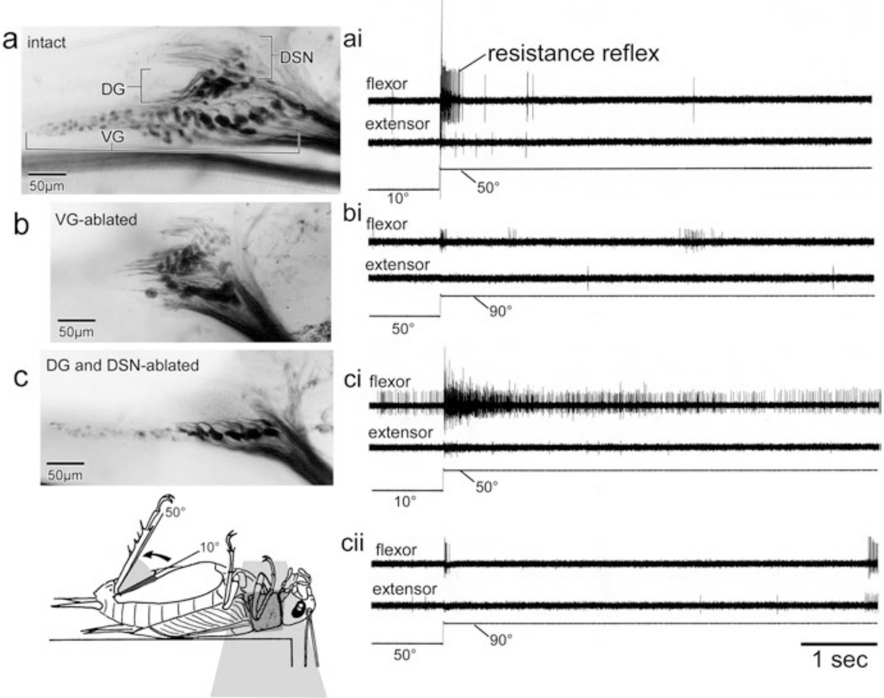

Fig. 8.7 Neurograms of the metathoracic flexor and extensor tibiae in intact and FCO-operated legs during flexed-leg TI. (**a**,**ai**) The leg with the intact FCO (**a**) shows a resistance reflex recruiting fast and intermediate units in the flexor muscle during tibial extension (8 s) from 10° to 50° (inset) but the resistance reflex is rapidly waned and catalepsy commences (**ai**). (**b**, **bi**) In the VG-ablated leg (**b**), the knee joint immediately relaxed to ca. 40° before the displacement. Resistance reflex to tibial extension does not occur except for weak intermediate exciter activation (b). (**c**, **ci, cii**) The DG plus DSN-ablated leg (**c**) shows a stronger and prolonged resistance reflex compared to that in the intact leg (**ci**). The additional cut of the FCO apodeme results in loss of resistance reflex (**cii**)

immediate loss of flexor tonus and disappearance of resistance reflex (Fig. 8.7cii), which were the same as the results for VG-ablated or ventral ligament-cut legs (Fig. 8.7bi).

Ablation of DSNs or cutting a nerve innervating a muscle tension receptor in the distal femur (Theophilidis and Burns 1979) resulted in no detectable deficiency in knee joint control (not shown), indicating that the resistance reflex and catalepsy remained normal (Nishino et al. 1999).

These results suggest that the flexor activation mediated by VG neurons is normally maintained under the condition of inhibitory control by DG neurons. The inhibition strength should be proportional with contraction force according to knee joint angles. DG neurons are, therefore, pivotal for immobilizing the tibia at a wide range of knee joint angles (0–100°), thereby inducing catalepsy (Nishino et al. 1999).

8.6.2 Suppression of Leg Movements

Flexed-leg TI can be repetitively conducted in restrained crickets in which movements of prothoracic legs are continuously hindered by applying a gentle pressure laterally with a clothespin (Fig. 8.8a). In crickets without restraint, a wind-puff stimulation applied to cerci (equipped with wind-sensitive hair receptors) during TI evoked an immediate righting response, which was expressed as recruitment of all motor neurons including fast exciters for all legs (see Fig. 8.3). However, in crickets under continuous restraint of prothoracic legs, a wind-puff stimulation evoked brief activation of intermediate exciters but not fast exciters. Immediately after the stimulus ceased, the cricket re-entered TI (Fig. 8.8b, bi). This observation indicates that an attempt to struggle causes isometric loading in prothoracic legs, thereby the cricket re-enters the TI via inhibition of active movements of all legs.

The intra- and intersegmental inhibition seen in intact crickets was impaired when prothoracic FCOs were bilaterally ablated. When the prothorax of the operated crickets was laterally compressed, TI occurred temporarily but a wind-puff stimulation resulted in prolonged struggling of mesothoracic legs and metathoracic legs (Fig. 8.8c, ci). Taken together with the finding that ablation of FCOs in all six legs led to a significant reduction in the proportion of TI to 22% of individuals (Nishino and Sakai 1996), I concluded that the FCOs are not the sole but major sense organs that inhibit leg movements.

8.7 Motor Neuronal Activity Characterizing TI

Apart from cessation of activity of fast and intermediate exciters during TI (Figs. 8.2 and 8.3), how small motor units such as slow exciters and common inhibitors (CIs) contribute to the postural maintenance during TI had been totally unknown. To tackle this problem, I focused on the accessory flexor in the metathoracic leg (Fig. 8.9a).

Two CIs (CI2 and CI3), analogous to those in locusts (Hale and Burrows 1985), innervated the accessory flexor, as shown in a cross section of the AFN (inset, Fig. 8.9a) and backfill staining of the AFN (Fig. 8.9b). The CIs indeed promoted relaxation of muscle fibers as shown by inhibitory synaptic potentials (IJPs) (Fig. 8.9c). Extracellular recording of the AFN by using a suction electrode in crickets revealed that CIs discharged tonically at 0.5–1 Hz during the resting state (Fig. 8.9c). When the cricket was provoked by touching or pinching the tarsus (tip of the leg), both CIs and slow exciters were co-activated, with magnitude of activation being larger with stronger nociceptive stimuli (Fig. 8.9d, e).

I succeeded in recording from slow exciters by coiling a thin copper wire (ø 22 μm) around the AFN. When the cricket was in quiescence, slow exciters tended to discharge at progressively higher frequencies as the knee joint was flexed (Fig. 8.10b, c, d). However, when the cricket was in strong TI (knee joint angle being slightly opened to 15° due to the operation), slow exciters fired at lower

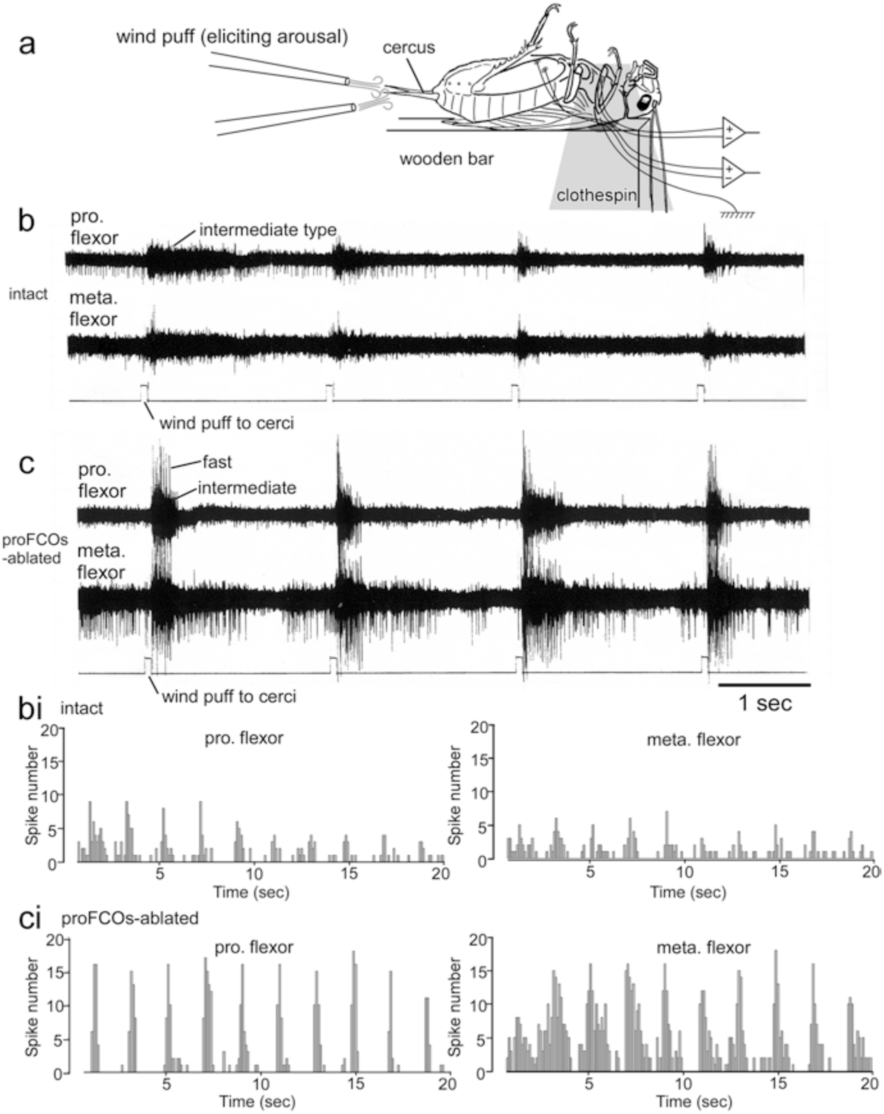

Fig. 8.8 Neurograms of prothoracic and metathoracic flexor tibiae in intact and operated legs during flexed-leg TI. A series of brief wind-puff stimulations with durations of 100 ms and intervals of 2 s were applied to wind-sensitive hairs on the cerci (**a**). Wind-puff stimuli resulted in larger excitation of flexor exciters in prothoracic FCO-ablated legs (**c**) than in intact legs (**b**), as manifested by peri-stimulus-time histograms (PSTHs) (**bi**, **ci**)

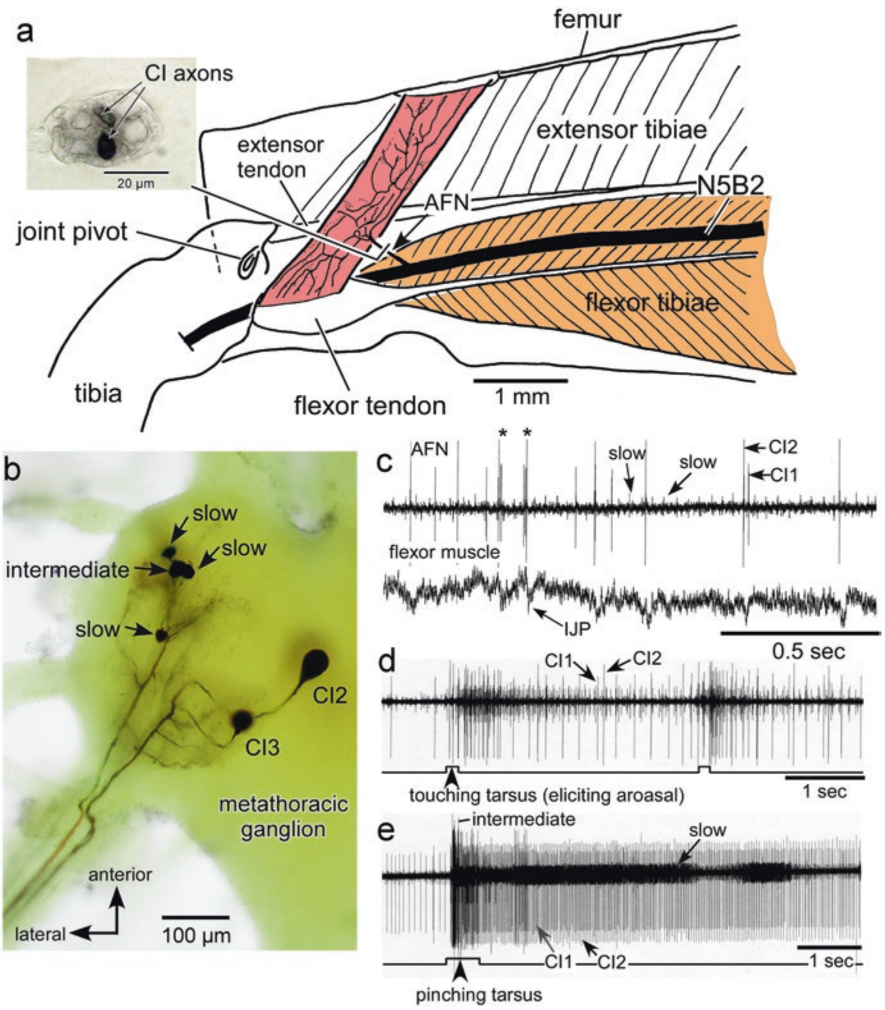

Fig. 8.9 Neuroanatomy and physiology of the accessory flexor. (**a**) *Camera lucida* drawing of the motor innervation in the distal part of the metathoracic femur, viewed anteriorly, showing that the accessory flexor (red) is inserted diagonally (about 45°) into the cushion, which is extended from the apodeme on which the main flexor (orange) attaches. Inset in A shows a cross section of the AFN in which two thick axons of common inhibitors (CI) are stained by backfilling of the main leg nerve (N5B2) in the distal tibia. (**b**) The metathoracic ganglion in which the AFN backfilled with $NiCl_2$ and silver-intensified shows that the accessory flexor is innervated by one intermediate exciter, three slow exciters, and two inhibitors. (**c**) Recording from motor neurons innervating the AFN (upper trace) combined with an intracellular recording from a fiber in the accessory flexor (bottom trace) show that spikes of two CIs evoke inhibitory junctional potentials (IJPs) in the muscle fiber. When the two CIs fired synchronously (asterisks), summation of IJPs occurred. (**d**, **e**) Recordings of motor neurons innervating the accessory flexor show that external disturbances such as touching the prothoracic tarsus ipsilateral to the recording site evoked longer activation of two CIs and slow exciters (**d**) and strong pinching of the same tarsus evoked a brief activation of intermediate exciters and subsequent long-lasting activation of CIs and slow exciters (**e**). In **e**, the tonic activation of CIs and slow exciters was sustained for more than 70 s with slowly adapting discharges (not shown)

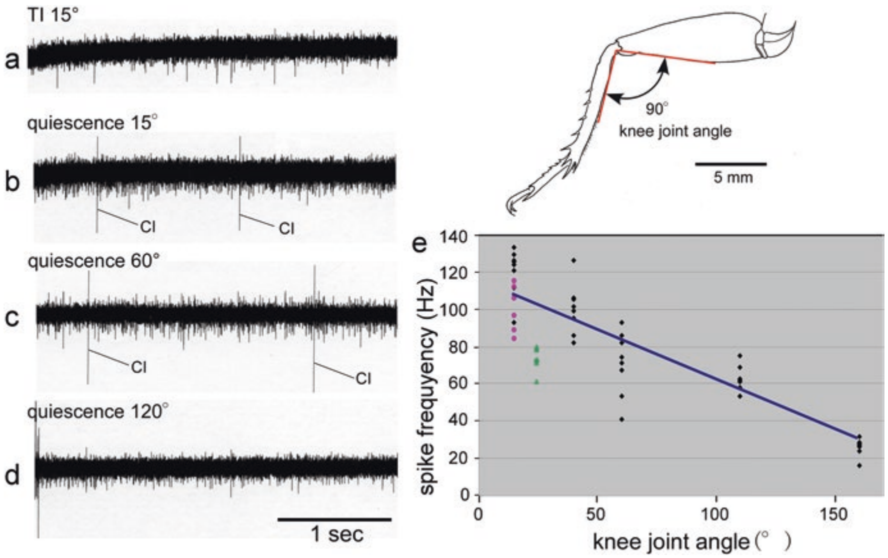

Fig. 8.10 Activity of slow exciters during quiescence and TI. (**a–d**) Activity of slow exciters when the cricket maintained the knee joint at various angles, showing that at least one CI unit fired sporadically at low frequency (0.5–2 Hz) during the quiescent state (**b–d**) but not during TI (**a**). (**e**) Average spike frequencies of slow exciters sampled for 5 s when the cricket exhibited strong TI (magenta dots), weak TI (green dots) or the quiescent state at various knee joint angles (black dots). Note that activity of slow exciters is larger when the knee joint is maintained at smaller angles and is rather suppressed during TI compared with those during the quiescent state at the same knee joint angle

frequencies (Fig. 8.10a; magenta dots in e) than those in the quiescent state at the same knee joint angle (Fig. 8.10b; black dots in e). This tendency was more prominent when the cricket was in weak TI (green dots in Fig. 8.10e) with a knee joint angle of 30°, in which slow exciters discharged at much lower frequencies than expected in the quiescent state (Fig. 8.10e).

CIs showed distinctive patterns of activity during TI and immediately after arousal. During strong TI, the normal 0.5–2 Hz firing of the CIs during quiescent state was suppressed, especially in the beginnning (Fig. 8.11d). Only eight spikes of CIs were identified in 42 s of the maintenance phase of TI (asterisks in Fig. 8.11a). Immediately after arousal, the cricket was motionless with the knee joint maintained at 120°, as manifested by no activity of intermediate exciters. Nevertheless, strong firing of CIs (at about 15 Hz) together with slow exciters occurred (Fig. 8.11a), possibly due to post-inhibitory rebound. As walking commenced, CI firing diminished rapidly while fast and intermediate exciters were recruited (Fig. 8.11a). On the other hand, during weak TI with the knee joint maintained at 95°, the CIs and the intermediate exciters fired at higher frequencies (Fig. 8.11b). Again, strong CI firing occurred just after arousal (Fig. 8.11b). The period of the marked activation of CIs coincided exactly with that in which explosive escape running occurred when the cricket was disturbed (Nishino and Sakai 1996). This is reasonable given that CI firing eliminates residual tension in slow

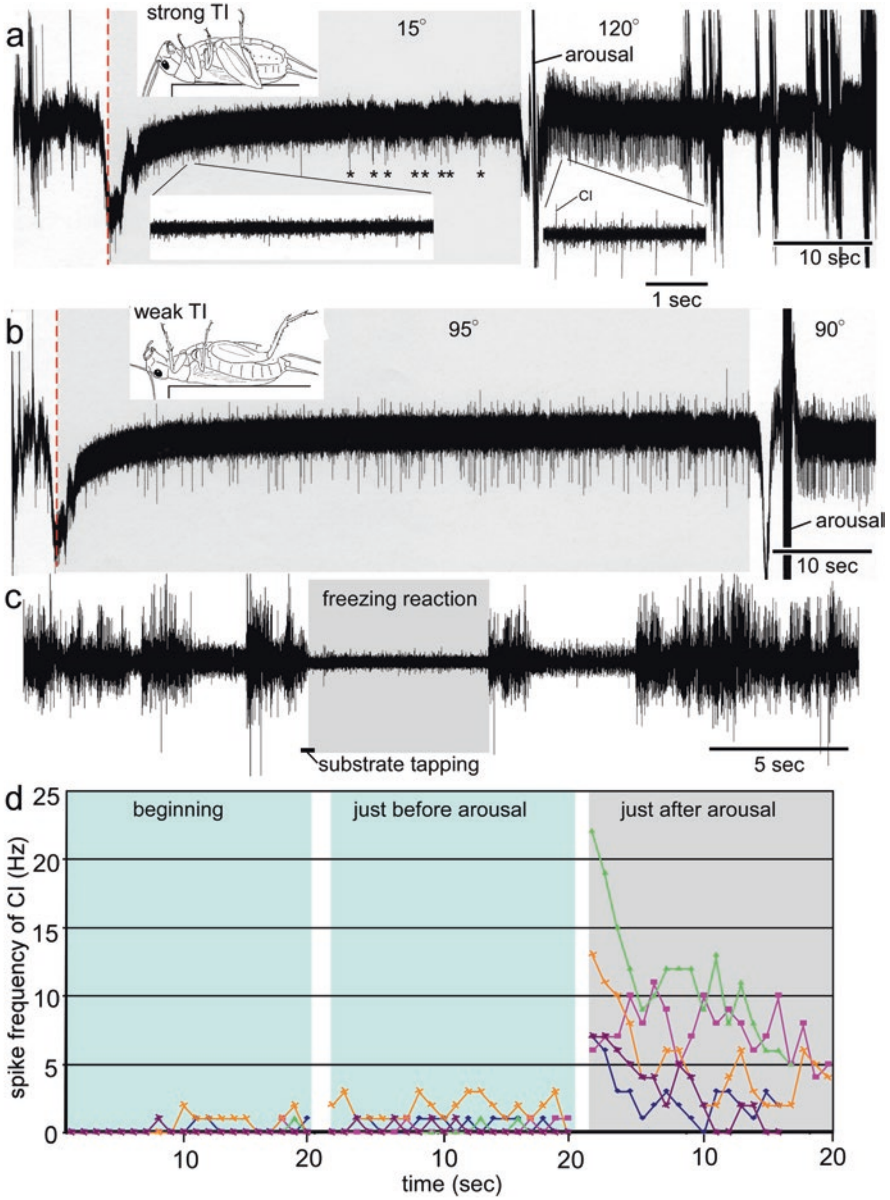

Fig. 8.11 Activities of common inhibitors (CIs) before, during, and after TI. The maintenance phase of TI is shaded by gray. (**a**) During strong TI (inset), activity of CIs was suppressed especially in the beginning of TI and then increased gradually towards arousal. Only eight spikes of CIs were identified in 42 s of the maintenance phase of TI (marked by asterisks). Strong recruitment of CIs occurred immediately after arousal despite lack of motion of the recorded knee joint at 120° (Note that intermediate exciters are inactive). The increased activity suddenly waned once the cricket started walking with recruitment of intermediate exciters. (**b**) During weak TI (inset), CIs started firing soon after induction of TI and firing occurred frequently during TI. Nevertheless,

muscle fibers and speeds up ongoing leg movements (Usherwood and Grundfest 1965; Burns and Usherwood 1979; Wolf 1990).

When a light tapping of the substrate was applied to a cricket when walking, a freezing reaction (Friedel 1999) was observed (Fig. 8.11c). The cricket in the freezing reaction assumed a fully alert posture with the body and antennae raised. In such a case, suppression of the activity of both exciters and CIs was evident (Fig. 8.11c), resembling the motor neuronal inhibition that occurred during TI.

In summary, the entire motor neuronal pool is held under active suppression during TI and feezing reaction, and flexor muscle rigidity is maintained by a weak discharge of slow exciters together with suppression of inhibitors. TI in the cricket does not depend on the absence of motor output, nor is there any evidence for a catch-like mechanism in the muscle (Hoyle and Field 1983). These characteristics are generally in good agreement with those derived from other insects during TI. In stick insects, only slow units were active, while most of the other units were inactive (Godden 1972). In the locust, there was little activity in any of the metathoracic muscles during TI (Faisal and Matheson 2001).

8.8 Neuronal Underpinnings Underlying the Induction and Maintenance of TI

Based on above results, I propose the most plausible picture of neuronal circuits of TI in the cricket, summarizing as follows (Fig. 8.12).

8.8.1 Sensory Aspect

FCOs act as “leg restraint detectors” and promote induction of TI. The key stimulus is likely to be low-frequency vibrations caused by isometric contraction of the flexor tibiae. The most plausible candidate for detection of vibratory signals is DG neurons in the FCO because projection fields of DG neurons largely overlapped with those of the subgenual organ specialized for detecting substrate vibrations (Nishino 2003). DG neurons mediate catalepsy by inhibiting the resistance reflex in the same leg and possibly inhibiting movements of other legs intersegmentally (Fig. 8.12a). The possibility of minor contribution of other COs such as thoracic COs (Pflüger

Fig. 8.11 (continued) strong recruitment of CIs occurred immediately after arousal when the knee joint was maintained at 90°. Large spikes of fast exciters were truncated. (**c**) Accessory flexor activity during a freezing reaction (shaded by gray). Activity of CIs and slow exciters is temporarily suppressed. (**d**) PSTHs of CI activity in the beginning, just before arousal, and just after arousal of TI. Results of five trials of TI derived from three individuals are plotted

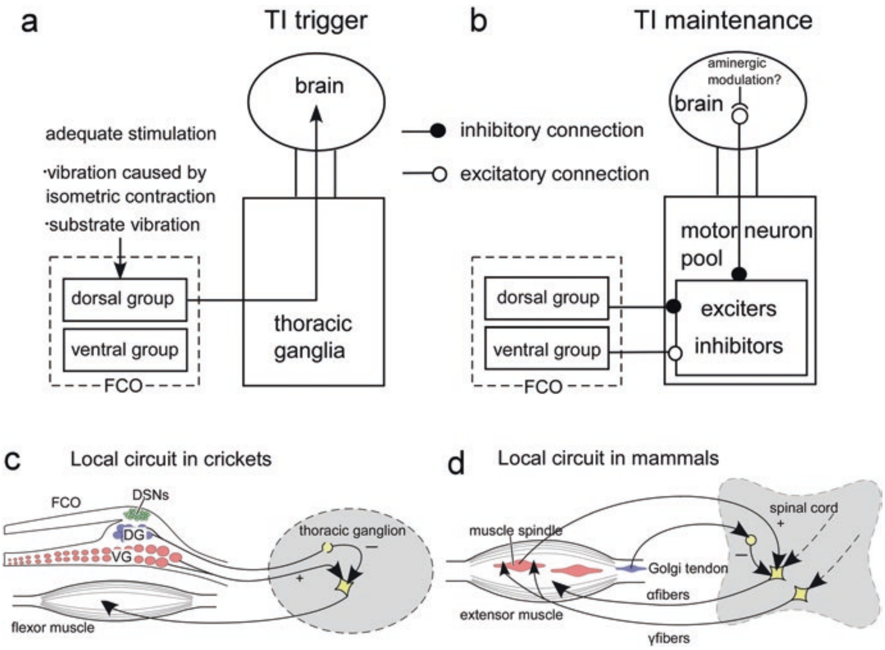

Fig. 8.12 Working hypotheses showing neuronal circuits that participate in triggering (**a**) and maintaining TI (**b**) and local circuits that underlie catalepsy (**c, d**). The local circuits that inhibit movements to imposed restraint (**a**) are incorporated into the whole body immobilization evoked by continuous activity of descending neurons originating from the brain (**b**). Aminergic neurons might modulate activity of the descending neurons (**b**). The resistance reflex of the flexor tibiae and its inhibition are mediated by two distinct neuronal groups within the same chordotonal organ in crickets (**c**), whereas the stretch reflex and its inhibition are mediated by muscle spindles and Golgi tendon organ in mammals (**d**)

and Field 1999) to induction of TI, however, cannot be ruled out (Nishino and Sakai 1996).

It is noteworthy that substrate vibrations detected by COs elicit TI or freezing reaction in many insects (Fig. 8.12a; Acheampong and Mitchell 1997; Takanashi et al. 2016). When the stimulus is novel and unexpected, even *G. bimaculatus* assumes TI in response to substrate vibrations (Nishino, unpublished observation). Regardless of whether vibrations are substrate-borne or muscle-borne, vibrations detected by COs might signal sudden, threatened stimuli to animals and inhibit their movements via similar sensory channels.

8.8.2 Central Nervous System

The cataleptic state that emerges in all appendages is maintained by continuous activity of descending neurons from the brain (Fig. 8.12b). A pair of descending neurons that result in the evocation of freezing by their tonic activity have been identified in the crayfish *Procambarus clarkii* (Bowermann and Larimer 1974) and fruit fly *Drosophila melanogaster* (Zacarias et al. 2018). Neurons that are involved in maintenance of TI with a characteristic posture have not yet been identified in any insects. Given that TI in the cricket occurs at any posture, descending neurons that evoke a freezing reaction might be involved in the evocation of TI.

8.8.3 Motor Aspect

The most reliable index characterizing TI is activity suppression of CIs, which is manifested by their rebound activation after arousal. At the motor neuronal level, TI resembles the freezing reaction in that both states entail muscle rigidity caused by suppression of CI activity. In both states, a weak tonus generated by weak flexor discharge is compensated by stiffness of muscles (especially the accessory flexor) caused by no activity of CIs, and thereby complete immobilization is achieved for camouflaging against predators (Chap. 2).

In contrast, CIs discharge continuously at low frequencies to eliminate basic muscle tonus during normal quiescence or a resting state. When the cricket is provoked by noxious stimulation during the resting state, activities of both CIs and slow exciters increase transiently. Therefore, activities of CIs and slow exciters appear to reflect the animal's arousal level.

8.9 Evolutional Perspective

TI that occurs under the condition of physical restraint comprises two temporal sequences: (1) cessation of ongoing movements and (2) prolonged maintenance of the restrained posture, the latter of which is recognized as TI. Although the first sequence has been often overlooked, many animals tend to pause in a situation when ongoing movements are physically hindered, which is also referred to as post-contact immobility (e.g., Sendova-Franks et al. 2020). For example, among closely related species of field crickets, *G. bimaculatus* enters TI easily, while *T. emma* becames motionless during restraint, but this is not sustained after removal of the restraint (see Fig. 7.6 in Chap. 7).

The initial motor inhibition is attributed to a local reflex loop mediated by COs. In crickets, resistance to an imposed restraint (i.e., resistance reflex) and its inhibition are mediated by VG neurons and DG neurons of the FCO in the same leg (Fig. 8.12c). In mammals, the functional parallels of the resistance reflex and its inhibition are stretch reflex and Golgi tendon reflex, and they are mediated by muscle spindles and Golgi tendon organs, respectively (Fig. 8.12d; Pflüger and Sillar 2013).

Mitigating excessive muscle load is a fundamental physiological function not only in animals under the condition of physical restraint but also in animals during locomotion. In locusts immediately after the final molt, the attachment of the extensor tendon to the exoskeleton is weak (Norman 1995). A forced co-contraction of the flexor and extensor tibiae in the fully flexed position causes permanent damage to the extensor tendon. Thus, newly molted locusts rarely jump, but instead show kicking (Norman 1995). In the crayfish *Procambarus clarkii*, isometric loading of the depressor muscle developed during walking resulted in inhibition of depressor motor neurons (Grote 1981). Such a reflex could potentially also mitigate the futile energy consumption. The reflex to prevent isometric loading of skeletal muscles, which is conserved in many animal phyla, therefore appears to be a primitive function incorporated into TI.

On the other hand, the proportion of individuals showing TI and the duration of TI are varied among closely related species, sometimes even among different trials of the same individual. Probably, both internal state of individuals and species-specific factors could affect the propensity of TI. Given that the proportion and duration of TI are amenable to artificial selection in the same beetle species (Miyatake et al. 2004), the change in these parameters seen in the same species could be attributed to the descending control of the brain. Aminergic neurons, whose activities change according to insects' internal state, are candidates that regulate the duration of TI (Nishi et al. 2010), potentially via modulating activity of descending neurons (Fig. 8.12b).

Species-specific differences in the duration of TI have been suggested to be associated with anti-predatory strategies (Humphreys and Ruxton 2018). Since insects are generally important food resources for vertebrate predators, strong selective pressure could promote TI to occur before capture by predators so that insects can hide or dazzle predator's sight before being consumed (Nakayama et al. 2012).

Given that selective pressure is first exerted on the peripheral sensory system (Boyan 1993), early predator detection could be achieved by functional modification of sense organs. Subgenual organs and tympanal organs, specialized for detecting substrate vibration and airborne sound, are derived from proprioceptive COs (Boyan 1993; Stumpner and von Helversen 2001). Intriguingly, holometabolous insects including moths and lacewings show immobilities when detecting ultrasounds with tympanal organs (Hoy et al. 1989; Chap. 5). This is one possible evolutional route from restraint-induced TI that mitigates isometric muscle loading to sound/vibration-induced TI for camouflaging against predators along with the acquirement of exquisitely vibration-sensitive COs during a long evolutionary process.

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Chapter 9
Catalepsy and Twig Mimesis in Insects and Its Neural Control

Ansgar Büschges, Hans-Joachim Pflüger, and Ulrich Bässler

Abstract Phasmids exhibit twig mimesis as defense mechanism against predators. Most detailed information on the neural basis of this particular behavior is existing for the femur-tibia joint of the stick insect leg. The neural network controlling the activities of the extensor tibiae and the flexor tibiae muscles of this leg joint generates the motor output for catalepsy. Catalepsy, an element of twig mimesis is characterized through extremely slow return movements in response to external perturbations. This property of the neural network governing the femur-tibia joint sets phasmids apart from other orthopteran insect species, e.g. locusts, which do not generate twig mimesis. Cybernetic and comparative analyses have shown that catalepsy is produced by an increased sensitivity of the belonging joint control network to movement velocity. This is achieved by the particular processing of sensory feedback signals about movements of the tibia provided by the femoral chordotonal organ, the main transducer of the femur-tibia joint. This chapter summarizes the present knowledge concerning the neural basis of catalepsy and twig mimesis in the stick insect.

Keywords Motor control · Resistance reflex · Motor neuron · Interneuron · Sensory neurons · Neural circuit

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9.1 Introduction

Posture and movement of animals' appendages are controlled by premotor networks controlling the activity of motor neurons innervating the muscles of the appendage. These networks controlled by the brain reside in the central nervous system of an animal close to the appendage (Orlovsky et al. 1999; Hooper and Büschges 2017): for example, legs of mammals are governed by premotor networks residing in the lumbar spinal chord; legs of insects are governed by neural networks residing in the respective segments, i.e. ganglia, of the thoracic nerve chord. In the resting animal these networks generate reflexes in the muscles of the appendages, i.e. the legs, to guarantee the desired posture of the animal. Here we describe the present knowledge about a neural network controlling posture and movement of a leg joint in phasmids, in which these networks have been specialized in order to allow the generation of twig mimesis, i.e. the defense mechanism towards predators.

9.2 Thanatosis and Catalepsy in the Stick Insect

In the bright light stick insects, *Carausius morosus* can assume several different postures: at one extreme, the femur-tibia joints are bent to different angles and the femurs stand out from the body (Fig. 9.1a), and at the other extreme, a stick posture is assumed by the animal with all femur-tibia joints being fully extended and the front legs stretched forward and middle and hind legs lying flat against the body pointing rearward (Fig. 9.1b). All intermediate postures between these two extremes can be observed while the overall passively imposed body postures are kept (e.g.

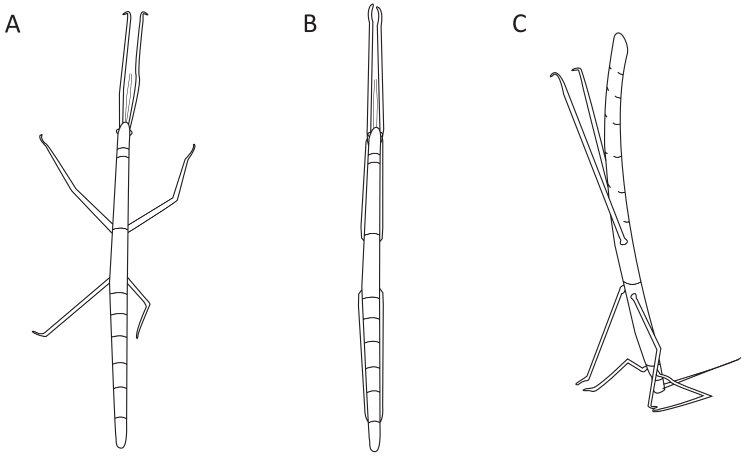


Fig. 9.1 Catalepsy in the stick insect. Diverse resting postures of the stick insect Carausius morosus (see text for details)

Fig. 9.1a, b, c). The stick posture in *Carausius* is called *thanatosis* based on the plausible assumption that thanatosis emphasizes the twig-like body shape of a stick insect in which no movement occurs and the animal appears to be a twig for an observer.

When a free leg of a resting stick insect without tarsal contact is forced into a new position, it will return towards the original position extremely slowly, being as slow as the hour hand of a clock. Therefore, it appears to the casual observer as if the leg would remain in the new position. This behavior is called catalepsy, because of its resemblance to the cataleptic (catatonic) state in humans and other mammals. Catalepsy suppresses fast movements in response to passive deflections that could be detected by a predator. It is therefore plausible to allocate catalepsy as part of twig mimesis in stick insects.

In the 70s, Ulrich Bässler and coworkers have addressed the question, whether thanatosis and catalepsy can be regarded as closely related behaviors or separate behavioral states. Depth of thanatosis was measured by quantification of the extensor forces generated by means of a force transducer in the almost fully extended position of the femur-tibia joint, i.e. at 170 deg. Depth of catalepsy was monitored by measuring the time of return movements of the tibia to the 90 deg-angle in response to a passive flexion from 170 deg to 50 deg and holding it there for 30 s before releasing it (Bässler 1972a, b; e.g. Fig. 9.2). Measuring both values, i.e. extensor force and return speed directly one after the other and plotting them against each other revealed a weakly negative correlation. This meant that the deeper the thanatosis the less pronounced is catalepsy (Bässler 1972b), indicating that thanatosis and catalepsy may not be considered as expressions of one behavior, as both of them may not fall into the same behavioral context. This conclusion was further corroborated by lesioning the proprioceptive sense organ of the femur-tibia joint, the femoral chordotonal organ. While this did not affect thanatosis, cataleptic behavior was no longer detectable (Bässler 1972b; Bässler and Foth 1982).

9.3 Neural Basis of Catalepsy in the Stick Insect

9.3.1 Anatomy of Sense Organs and Muscles Controlling the Femur-Tibia Joint

Due to its anatomical advantages the femur-tibia joint of the stick insect was chosen to study the neural basis of catalepsy (summary in Bässler 1983): the fact that the pivot of the femur-tibia joint is oriented exactly rectangular to the plane formed by the femur and the tibia was an important aspect. It allows measuring the femur-tibia angle in a plane with high resolution and without optical distortion.

The femur-tibia joint is controlled by two antagonistic muscles, the flexor tibiae and the extensor tibiae. As common for many orthopteran insects these leg muscles are innervated by excitatory and inhibitory motoneurons and at least one efferent

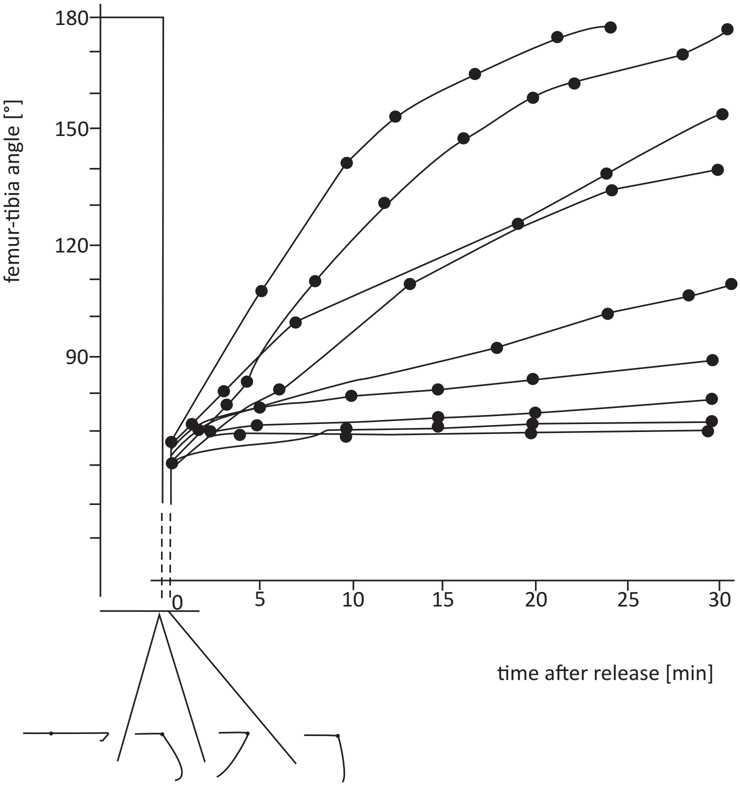
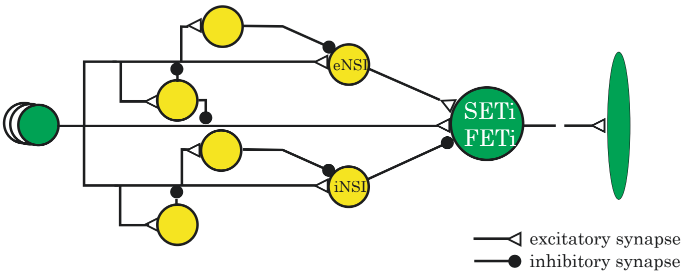


Fig. 9.2 Catalepsy and tibial return movements. Return movements of the tibia to the 180 deg starting position after being passively bent to 50 deg for 30 s. Nine randomly selected examples of intact legs (modified after Bässler 1972b)

modulatory neuron: The extensor tibiae is innervated by only three motoneurons, i.e. one slow motoneuron (SETi), one fast motoneuron (FETi), and the common inhibitor I (Bässler and Storrer 1980) and in addition by modulatory dorsal unpaired median efferent neurons, called DUM-neurons (Mentel et al. 2008; for review see Bräunig and Pflüger 2001). The flexor tibiae is innervated by some 15 excitatory, two inhibitory motoneurons, the common inhibitor II and III (Storrer et al. 1986; Debrodt and Bässler 1990; Goldammer et al. 2012) and as well modulatory DUM-neurons (see above). Among the excitatory motoneurons innervating the flexor muscle are slow, semi-fast, and fast motoneurons, which show sequential recruitment from slow to fast upon activation of the flexor tibiae muscle with increasing contraction forces generated (Gabriel et al. 2003). Because of its simpler innervation the extensor tibiae lent itself for studying the neural basis of catalepsy in the femur-tibia joint (Fig. 9.3).

Movement and position of the tibia are measured by three sense organs, the femoral chordotonal organ (fCO) situated close to the proximal end of the femur and



NEURON TYPE	flexion sensitive fCO afferents	spiking interneurons	non-spiking interneurons	motor neurons	extensor muscle fibers
ACTIVITY	action potentials upon tibial flexion	activation upon sensory input	change in membrane pot. upon tibial flexion	depolarization upon tibial flexion	generation of force in response to tibial flexion

Fig. 9.3 Schematic presentation of the identified information flow of sensory signals about tibial movement in the neural network governing the activity of the femur-tibia joint in the stick insect. The scheme shows the principle connectivity for the distributed processing of flexion signals in the neural network between sensory afferents and the two excitatory extensor motor neurons (slow extensor tibiae (SETi) and fast extensor tibiae (FETi)) and the fibers of the extensor muscle for three pathways: (1) direct monosynaptic pathways between fCO afferents and the extensor motor neurons, (2) polysynaptic pathways providing synaptic drive to nonspiking interneurons exciting extensor motor neurons (eNSI), and (3) polysynaptic pathways providing synaptic drive to nonspiking interneurons inhibiting extensor motor neurons (iNSI). For references and details see text

connected to the tibia via a long apodeme, and two single sensory cells, the RDPL and the RDAL located close to the tibia (Coillot and Boistel 1968; Bässler 1977; summary in Bässler 1983). The latter two play no obvious role in the control of tibial muscles. However, the fCO has been shown to serve as sensor in the control of the tibia (summary: Bässler 1983). It is two partite with a ventral part consisting of ca. 80 sensory neurons and a dorsal part encompassing ca. 420 sensory neurons (Füller and Ernst 1973). While the sensory neurons of the dorsal part monitor vibration of the tibia (Field and Pflüger 1989; Sauer and Stein 1999), the sensory neurons of the ventral part monitor position, velocity, and acceleration of tibia movements, either purely or in combinations (Hofmann et al. 1985; Hofmann and Koch 1985; Büschges 1994). fCO sensory neurons show considerable adaptation, e.g. sensory units responding to velocity and position of tibial movements (Sauer et al. 1996). This is at least partially due to sensitivity of fCO sensory neurons depending on the history of discharge controlled by a calcium-dependent adaptation mechanism (DiCaprio et al. 2002). Load on the femur and the tibia is measured by cuticular sensors, the so-called campaniform sensillae situated on the proximal femur and tibia (e.g. Hofmann and Bässler 1982, 1986; Zill et al. 2013, 2017). These load sensors are activated upon resisted forces generated, for example, by activity of the tibial muscles or gravity. They can, therefore, be neglected for the generation of unrestrained return movements of the tibia as generated in the generation of catalepsy.

9.3.2 Catalepsy in the Femur-Tibia Control Loop and Its Neural Basis

Catalepsy in the femur-tibia joint was quantitatively described by studying return movements of the tibia in experimental animals, which express thanatosis, i.e. showing a fully extended tibia (Fig. 9.3). This holds both for *female Carausius morosus* and *Cuniculina impigra* (Bässler and Foth 1982).

By selectively stimulating the fCO in an otherwise semi-intact, restrained experimental animal and recording the activity of tibial motoneurons and muscles it was found that the underlying neural mechanism for the generation of catalepsy arises from the fact that velocity and movement signals from the fCO strongly affect tibial motoneuron activity, however with differing times and gain of influence. Presently, the following picture applies: when the tibia is flexed by passive displacement and return of the tibia is allowed after some time, then the initial fast return movement is generated by a still effective resistance influence elicited in extensor motoneurons and muscles by fCO signals signaling flexion of the tibia. This influence has a rather short time constant of action and consequently decays relatively fast. At the same time the generated extension movement elicits activation of the antagonistic flexor tibiae, serving in addition in slowing down the return movement. The slow part of the return movement of the tibia results from the balance between the persistent positional feedback about flexion of the tibia activating the extensor motoneurons and muscle and the counteracting force generated by the flexor muscle upon the ongoing extension movement, elicited by fCO-signals about joint extension.

This action of the neural control system arises from the following properties: (1) a high gain with which signals about movements, i.e. position and velocity, of the tibia from the fCO are processed in the premotor network of the femur-tibia joint, (2) the relative dominance of velocity sensitivity over position sensitivity of the neural control system, and (3) the long time constants of decay for the processing of low velocity signals as compared to high velocity signals. Together, these serve in giving the neural control system of the tibial motoneurons the properties of a low pass filter, a characteristic the system needs to generate the very slow movements generated during catalepsy (for summary see Bässler 1983, 1993). Individual properties were traced to the level of their origin in the neural components involved, e.g. sensory neurons, interneurons, and motoneurons (Fig. 9.3; e.g. Driesang and Büschges 1993; Büschges and Wolf 1995; Sauer et al. 1996): by means of intracellular recordings. Bässler and coworkers studied the network architecture underlying the processing of sensory information from the fCO. Interestingly, they did not find evidence for the existence of labeled lines with respect to a given motor output generated, e.g. a resistance reflex in response to passive deflections of the tibia. In contrast they found a principle of information processing and network architecture processing sensory signals in a distributed fashion by means of parallel direct and polysynaptic neural pathways between sensory neurons in the fCO and the tibial motoneurons (e.g. Büschges 1990; Sauer et al. 1996). These parallel pathways generate the motor output based on their antagonistic nature with individual pathways not only supporting, but also opposing the motor activity generated. Thus, the motor activity generated is always the balance between the action of individual pathways. Due to its similarity to the generation of political decisions in a democratic society Bässler coined the term "parliamentary principle" for it (Bässler 1993). All in all Bässler and coworkers identified five levels of antagonistic interaction in the control of the femur-tibia joint: (1) fCO afferents of a given sensitivity influence each other by means of presynaptic inhibition (Fig. 9.3; Sauer et al. 1997), (2) premotor interneurons receive in parallel excitatory and inhibitory synaptic inputs from the same class of fCO signals, e.g. from velocity sensitive sensory neurons (Fig. 9.3; Sauer et al. 1995, 1996), (3) the membrane potential of tibial motoneurons is affected by antagonistic synaptic inputs from direct and polysynaptic pathways from the fCO (Fig. 9.3; Büschges 1990; Sauer et al. 1996), (4) the membrane potential of the tibial muscle fibers is affected by the parallel action of excitatory and inhibitory motoneurons (Bässler and Stein 1996), (5) the movement of the tibia results from the ongoing balance between the antagonistic actions of the respective muscles, i.e. the flexor and the extensor tibiae (for summary see: Bässler 1993; Büschges 1995). This means that all the different outputs use a common neuronal network. Only the internal parameters need to be changed.

Comparative studies on other orthopteran insects expressing catalepsy (proscopiids: *Prosarthria*; Wolf et al. 2001) and lacking catalepsy (locusts; Ebner and Bässler 1978; Büschges and Wolf 1995) have corroborated the notion that these are indeed the crucial factors for an insects capability of generating catalepsy. The different outputs apparently use the same neuronal networks (see above). It may be thus plausible that this is also the case for thanatosis, in other words, that thanatosis is the output of a network with parliamentary, i.e. distributed, structure. If this is true, there would be no specialized neural circuits for thanatosis but it rather would depend on special internal (and external) conditions of a particular behavioral context.

9.4 General Summary

In summary, it presently appears, that even though the ecological relevance renders similarities thanatosis and catalepsy are different behaviors. By means of a highly specific processing of proprioceptive signals catalepsy is aiding the generation of slow movements necessary for twig mimesis thereby allowing for camouflage. Thanatosis, on the other hand, appears to be an active component of behavioral camouflage.

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Chapter 10
Descending Neuron for Freezing Behavior in *Drosophila melanogaster*

Shigehiro Namiki

Abstract Freezing is a defensive behavior characterized by immobility, which makes detection by predators difficult. Flies show freezing behavior in response to visual stimulus like looming, which is relevant to the attack of predators. Brain control of freezing behavior is largely unknown. Here, I introduce the analysis of the neuronal mechanism, using a set of driver lines for a population of descending neurons, which is a bottleneck of the nervous system, connecting the brain and ventral nerve cord in *Drosophila*. A pair of descending neurons has been identified as a critical component for this behavior. I also discuss the connectivity of the neurons.

Keywords Command · Escape · Brain · Giant fiber · Descending neuron · Looming · Channel rhodopsin · Optogenetics

10.1 Tonic Immobility and Freezing

Defense behaviors are behavioral actions that aim to minimize the chances of being predated. When faced with a threat, insects can show different types of defensive behavior. One of these is tonic immobility (TI), where insects show a motionless posture lasting from seconds to hours. TI is triggered by physical contact with or close proximity to a predator (Humphreys and Ruxton 2018). Although the sensory and motor mechanisms have been investigated, the neuronal mechanisms are largely unknown.

Another kind of defensive behavior is freezing, where insects show a cessation of all movements, except those associated with respiration and vision (Misslin 2003). Because movement acts as a releasing stimulus for predatory attack, freezing

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contributes to the prevention of detection by predators (Eilam 2005). Freezing behavior has been observed in a wide variety of insects such as crickets (Adamo et al. 2013) and moths. Locusts also show freezing behavior in response to vibration stimuli on the platform (Friedel 1999).

Freezing behavior should be distinguished from TI. Whereas freezing occurs much earlier in the sequence of a predatory attack, TI occurs at a later stage of defensive behavior. Unlike the case of TI, freezing behavior is not necessarily associated with a specific posture and usually keeps the posture when detecting the presence of a predator. In addition, responsiveness to the external sensory stimuli is reduced in TI, whereas it is functionally unchanged in freezing behavior.

Both TI and freezing behavior are different options in defensive behavior. Despite the differences, freezing shares several behavioral features with TI. Both behaviors affect muscle activity throughout the body. Furthermore, both can be driven by the brain (e.g., visual stimulus of predator), and the brain control of the behaviors is mediated by a population of descending neurons (DNs), which connect the brain and body ganglia. Indeed, studies using surgical experiments suggest the importance of the brain in maintaining TI in some species.

Although TI is not reported in *Drosophila*, some female flies of the family Asilidae, *Efferia vapipes*, show a form of TI that prevents males from mating with them (Dennis and Lavigne 1976). The candidate neuronal pathways for TI are generally unknown, but those for freezing behavior have been reported recently. In this chapter, I first describe the descending neural command for freezing behavior identified in crayfish. Then, I discuss a recent work on the descending neuronal component relevant for executing freezing behavior in *Drosophila* (Zacarias et al. 2018).

10.2 Defensive Behavior and Candidate Command for Freezing in Crayfish

Freezing behavior has been observed in many crustaceans (Pereyra et al. 1999; Oliva et al. 2007). Juvenile crayfish exhibit either escape or freezing in response to predatory attacks. In the case of escape, crayfish exhibit tail-flip movement and move away from predators. Liden et al. 2010 examined the behavioral output of crayfish in response to an approaching shadow (Liden et al. 2010). They presented a shadow on a white paper and captured the crayfish movement in a narrow tunnel. They also measured the field potentials generated during crayfish response. Escape and freezing are exclusive to all types of stimulus tested in the study, and the occurrence varies depending on the speed of the approaching shadow. Animals exhibit fewer escape and more freezing behaviors in response to fast-approaching shadows. In contrast, animals exhibit more escape and fewer freezing behaviors in response to slow-approaching shadows. The variability of behavioral output is explained by cost–benefit relationship. Whereas tail-flipping is an effective strategy for the escape response (Herberholz et al. 2004), the energetic cost is high (Webb 1979). When a predator approaches at velocities that make escape impossible, animals show freezing along with a reduction in the energetic cost.

The neuronal circuits for escape response have been identified in crayfish thus far. For example, it is known that tail-flipping is driven by a pair of medial giant interneurons (MG) (Liden and Herberholz 2008). The MG is activated when there is moving visual stimuli or tactile input. Single action potential in the MG is sufficient to activate the escape response.

Although neuronal mechanisms for freezing remain unclear, a candidate neuron for freezing behavior has been reported (Bowerman and Larimer 1974). A movement-suppressing command fiber called the "Statue fiber" has been reported in descending system in crayfish *Procambarus clarkii*. In the study, crayfish is secured by the branchiostegite to the end of a rigid object, and one side of the circumesophageal connective is de-sheathed and small bundles of fibers are stripped from the connective and suspended on the electrodes for stimulation. The position of the axon running the connective is relatively conserved, and researchers can create a cross-sectional map of axon for individual neurons (Wiersma 1958). This allows recording from a target neuron via electrodes. The animal is positioned over the outer edge of a freely rotating horizontal walking wheel. The nerve bundle is stimulated with electric pulses and the behavior is recorded by camera. The activation of this unit terminates the ongoing activity by freezing irrespective of position. The position is maintained for the stimulus duration, which is reminiscent to naturally occurring freezing response.

10.3 Defensive Behavior to Looming Object in Flies

When visual stimuli such as looming are presented, flies show defensive behavior including jumping, running (increasing speed), and freezing. Flies also show a sequence of defensive behavior in response to a natural threatening stimulus such as a looming object (Hammond and O'Shea 2007). Looming stimulus elicits a sequence of actions: freezing, posture adjustment, wing elevation, and jump (Card

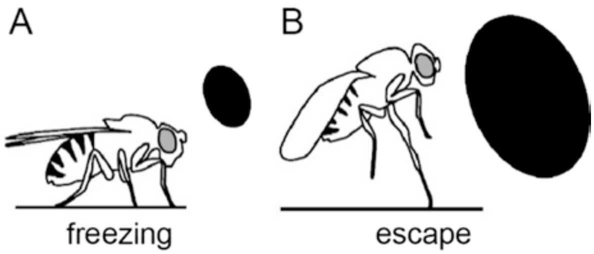

Fig. 10.1 Behavioral sequence of fly to looming stimulus. (**a**) When flies detect small moving object, they exhibit freezing behavior. (**b**) When the object shows expansion, flies show escape response

2012) (Fig. 10.1). The sequence of each action is flexible, suggesting that escape behavior does not have a fixed action pattern.

Neuronal circuits for jumping behavior have been investigated (Trimarchi and Schneiderman 1995; Card and Dickinson 2008a; von Reyn et al. 2014). Simple visually elicited jump response is mediated by the giant fiber neuron (Allen et al. 2006). Single action potential of the giant fiber is sufficient to trigger the full sequence of jump response. However, the action of the giant fiber does not induce other behavioral modules of postural adjustment and wing elevation. Other neuronal pathways must be involved, but such neuronal components have not yet been anatomically identified. Escape response that does not require giant fiber activation is reported (Holmqvist 1994). Another study electrophysiologically characterized the unit that are responsive to the looming stimulus (Fotowat et al. 2009). These studies indicated the presence of a parallel neuronal pathway for controlling escape behavior.

In addition, flies also exhibit freezing behavior (also referred to as "pause") in response to visual stimuli such as moving shadow (Gibson et al. 2015) and looming (Card and Dickinson 2008b). Moving shadow is a stimulus where a predator (e.g., dragonfly) is flying around the fly, while looming is a stimulus where a predator approaches the fly. To the looming stimuli, flies display stereotyped responses beginning with an initial freezing period lasting less than a second before escape behaviors are initiated (Card 2012). Freezing behavior in *Drosophila* is also reported in response to translational motion of a small fly-sized robot moving in the same plane as the fly (Zabala et al. 2012). In contrast to escape behavior, the neuronal components for freezing behavior are less investigated.

10.4 DNp09: Descending Neuron for Freezing

Visual signal of looming is processed at the optic lobe in the brain; thus, freezing behavior must be controlled by the brain. A population of DNs is a good target in investigating the neuronal components for freezing behavior (Fig. 10.2). DNs connect the brain and ventral nerve cord (VNC), which is analogous to the vertebrate spinal cord, located in the thoracic segment. Hence, this population is a bottleneck of the information flow in the central nervous system. However, no systematic data for individual neuron morphology has been available in any insects until recently. Namiki et al. 2018 identify ~100 types of DNs in female *Drosophila* (Namiki et al. 2018) and create a set of driver lines which selectively labels individual DN by using split-GAL4 technique (Luan et al. 2006). The authors performed photoactivation at the neck connective and selectively labels descending and ascending neurons. The number of labeled cell bodies, as an estimate of the total number of DNs, was counted ~350 at maximum. Among these, a population of DNs arising from optic glomeruli has been identified. For example, the giant fiber (also termed as DNp01) receives input from a specific set of visual glomeruli: lobula columnar neuron type 4 (LC4) and, lobula plate/lobula columnar neuron type 2 (Klapoetke

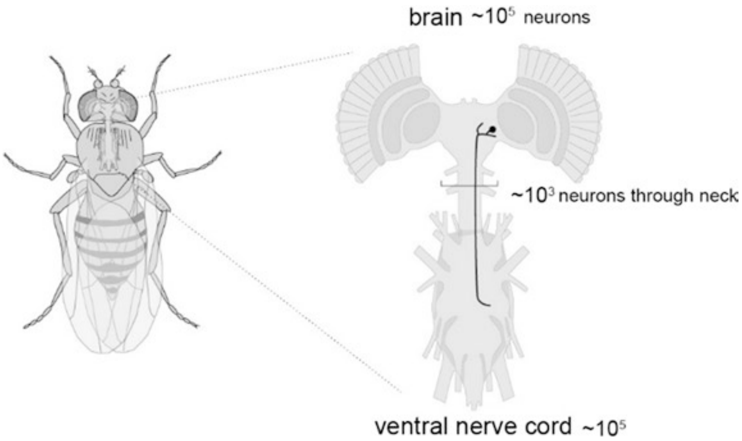

Fig. 10.2 Schematics of descending neurons connecting the brain and ventral nerve cord. Fly's body (left) and the structure of central nervous system are shown (right). Although the number of neurons in the brain and ventral nerve cord is the order of fifth power, the number of neurons running through the neck connective is the order of third power

et al. 2017; von Reyn et al. 2017). Seven DNs arborizing the same glomerulus to the giant fiber were identified (Namiki et al. 2018).

A candidate DN for freezing behavior has been identified in a recent work in *Drosophila*. Using the driver lines for DNs established, Zacarias et al. 2018 examined the silencing neuronal activity with inwardly rectifying K^+ channels (Zacarias et al. 2018). The movement of unrestrained flies was monitored in the experiment (Fig. 10.3). Through the screening, the authors found that silencing a specific DN, called the DNp09, significantly reduced the probability of freezing behavior in response to the looming stimuli. Furthermore, DNp09-silencing increased the probability of jumping compared in control flies, suggesting the presence of an inhibitory mechanism in the jump pathway. Optogenetic activation of DNp09 with channelrhodopsin induced freezing, and the effect persists during activation. Running at the initial phase was often observed, suggesting DNp09 is also involved in locomotion. The probability of DNp09-activation inducing freezing can be deduced as walking speed increases, indicating this behavioral choice; freezing/running is dependent of the walking speed (Zacarias et al. 2018). It would be interesting to study the neuronal mechanism to drive distinct behavior by the same DNs.

10.5 Optic Glomeruli

The neuroanatomy of DNp09 is shown in Fig. 10.4. DNp09 has wide field innervation in the brain, including the ventral protocerebrum and the cell body is located on the posterior brain surface. The innervation with smooth appearance is located in the wedge, inferior clamp, gorget, and ventral protocerebrum (VLP). The

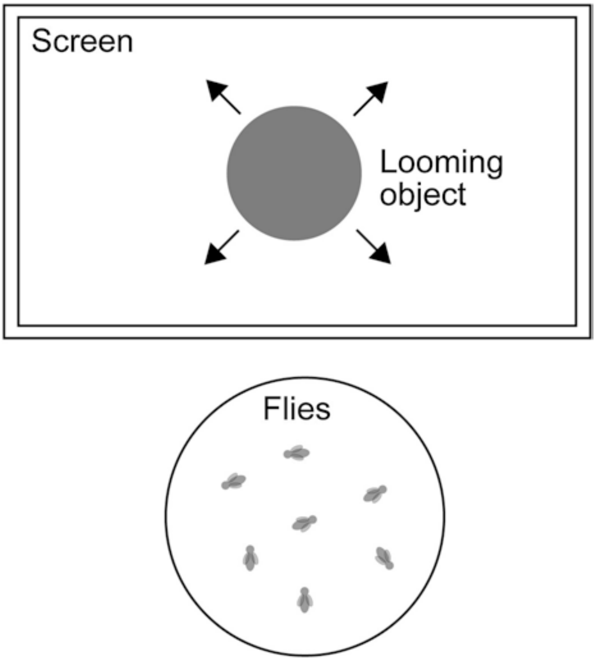

Fig. 10.3 Simplified schematics of experimental setup used in Zacharias et al. 2018. Flies were placed in a covered walking arena. A computer monitor above arena showed a looming stimulus

innervation with blebby appearance is located in the superior/inferior posterior slope and gnathal ganglia (GNG) and the terminal exhibits anti-synaptotagmin immunoreactivity. The VLP is an array of optic glomeruli, each of which receives input from the lobula complex. Optic glomeruli process ethologically relevant features of the visual object. DNp09 arborize one of the glomeruli which receive the input of lobula columnar neuron type 9 (LC9), whose function has not yet been characterized. The functional connectivity between LC9 and DNp09 has been confirmed by combination of optogenetics and calcium imaging (Bidaye et al. 2019). Wu et al. 2016 showed that optogenetic activation of visual projection neuron population LC6 also drives long-mode escape jump (Wu et al. 2016). Large-scale behavioral screening study combined with machine learning observes increasing jump response for GAL4 lines labeled LC9 and LC10, as well as LC6 (Robie et al. 2017). The wedge is connected with the antenno-mechanosensory motor center, receiving input from the Johnston's organ (Kamikouchi et al. 2006; Lai et al. 2012). Because flies also show freezing behavior in response to vibration stimulus (Howard et al. 2019), DNp09 innervation in the wedge may mediate vibration-sensitive freezing behavior. The function of other protocerebral regions, inferior clamp, and gorget is unknown.

In addition, the optic glomerulus LC11 is a candidate population for presynaptic partner of DNp09. A class of lobula columnar cells that is relevant to freezing

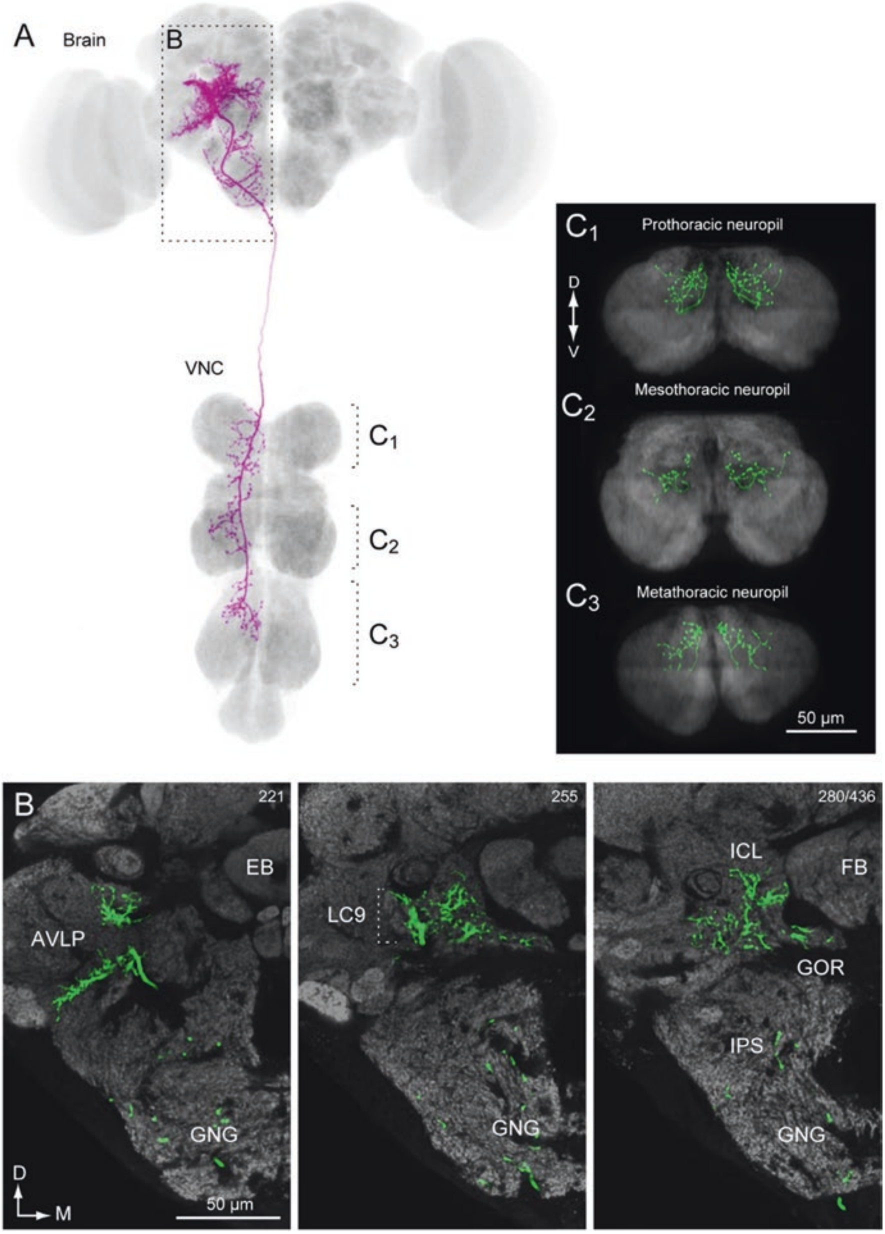

Fig. 10.4 Morphology of DNp09. (**a**) Morphology of DNp09 and the brain. The neuron and neuropil are shown with magenta and gray. (**b**) Three consecutive confocal stacks of the neuronal innervation in the brain. *AVLP* anterior ventral protocerebrum, *D* dorsal, *EB* ellipsoid body, *FB* fan-shaped body, *GNG* gnathal ganglia, *GOR* gorget, *ICL* inferior clamp, *IPS* inferior posterior slope, *LC9* lobula columnar neuron type 9, *M* medial. (**c**) Axonal projection of DNp09. Coronal sections for prothoracic, mesothoracic and metathoracic segments are shown. *D* dorsal, *V* ventral

behavior is identified recently. LC11 is known to respond to small moving objects, and silencing the LC11 does not affect object avoidance behavior (Keleş and Frye 2017). Tanaka and Clark investigated the function of LC11 using silencing, imaging, and behavior on the track ball. In calcium imaging, LC11 neurons respond to moving small objects. Silencing LC11 reduced the probability of stopping behavior, whereas optogenetic activation of LC11 briefly increased stopping probability. These suggest that LC11 is sufficient to trigger the freezing behavior. Functional connectivity from LC11 on DNp09 has been confirmed (Bidaye et al. 2019). Summary connection scheme of DNp09 is shown in Fig. 10.5.

10.6 Axonal Projection of Freezing Descending Neuron

DNp09 axon descends through the ipsilateral neck connective and projects into leg neuropils in the VNC (Fig. 10.4c). Dense innervation with blebby appearance is present in all regions: foreleg, middle leg, and hind leg neuropils. These blebby terminals exhibit anti-synaptotagmin immunoreactivity. In addition, DNp09 also projects to the region called the tectulum, a bundle of descending and ascending neurons running through the central zone of the VNC (Power 1948), and its function is unknown.

DN terminals in the leg neuropils could be sorted into two major types in Namiki et al. 2018: DNs projecting to the dorso-medial part of each neuropil (type-I) and DNs penetrating through the neuropil via pathway passing through the core of leg neuropil called the oblique tract (type-II). For example, giant fiber and other three DNs (p02, p05, and p11) arising from the LC4 glomerulus project to leg neuropils, and all of these DNs are classified as type-II (Namiki et al. 2018). Innervation

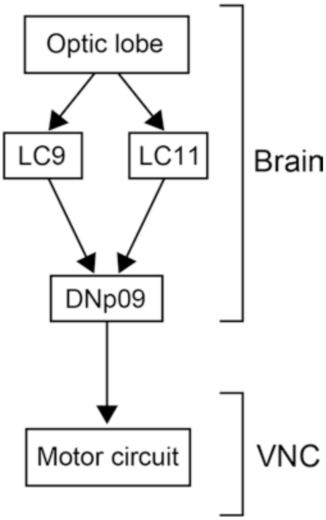

Fig. 10.5 A schematics of connectivity for DNp09. Candidate neuronal components related to freezing behavior are shown. The optic lobe supplies visual input onto optic glomeruli (LC9 and LC11). The optic glomeruli supply the DNp09, which send information to the motor circuit in the VNC. The neuronal components for freezing behavior are unknown in the VNC

pattern of DNp09 is classified as type-I, and most of the blebby terminals are present in the medio-dorsal portion. The connectivity of type-I DNs with downstream neurons is still unclear.

A recent study suggests that the serotonergic system in the VNC helps in facilitating an intermediate and stimulus-independent pause response when flies are startled (Howard et al. 2019). The study performed a silencing experiment using tryptophan hydroxylase (TRH)-Gal4 line. Although the individual neuron morphology in the Gal4-lines has not yet been analyzed, the line may contain the candidate downstream neurons of DNp09.

In addition, DNp09 also have presynaptic terminals in the GNG, which contains the largest number of DNs projecting to the leg neuropil (Namiki et al. 2018). There is a possibility for the information flow of freezing behavior: DNp09-GNG-Leg neuropils.

10.7 Neuromodulation of the Startle Responses

Howard et al. 2019 investigated the effect of neuromodulation on walking and startle response using neurogenetics in *Drosophila* (Howard et al. 2019). Gal4 line which labels serotonergic system in the entire nervous system is used. The line was crossed with teashirt-GAL80 (Rubinstein et al. 2010), so that the expression in the brain is suppressed. The neurons in the leg neuropils were densely labeled, suggesting the contribution of leg motion. This technique enables the manipulation of neuronal activity which is specific to the VNC. Among neuromodulators examined, they found the activation of serotonergic system to cause speed change in flies. When the serotonergic system was activated, walking slowed down; whereas when the system was genetically inhibited, walking became faster. Also, silencing serotonergic system altered how flies respond to being startled, taking longer time to exhibit escape response. These results suggest that serotonergic system in the VNC serves a role in freezing response. However, flies are still able to show startle response, suggesting that there is another pathway for freezing behavior. Also, the serotonin effect on startle response is observed both in visual and vibration stimuli; neurons for leg control are shared by both systems.

10.8 Similarity Between Freezing in Flies and Fears in Mammals

Whether flies have emotion like human remain unclear. There is no generally accepted definition for emotion. Anderson and Adolphs describe emotion as a type of internal brain state with central general properties than can exist independently of subjective, conscious feelings, which can only be studied in humans (Anderson and

Adolphs 2014). In their definition, the emotion is divided into four building blocks: scalability, persistence, valence, and generalization. To examine these characteristics, Gibson et al. 2015 created a new behavioral assay where flies are confined in enclosed areas and repeatedly exposed to an overhead translational shadow (rotating paddle) (Gibson et al. 2015) (Fig. 10.6). Repeated presentation of shadows resulted in graded (scalable) and persistent increase of freezing behavior. The repetitive shadow also resulted in dispersal of feeding flies, suggesting the presence of negative valence and context generalization. The authors concluded that freezing behavior of flies in response to repetitive shadows expresses an internal state exhibiting emotion primitives, which may be analogous to fear in humans.

10.9 Future Work

Identification of neurons involved in freezing behavior is helpful in understanding the neuronal mechanism. However, most of the neuronal components of the pathway for freezing behavior are currently unknown. The missing link can be identified using a fruitful genetic tool in *Drosophila*. Using driver lines which label DNp09 (Namiki et al. 2018), it is possible to identify potential upstream and downstream neurons with transsynaptic tracer (Feinberg et al. 2008; Talay et al. 2017). In addition, cell-type specific driver lines are being created for various brain regions (Aso et al. 2014; Wolff et al. 2015; Wu et al. 2016). For example, activation screening with optogenetic tools such as channelrhodopsin to search lines with freezing phenotype, in which unknown neurons for freezing behavior are present. Also, a synapse-resolution electron microscopic data is recently published for use in anatomical approach (Maniates-Selvin et al. 2020). Searching neurons which have

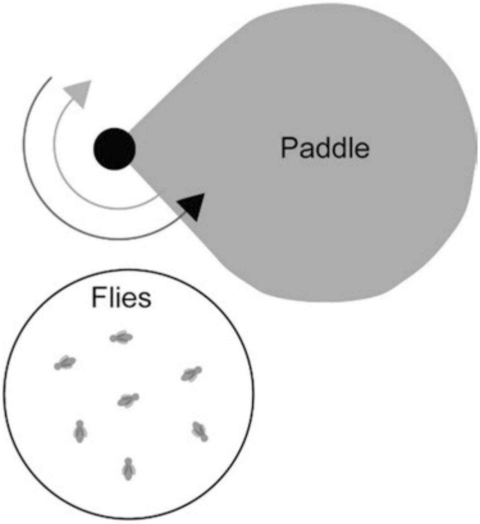

Fig. 10.6 Experimental setup used in Gibson et al. 2015. Flies are in the Petri dish and the paddle show rotation, which provide moving stimulus to flies

synaptic contact with DNp09 is another way to identify the pathway for freezing behavior.

Because a fly's freezing behavior shares basic features with defensive behavior in mammals, suggesting the presence of shared neuronal mechanisms called emotion primitive (Anderson and Adolphs 2014) will contribute in understanding the neural mechanisms in mammalian brain.

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Chapter 11
Immobility Behaviors in Fish: A Comparison with Other Vertebrates

Masayuki Yoshida

Abstract There are multiple types of immobility behaviors in the defense cascade in fish. Each immobility behavior has different functions depending on the imminence of the threat. The three major types include immobility during the orienting response, freezing, and tonic immobility (TI). The orienting response is elicited upon detection of any salient stimulus where the animal shows attentive immobility to maximize the monitoring of the environment and assess the seriousness of the situation. Freezing immobility is to minimize the possibility of detection by a predator. However, freezing is not merely a passive state but a continuous monitoring of the environment to seek a chance for escape. These two types of immobility behavior in fish share basic manifestations and biological similarities with those in land vertebrates. Only a limited number of reports show that TI in fish is functional in actual threatening situations, suggesting that TI is not a general defensive behavior in fish. Nevertheless, lines of evidence support the idea that the contribution of reflexive components in TI induction is conserved among vertebrates. Artificially induced TI is practically useful for safely manipulating sharks in the field. In some fish species, immobility-like behavior is used for feigning death or illness as a predatory tactic, instead of defensive behavior, to attract prey fish.

Keywords Death feigning · Defense cascade · Fish; freezing · Immobility behavior · Orienting response · Tonic immobility · Vertebrates

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11.1 Introduction

Immobility behaviors occur when animals face attentional and/or emotional situations that usually involve a predation risk. Animals stop ongoing behaviors to evaluate the situation or stimuli that are potentially significant for their survival. This is the first step of the defense cascade called the orienting response (Fig. 11.1). If the situation is found out to be threatening, the cascade proceeds to the next step: either fleeing or freezing. Then, if there is a further increase in the imminence of the threat and escape is not an option, animals fight back or show tonic immobility (TI). Various states of the defense cascade in mammals are briefly described in Chap. 1 of this book by exemplifying the case of a rat encountering a cat.

Thus, there are multiple types of immobility responses in the defense cascade, and each type has a different function depending on the context or the seriousness of the threat. In contrast to somewhat straightforward and explicable functions of orienting and freezing immobility, the biological significance of TI is not that clear, especially in fish, although it apparently often occurs in the terminal phase of antipredator behaviors.

In this chapter, I briefly overview the characteristics of three types of immobility behaviors, orienting, freezing, and TI, in vertebrates, mainly mammals, as components comprising a cascade of defensive behavior. For each type of immobility behavior, attempts are made to present examples in fish. Although a considerable amount of work on fish in this field has been published, the comparative biology of immobility behaviors in fish has not been thoroughly discussed.

11.2 Orienting Immobility

11.2.1 General Orienting Response in Vertebrates

The first step of the defense cascade is the orienting response that accompanies the immobility state. This step is activated upon detection of unfamiliar signs such as any disquieting sounds, vibrations, or odors. The cessation of ongoing or background stimuli can also be a salient sensory input to elicit an orienting response. If the novel or salient stimulus turns out to be of no significance, the orienting response wanes as the stimulus is repeated. This waning is not due to sensory adaptation but habituation of the response itself. Habituation is considered to be one basic type of learning (Petrinovich 1973). During orienting, the animal stops ongoing activity and reduces motion, which is referred to as "attentive immobility" (Volchan et al. 2017). An animal exhibiting the orienting response maximizes the monitoring of the environment by attentive immobility in which the posture is stabilized and muscle tone is increased (Kozlowska et al. 2015).

The orienting response is subdivided into two components: non-directional arousal, and directional attention towards the source of the arousing stimulus (Barry

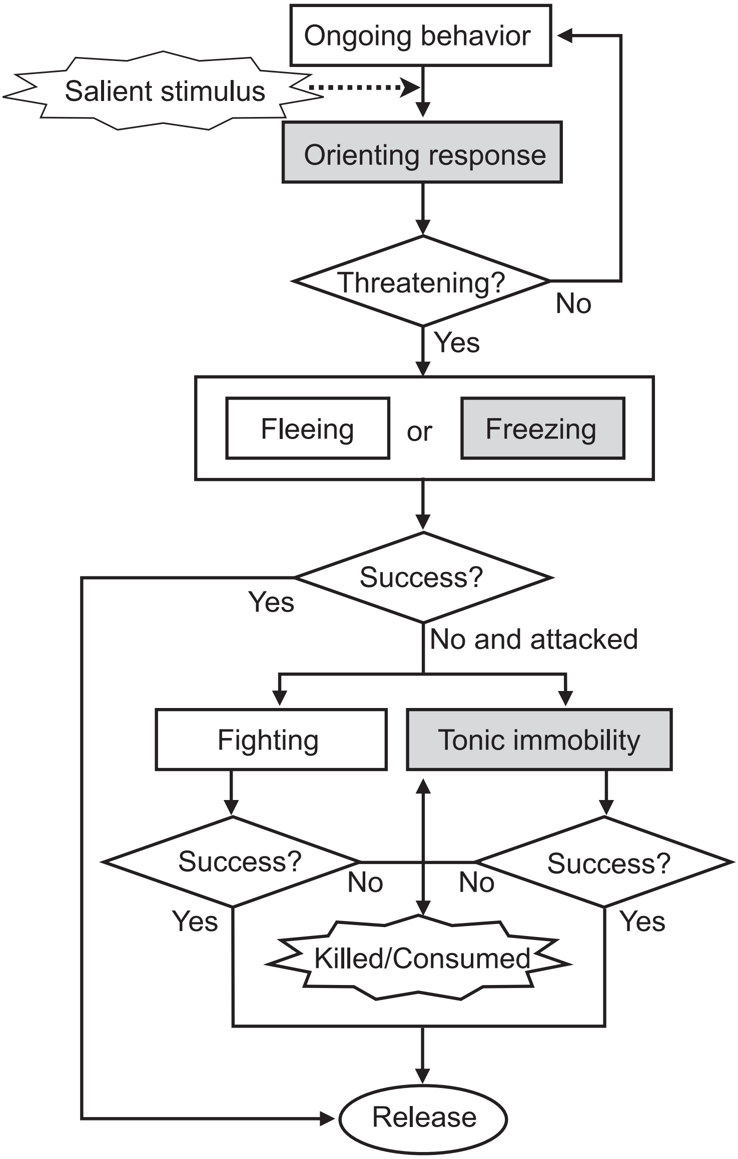

Fig. 11.1 A schematic representation of the defense cascade. Shaded behaviors include immobility states. See also Fig. 3.1 of Chap. 3

1984; Nicol and Laming 1993). During arousal, increased sensitivity of the sense organs is observed (Lynn 1966; Laming 1989). In humans, auditory sensitivity is enhanced by a preceding visual stimulus (Thompson et al. 1958) and vice versa (Watkins and Feehrer 1965). Increased sensitivity to a stimulus or facilitation of sensory neural activities during arousal has also been shown in other vertebrates, including fish (Laming 1989; Laming and Bullock 1991). Thus, the arousal state in the orienting response facilitates all sensory modalities to spot the source of the arousing stimulus.

In addition to sensory facilitation, physiological changes accompanying behavioral arousal include alterations in cardiac and respiratory activities. Heart rate and respiration rate decrease in the orienting response, especially during arousal, in various mammal species, including humans, dogs, sheep, and cats (Lynn 1966). The second state in the orienting response, attention, is aimed at the spotted source of the arousing stimulus.

Orienting should be comprehended integratively with motivation followed by appetitive or defensive emotional responses (Bradley 2009). This response is a part of the assessment process to examine a salient stimulus or a circumstance potentially related to positive (appetitive) or negative (fear or anxiety) emotions. Thus, the orienting response is not only for eliciting the defensive behavior but also for promoting an appetitive behavior, especially in predatory animals.

Immobility in the orienting response can also reduce the possibility of being noticed by a predator or by pray. As mentioned in Chap. 1 of this book, if the arousing stimulus is assessed as being originated from a threatening source, or predator, the next step of the defense cascade, flight or freeze, will take place.

Immobility during the orienting response shares some common features with freezing as a fright response: in both states, reduced motion and raised attentiveness are the characteristics. However, it should be noted that the substantial difference is that orienting immobility is a state of maximizing information gathering, while freezing is primarily a defensive/concealing reaction with heightened attention: the posture in orienting is usually upright or accompanied by small postural adjustments for redirection, while in freezing the animal commonly shows a tense crouching posture. Nevertheless, in many cases it is difficult to divide these two states clearly since the fright response can occur immediately upon the detection of a threat or if the novel stimulus is sufficiently intense (Lynn 1966; Laming and Savage 1980; Roelofs 2017).

11.2.2 Orienting Response in Fish

In fish, during the orienting response, there is an increase in muscle tonus, which is apparent from erected dorsal, abdominal, and anal fins, and an expanded tail fin (Fig. 11.2). Periodical twitching or continuous undulation of the pectoral fins is common, stabilizing the body in the water column. A decrease in heart rate and ventilation is also observed during the orienting response (Laming and Savage

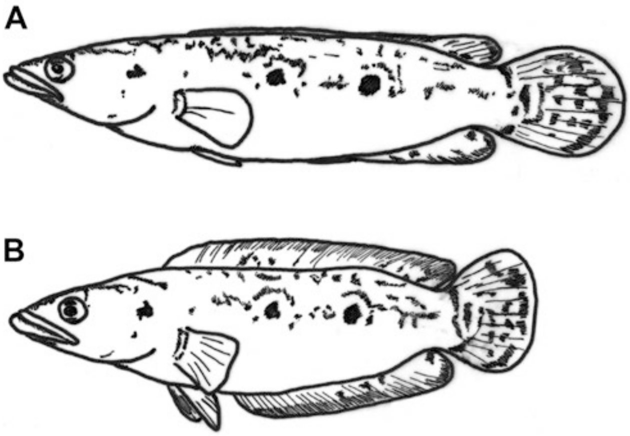

Fig. 11.2 Orienting response of the snakehead *Channa pleurophthalma*. (**a**) Resting posture. (**b**) A typical orienting response showing erected dorsal, abdominal, and anal fins, and expanded tail fin. Viewed from slightly diagonally from the front

1980; Rooney and Laming 1986). Fish shows quick habituation to an arousing stimulus when the stimulus is presented repeatedly (Laming and Savage 1980; Nicol and Laming 1993; Yoshida et al. 2004).

Attention, the latter phase of the orienting response, has been suggested to have the reverse physiological effect: increased heart rate (Laming and Bullock 1991) and attenuated sensory activity (Laming and Brooks 1985; Laming and Bullock 1991; Nicol and Laming 1993). It can be said, as in mammals, that during nondirectional arousal, general facilitation of the sensory system and cardiorespiratory deceleration occurs, and in directional attention, selective inhibition of sensory input and cardiorespiratory acceleration takes place (Laming 1989).

The orienting response is apparently not obvious in some fish species. For example, although zebrafish have been widely used for studying flight (erratic swimming) or fright (freezing) responses, they do not show apparent behavioral arousal and attentive immobility as observed in some other fishes such as goldfish. A recent preliminary paper considers an approaching behavior towards novel visual stimuli as an orienting response (do Nascimento et al. 2019). In this case, however, the abrupt cessation of ongoing activity as in behavioral arousal and following attentive immobility, which are two major components of the orienting response, was not reported. Immobility as a part of the orienting response in fish could be a characteristic of species that show stable hovering in the water column.

11.3 Freezing Immobility

Following the initial risk assessment in the orienting response when the animal detects cues of an actual threat such as a predator, if not spotted, the animal exhibits the next stage of the defensive cascade in a species-specific way. If the threatening source is perceived as relatively distal, the animal at this stage has two alternative choices for a fear response, fleeing or freezing. Attentive freezing immobility is a common adaptive behavior to avoid detection by a predator (Whishaw and Dringenberg 1991; Eilam 2005; Chap. 1 in this book).

11.3.1 General Features of Freezing in Vertebrates

Immobility during freezing behavior is not merely a defenseless/motionless state, but rather an active preparatory stage with heightened alertness that facilitates sensitivity for the encounter of the threat (Eilam 2005; Gladwin et al. 2016; Rösler and Gamer 2019). In regard to the enhanced sensitivity and serving as an action-preparation state rather than a passive motionless state, attentive freezing immobility shares features with the orienting response (Gladwin et al. 2016; Roelofs 2017). Thus, freezing can also be a continuum of orienting-related immobility (Gabrielsen et al. 1985). Internally, fear elicited by the detection of threatening stimuli during the orienting response or by perceiving a learned cue related to an aversive situation seems to underlie attentive freezing immobility (LeDoux 2000).

Many factors including the anxiety level (Frank et al. 2006), context of the threatening situation (Eilam 2005; Blanchard et al. 2011), distance from the threat (Blanchard et al. 2011), and availability of escape routes (Blanchard et al. 1990) affect the decision of the dichotomy to flee or freeze (Eilam 2005; Hagenaars et al. 2014). Individuality or personality within species, either predisposed or experience-dependent, is also a factor contributing to the variety of fear responses (Gabrielsen et al. 1985; Budaev 1997; Eilam et al. 1999; Huntingford and Giles 2010).

The word “freezing” has been used to refer to a wide range of defensive immobility behaviors and is interchangeably used to describe different kinds of immobility responses to a threatening condition (Volchan et al. 2017; Chap. 1 in this book). Unlike other immobility states including TI, freezing is activated at an intermediate level of a threat to avoid detection by a predator. However, the anxiety context also elicits freezing. Although lines of evidence indicate a distinction between fear and anxiety, the behavioral manifestation of these emotional states might share some features including freezing in regard to the seriousness of a defensive situation.

Parasympathetic dominance, in addition to the systemic increase in muscle tonus, seems to be a major characteristic during freezing, causing cardiac deceleration in various vertebrates including humans. However, since freezing consists of both sympathetic and parasympathetic features, physiological changes vary depending on which system is dominant in the context even in the same species. In the case of classical conditioned fear, conditioned cardiac responses in similar classical fear conditioning paradigms differ in direction depending on both the animal species and condition (LeDoux 2000); restrained rabbits and rats (Kapp et al. 1979; Supple and Leaton 1990a) show bradycardia, whereas unrestrained rat (LeDoux et al. 1983; Supple and Leaton 1990b) and baboons (Smith et al. 1979) show tachycardia.

11.3.2 Freezing in Fish

Freezing immobility is also common in fish although studies specifically focusing on this behavior are not abundantly available compared to those for land vertebrates.

Since the finding of the "schreckstoff" by von Frisch (1938), alarm-substance-elicited fright reactions in ostariophysian fishes have attracted much attention (refer to Pfeiffer (1977) for a classical review; Brown et al. 2011). Along with fleeing and gathering, freezing is a major component of the fright reaction when exposed to an alarm substance released from the injured skin of conspecifics.

Freezing immobility in fishes is also commonly observed as a defensive behavior in prey fish visually exposed to a predator; ex. Nile tilapia *Oreochromis niloticus* (Cichlidae) (Barreto et al. 2003), darters *Etheostoma flabellare* and *E. spectabile* (Percidae) (Radabaugh 1989), three-spined stickleback *Gasterosteus aculeatus* (Gasterosteidae) (Messler et al. 2007), guppy *Poecilia reticulata* (Poeciliidae) (Templeton and Shriner 2004), and zebrafish *Danio rerio* (Cyprinidae) (Bass and Gerlai 2008).

With or without preceding orienting behavior, on detecting an imminent threat, a fish shows either apparently disoriented rapid swimming (erratic movement) or freezing immobility. These two fear-related behaviors frequently alternate multiple times: erratic movement followed by freezing and vice versa.

It seems that there are two types of freezing immobility in fish: one manifests when facing a threat and the situation shows the possibility of escape, and the other occurs when the success of escape is unlikely. Exposure to an alarm substance or a novel situation corresponds to the former case. Freezing in such cases accompanies cardiac and ventilatory accelerations (Laming and Savage 1980; Holopainen et al. 1997; Barreto et al. 2003). This is apparently in preparation for, or recovery from, erratic movement or rapid escape from the threat where intense motor activity is demanded. On the other hand, restrained fish have been reported to show cardiac and ventilatory decelerations as in classical conditioned fear responses (Davis and Holmes 1971; Yoshida et al. 2004).

Thus, similar to mammals, the probability of the chance of escape seems to be a major factor determining the physiological response during freezing immobility in fish. As Volchan et al. (2017) discussed for mammalian cases, freezing should be subdivided into two different immobility states, attentive immobility and immobility under attack. The former is elicited by the detection of a potential threat and is ready to switch to another anti-predator behavior by monitoring the environment.

The latter occurs in case the prey detects signs of imminent attack and tries to reduce the possibility of an actual attack by the predator. The underlying autonomic balance between the two types of freezing behavior in fish has yet to be investigated.

The occurrence of the freezing immobility as an anti-predator behavior varies depending on environmental and physiological conditions. Trinidadian guppies *P. reticulata* inhabiting shallow riffles tend to freeze on the bottom upon potential aerial threat, while guppies inhabiting heavily vegetated streams hide under shelter in response to an aerial threat to reduce the risk of detection (Templeton and Shriner 2004). Male orangethroat darters *E. spectabile* in breeding states are likely to flee and less likely to freeze compared with those in non-breeding states when threatened (Radabaugh 1989). This is suggested to be due to the high-contrast vivid coloration of the male darter in the breeding state that lessens the cryptic effect of body coloration (Radabaugh 1989). The satiation level also affects the tendency to freeze in response to conspecific alarm substances. Although food-deprived Brazilian catfish *Pseudoplatystoma corruscans* show fright responses to exposure to an alarm substance the same as in fed fish, the freezing component of the response is abolished in the hungry fish (Giaquinto and Volpato 2001).

In addition to fear responses, in which the fish detects an apparent cue of a threat, elevated anxiety also elicits freezing immobility as in the case in mammals. Freezing immobility, together with other fear/anxiety-related behaviors, has been well documented for zebrafish in the field of translational neurobehavioral research involving the evaluation of anxiogenic or anxiolytic effects of compounds (Kalueff and Cachat 2011; Kalueff et al. 2012; Kalueff et al. 2013).

11.4 Tonic Immobility

The definition and biological significance of TI in animals in both vertebrates and invertebrates are described in Chap. 1 of this book. In this chapter, I briefly mention the general features of TI in vertebrates (mainly mammals) and introduce cases in fish, for which the biology of TI has been less studied compared with other animal groups.

11.4.1 General Features of TI in Vertebrates

Tonic immobility is a reflexive and involuntary reaction manifesting at the terminal phase of the defense behavior cascade (Kozlowska et al. 2015; Volchan et al. 2017; Kuiling et al. 2019). TI as an anti-predator response is seen in all classes of land vertebrates. This behavior is characterized by a subdued response to sensory stimulation and systemic catalepsy. Cardiac and respiratory decelerations seem to be common in stably sustained phase of TI (McDonald 1974; Nash et al. 1976; Giannico et al. 2014) although it is still controversial. Some studies have reported cardiac acceleration especially during induction period and/or initial phase of the TI (Nash et al. 1976; Gentle et al. 1989; Valance et al. 2008). The increased heart rate is probably due to physical and emotional stress caused by the procedure. Thus, it should be noted that autonomic balance in TI varies depending on the context of the induction and on the course of the TI.

During TI, although unresponsiveness to external stimuli is obvious, the central processing of sensory information appears to be unsuppressed and animals are capable of sensory association (Klemm 1966, 1971; Gallup et al. 1980). Neural substrates involving tonic immobility in mammals are comprehensively reviewed by Klemm (2001). Humans also are aware of their circumstances and retain a memory during TI under extreme threats where no other responses are possible (Kozlowska et al. 2015).

The adaptive value of TI is apparent as distinctively described for ducks and foxes (Sargeant and Eberhardt 1975) and in Japanese quails and cats (Thompson et al. 1981). In the terminal phase of an attack by a predator, the chance of survival of the prey is only expected when the predator loosens its grip or loses interest in the dead prey.

Although TI is apparently a fear-emotion-driven and/or reflexive terminal defensive response widely observed in various land vertebrates elicited usually by physical contact by a predator, there are a couple of cases that stand out from the others. Two famous "death-feigning" TI cases are of *Didelphid* opossums and *Heterodon* snakes as seemingly exaggerated performances. The North American opossum *Didelphis virginiana* shows a characteristic TI response, or death feigning, by violent provocations such as "grab and shake" by hands or by dogs (Francq 1969; Gabrielsen and Smith 1985). Francq (1969) reported that TI can also occur, if not typically, without tactile contact. In such cases, following the freezing response, the animal falls onto its side with a curled body and tail. Typical behavioral features during TI in the American opossum include open eyes, open mouth, extended tongue, salivation, urination, and defecation (Francq 1969; Gabrielsen and Smith 1985). Physiologically, a decrease in heart rate, respiration rate, and body temperature are observed (Gabrielsen and Smith 1985). As a defensive behavior, death feigning in the American opossum appears to be significant, since *"Despite our strongest encouragement, our dog invariably lost interest in the opossum once it entered this* (tonic immobility) *state"* (Gabrielsen and Smith 1985). It should be noted that not all *Didelphis* species show such remarkable death feigning: it is common in *D. virginiana*, rare in *D. albiventris*, and not reported in the others (Hunsaker and Shupe 1977; Kimble 1997).

Hognose snakes, or *Heterodon* species, are another example famous for their remarkable death feigning. Jones (1894) reported the stereotypical death feigning behavior of *Heterodon platirhinos* as follows: *"… at first tried to escape, but on being captured it turned on itself with mouth wide open, head thrown back sharply, and tongue limp and protruding."* During death feigning, the body is quite limp, and notably, when it is turned to the right position, the snake rolls back again and lays on its back. Upon induction of death feigning in *Heterodon* species, a marked bradycardic response has been observed immediately after rolling onto the back (McDonald 1974).

During TI, either elicited by physical contact or by external cues, animals are monitoring the presence of a predator. They apparently use visual information to assess the situation since the gaze by the predator or experimenter prolongs the duration of the immobility compared to just a presence of the threat (Hennig 1977; Gallup et al. 1972; Burghardt and Greene 1988). Given these lines of experimental and empirical evidence, it is almost irresistible to think that the occurrence and/or maintenance of some forms of TI involves intended behavior of the animals.

11.4.2 TI in Fish

Tonic immobility has often been used as a practical technique to keep fish quiet and still during various measurements and manipulations. It has been reported for more than 20 fish species, including both chondrichthyan and actinopterygian fishes, that the TI state is artificially inducible (Whitmann et al. 1986; Henningsen 1994; Brooks et al. 2011; Kessel and Hussey 2015). Quantitative case reports for artificial induction of TI in actinopterygian fishes are relatively scarce, and many of them were published in the early twentieth century (Whitman et al. 1986), compared with the availability of recent reports in chondrichthyans (elasmobranchs), especially the sharks. Since observations of TI in natural conditions are rare, the commonality and significance of TI as a defensive behavior in fish are yet to be determined.

11.4.2.1 Elasmobranchs

Tonic immobility can be artificially induced in most sharks and rays examined to date (Whitman et al. 1986; Henningsen 1994; Brooks et al. 2011; Kessel and Hussey 2015). Quick dorsoventral inversion and holding the fish in this posture for seconds to minutes make the shark act "limp", and the limp response can be an indication of successful induction of the TI state (Whitman et al. 1986). Thus, the condition of the skeletal muscles during TI in sharks is muscular hypotension, contrary to the increase in muscle tension or catalepsy in the case of TI in land vertebrates. Decreased rate of respiration and deep ventilatory movement are other characteristics during TI in elasmobranch fishes (Henningsen 1994). In one case for the Halmahera walking shark *Hemischyllium halmahera*, in addition to inversion, massaging the snout, where ampullae of Lorenzini are densely distributed, can be applied to induce TI although the effectiveness of the massaging has not been verified (Mukharror et al. 2019).

In the zebra shark *Stegostoma fasciatum*, it is known that TI is immediately induced by applying pressure to or grabbing the distal end of the tail (Henningsen 1994; Williamson et al. 2018). It has been suggested that the induction of the immobility state in zebra sharks is used in courtship where biting of the distal end of the

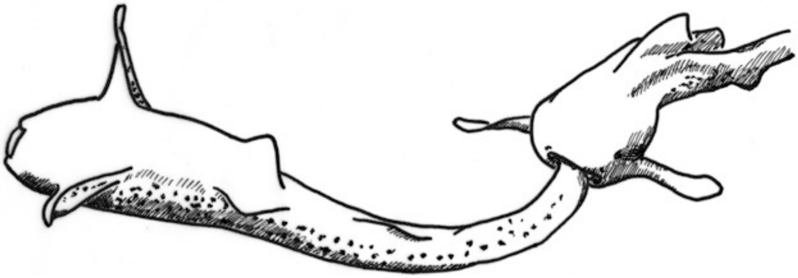

Fig. 11.3 Tonic immobility in a female zebra shark (left) induced by tail biting by a male (right) during courtship. Adapted from Fig. 1 in Williamson et al. (2018): Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/)

female's tale by the male makes the female enter a state of immobility (Williamson et al. 2018) (Fig. 11.3). However, as Williamson et al. (2018) reported, no differentiation in this type of immobility reaction between the sexes was found.

In the zebra shark, TI, other than in situations of courtship behavior, possibly functions as an anti-predator defense as in other taxa, because the distal end of the remarkably long tail fin would be a likely place for predator attack, and pressure to this part can induce TI (Williamson et al. 2018). However, the case of the zebra shark is exceptional. In other sharks, dorsoventral inversion and holding the posture for tens of seconds is the usual way to induce TI. In predatory attacks, it seems unlikely for an adult shark to be kept dorsoventrally inverted for such a long period, reducing the adaptive value of the immobility as a terminal defense, although when attacked by a predator, strong and forcible restraint might enhance the induction of TI. Thus, the significance of TI in elasmobranchs, especially sharks, is not yet clear.

Nevertheless, the significance of TI in sharks is obvious from a practical point of view. Artificially induced TI in sharks provides us with a time window for various measurements and manipulations such as the implantation of transmitters (Holland et al. 1999). Kessel and Hussey (2015) have argued for the benefits of TI over chemical-based anesthetics for sharks, especially in the field. In the field, it is obviously impractical to use a chemical anesthetic agent to obtain sufficient depth (stage IIb or III in the stages of anesthesia (McFarland 1959)) for surgery of large aquatic vertebrates. In contrast, the induction of TI requires a relatively short period of postural restraint, and the instant recovery from the immobility state reduces post-release risks (Kessel and Hussey 2015).

Cardiac deceleration during tonic immobility in sharks has been shown to be associated with, and caused by, a decrease in ventilation, indicating that the integrity of the brainstem controlling cardiorespiratory function is kept intact during TI (Davie et al. 1993). Although physiological perturbations associated with the disruption of respiration, which can be compensated for by artificial irrigation of the gills, are not negligible (Brooks et al. 2011), the negative effects of TI appear to be acceptable compared to anesthesia by chemical agents.

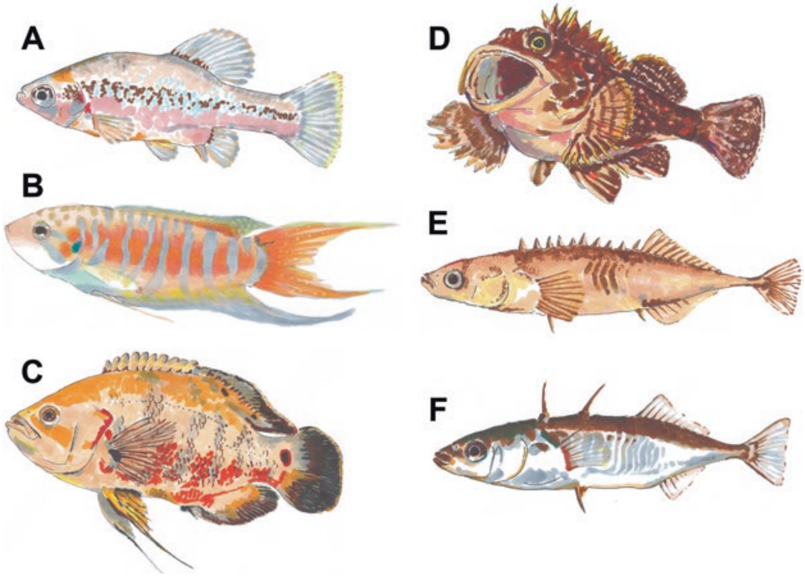

Fig. 11.4 Some fish species that show tonic immobility as a defense behavior. (**a**) butterfly splitfin *Ameca splendens*. (**b**) Paradisefish *Macropodus opercularis*. (**c**) Oscar *Astronotus ocellatus*. (**d**) A rockfish *Sebastiscus marmoratus* showing a resistive rigid posture. (**e**) Ten-spined stickleback (aka ninespine sticklepack) *Pungitius pungitius*. (**f**) Three-spined stickleback (aka threespine stickleback) *Gasterosteus aculeatus*. Reference materials were partly given by courtesy of Kamihata Fish Industries LTD

11.4.2.2 Teleosts

Tonic immobility caused by mimicked predatory attack or in high anxiogenic situations has been reported for the juvenile butterfly splitfin *Ameca splendens* (Goodeidae) (Fitzsimons 1973), paradisefish *Macropodus opercularis* (Osphronemidae) (Kabai and Csányi 1978), and oscar *Astronotus ocellatus* (Cichlidae) (Crawford 1977) (Fig. 11.4a, b, and c). TI is easily inducible in all of these species by an abrupt approach of a potentially threatening object like a human hand. For butterfly splitfin and paradisefish, introducing the fish into a novel tank, which is a highly anxiogenic situation, has been reported to be enough to induce TI. However, Kabai and Csányi (1978) described that immobility in paradise fish is often interrupted for a few seconds by intensive tail beats, suggesting that the immobility in this situation is a variation of freezing behavior, which is commonly intermitted by short periods of erratic movements (see above). Tonic immobility induced by a visual threat or other stressful events in oscars more resembles artificially induced TI in sharks. Oscars in a state of TI show slow and deep respiration (Crawford 1977).

For the juvenile butterfly splitfin, the significance of TI as a defense behavior is clear from fish showing TI surviving longer in the presence of predatory fish than individuals showing less TI behavior although detailed information is not available (Fitzsimons 1973). In this species, the posture of the immobility is characterized by systemic rigidity including a widely opened operculum, full expansion of the paired and median fins, strongly curved body, and mouth gape (Fitzsimons 1973). Thus, it can be said that the function of the TI in the splitfin is similar to that in the toadfish as described below.

Another notable case of the IT in teleost fish has been reported for Brazilian seahorses *Hippocampus reidi* (Freret-Meurer et al. 2017). In this species, TI was induced by a manipulation of the individuals by hands of the experimenter, either in the field or in the laboratory. During the immobility, seahorses rigidly curled their head and tails toward the abdomens, while some of the laboratory-kept individuals showed a stretched posture, and no breathing or eye movements were observed (Freret-Meurer et al. 2017). As swimming ability of seahorses is limited and they are not powerfully armed, a functional meaning of the immobility in the seahorses in threatening condition is probably identical to that in generally observed TI in insects: avoiding further attack by a predator. Since only a small portion of the individuals showed such characteristic TI postures in response to the handling (5 of 342 and 3 of 66 individuals in the field and in the laboratory, respectively), it is yet to be determined that this response is common in seahorses in the situation of actual predatory attack.

The toadfish *Opsanus beta* (Batrachoididae) shows a similar rigid posture including stiffened fins and maximally distended operculum in response to potentially threatening visual stimuli (Gunter and McCaughan 1959). Although the authors mention this immobility posture as a cataleptic state, the heightened systemic muscular tonus would have a tendency to repel external mechanical force. Many rock-dwelling scorpaeniform fishes show similar behavioral responses against predatory attack (Fig. 11.4d). Although this response is considered to be a defensive behavior, the function is unlikely to be the same as that of TI in many other vertebrates. Rigidly erected spiny fins and a widely opened thorny operculum would make a predator hesitate to further attack or ingest, and when the prey fish is in a gap or crack among rocks, the posture helps to resist an attempt by the predator to pull the prey out. An effectiveness of rigid postures with elected spines in prey fishes against consumption by a predator has been demonstrated in sticklebacks *Gasterosteus aculeatus* and *Pygosteus pungitius* (syn. *Pungitius pungitius*) (Hoogland et al. 1956) (Fig. 11.4e, f). When seized by a predator, a pike *Perca fluviatilis* or a perch *Esox lucius* in this case, sticklebacks rigidly erect their spines and keep motionless. The predator rejects the prey, and, after a small number of experiences eventually become negatively conditioned to avoid the sticklebacks (Hoogland et al. 1956). Supportedly, three-spined stickleback *G. aculeatus*, which have large stiff spines, is superior in the protection than ten-spined stickleback *P. pungitius* having smaller spines (Hoogland et al. 1956). In these context, this type of immobility posture in fish shares features with the death feigning posture in the pygmy grasshopper *Criotettix japonicus* (Honma et al. 2006; Chap. 3 in this book). Rigid spiny shapes, however, may not always protect the prey animals, and the validity apparently depends on its shape and relative size of the prey animals. Nishino and Sakai (1996) have reported that crickets showing TI in the mouth of a lizard were not rejected even though the crickets possess spines on the legs. Even from a limited number of observations, the manifestation of TI induced by threatening situations varies depending on teleost species. Limited information of physiological conditions during those immobility states makes it difficult to compare further with land vertebrates.

As in elasmobranchs, TI can be induced in the teleost goldfish *Carassius auratus* by quick dorsoventral inversion and firm restraint for tens of seconds (Davis and Holmes 1971; Yoshida unpublished) (Fig. 11.5). Gentle, rhythmic strokes with the fingertips in the area of the anterior abdomen facilitate the induction of immobility (Davis and Holmes 1971). Applying mild pressure to the anterior abdomen is also effective.

An apparent limp response is seen in goldfish during induced immobility, and the muscular tonus is greatly reduced. During TI, goldfish is obviously capable of sensing external stimuli, since the fish responds to arousing events such as tactile and visual stimuli with phasic reduction in ventilatory frequency although righting or escaping movements are not elicited. Furthermore, classical conditioned respiratory deceleration is achieved even in the immobility state (Davis and Holmes 1971), indicating basic central circuits involving sensory processing and association with stimuli are kept intact even during induced TI (Table 11.1).

Generally, catalepsy is not apparent in fish during TI. Rather, a limp response is characteristically observed in TI in both elasmobranchs and teleosts induced by postural manipulation and restraint, which are also commonly used to induce TI in land vertebrates.

11.4.2.3 Death and Illness Feigning in Fish as a Hunting Tactic

So far, there are three species that have definitely been reported to show death or illness feigning as a part of predatory behavior (McKaye 1981; Gibran 2004; Tobler 2005). The first quantitative description of death feigning was done for the African

Fig. 11.5 Induced tonic immobility in goldfish. The goldfish was placed in a V-shaped holder with a dorsoventrally inverted posture and restrained for tens of seconds to induce a tonic immobility state

Table 11.1 Summarized features of three types of immobility behaviors in vertebrates

	Primary defense		Secondary defense
	Orienting immobility	Freezing immobility	Tonic immobility
Situation	Detection of unfamiliar signs	Detection of an actual threat	Imminent attack or physical contact by a predator
Behavior	Stabilized, upright posture	Motionless, tense crouching	Lying Unresponsiveness to external stimuli[a]
Physiology	Facilitation of sensory system Increased muscle tone Decrease in heart/respiration rate	Facilitation of sensory system Increased muscle tone Decrease or increase in heart/respiration rate[c]	Paralysis[b] Decrease in heart/respiration rate[d]
Major function	Maximizing the monitoring of the environment	Minimizing the detection by a predator	Suppression of further attack by a predator

[a] Central processing of sensory information is ongoing

[b] Catalepsy is common in mammals; accompanying "limp" or rigid posture in fishes

[c] Varies depending on animal species and the context (see Sect. 11.3.1)

[d] Beginning of TI frequently accompanies cardiac acceleration (see Sect. 11.4.1)

cichlid *Nimbochromis livingstonii* (McKaye 1981). This predatory cichlid drops through the water column to the bottom and lays on its side, and occasionally its abdomen is buried partially in the sand. The behavior and color pattern of *N. livingstonii* mimic a dead fish and attract scavenging small fishes, which are potential prey of this predatory cichlid (McKaye 1981).

The Central American cichlid *Parachromis friedrichsthalii* has also been observed to show death feigning (Tobler 2005). Upon feigning death, *P. friedrichsthalii* sink to the bottom slowly and turn over on their side. Scavenging fishes are attracted by this death feigning fish and even pick on the body, making the fins frayed (Tobler 2005). Attack of the predator occurs when the small scavenging fish comes close enough. Tobler (2005) has suggested that feigning death hunting strategies have evolved at least twice within the cichlids; in Africa and in Central America.

In addition to the two freshwater species, a marine species, the comb grouper *Mycteroperca acutirostris* that inhabits southwestern Atlantic coastal waters, has been reported to show death/illness feigning (Gibran 2004). In limited sightings of the feigning, a juvenile comb grouper lays on its side on the bottom with occasional undulating of its body, apparently mimicking an ill or injured state (Gibran 2004). This death/illness feigning is successful enough to attract small fish, some of which are eventually captured by the predator (Gibran 2004).

Feigning death or illness as a hunting strategy is probably related to ambush and stalking tactics (Gibran 2004; Tobler 2005). In clear water, without cover for ambushing or stalking, it is difficult for a predator to approach its prey, and hence such an environment might promote the evolution of alternative hunting strategies like death or illness feigning to attract prey to within range of successful attack (Gibran 2004, Tobler 2005).

11.5 Significance of Immobility Behaviors in Fish

The adaptive significance of some immobility behaviors that manifest during orienting and freezing in the course of the defense cascade in fish is apparent as in other vertebrates. Orienting immobility maximizes information gathering and the assessment of the seriousness of a threat. Freezing immobility minimizes the possibility of detection by a predator while the animal continues to monitor the environment and wait for an opportunity to flee or hide. However, the adaptiveness of TI is not always clear when it comes to defensive mechanisms prevalently used in various fish species. That is partly due to the limited observations of TI in the terminal phase of the defensive cascade.

Fear emotion underlies multiple steps in the defense cascade: as the fear level increases, the cascade proceeds (Marx et al. 2008). Tonic immobility, including that in humans, is probably one form of emotional expression associated with extreme fear although the involvement of reflexive components cannot be excluded. The method of artificial induction of TI in fish is similar to that which is effective for land vertebrates: quick dorsoventral inversion and restraint, suggesting that the contribution of the reflexive component in TI induction is conserved through vertebrate evolution.

In the case of fish, however, TI that is functionally identical to that in land vertebrates might not be common. Many predatory fish suck a prey as a whole in one gulp, and hence limped, not cataleptic, musculatures as seen in artificially induced TI is unlikely to work in the natural environment. Immobility of a prey fish after attack can even facilitate the adjustment of the direction and ingestion by predators including birds. In this respect, the rigid thorny/spiny posture of some rockfishes and sticklebacks facing a threat is likely to be adaptive anti-predator behavior although this behavior can occur even in the early phase on encountering a predator.

Death or illness feigning in some predatory fish to attract small prey fish is another immobility-like behavior that is probably a form of an elaborated ambush or stalking tactic.

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