Mediating tactually guided behaviour: State-dependent and sensory modulation of activity in an identified descending interneuron

Gaëtan Lepreux

Mediating tactually guided behaviour: State-dependent and sensory modulation of activity in an identified descending interneuron

Dissertation zur Erlangung des akademischen Grades
des Doktors der Naturwissenschaften
Doctor rerum naturalium

vorgelegt von

Gaëtan Lepreux

Supervised by:

Prof.Dr. Volker Dürr

Department of Biological Cybernetics
Center of Excellence in Cognitive Interaction Technology (CITEC)
Bielefeld University, Germany

2020

Summary

Locomotion in natural environment is a complex task, particularly without visual cues. A universal sense that can be used for solving this problem is touch. In insects, the antennae are the main sensory organ of touch. They are actively movable and allow animals to maneuver in their surroundings. Stick insects of the species *Carausius morosus,* have poor vison, are wingless, live in cluttered environment and show remarkable climbing abilities. They mostly rely on information gathered by their antennae to find footholds or to cross gaps. They also show a tactually induced reach-to-grasp behaviour with targeted movement of a front leg towards the position of antennal contact. The underlying mechanism is fast and relies on the information transfer from the antennae to thoracic locomotor networks. Descending interneurons that potentially contribute to this behaviour have been identified and provide a nice substrate for the study of control of locomotion in response to tactile cues. However, the properties of these neurons, their role in the control of this behaviour is not well understood. In an attempt to fill this gap, I studied the spiking activity of the pair of contralateral ON-type velocity sensitive descending interneurons (cONv) of the stick insect *Carausius morosus*.

In chapter one I introduce general aspects of adaptative locomotion and the role of descending neuronal information. I expose the complexity of the control of locomotion under history- and context- dependent neuronal activity. I argue that stick insects are particularly suitable model to study these aspects because they show a reach-to-grasp movement of the front legs in response to antennal contact.

In chapter two, I use bilateral extracellular neck-connective recordings and analyze the modulation of spontaneous activity of cONv in resting animals, during antennal movement or substrate vibration. I found that the spontaneous activity of cONv is asymmetrically reduced during antennal motion whereas substrate vibration reduced the spontaneous activity of both cONv in a similar fashion. I show that cross-modal interactions occur in this system and relate to the effect of substrate vibration on the reduction of spontaneous to the footfall frequency during walking. I argue that walking-induced vibration reduces the spontaneous activity of cONv, thus reducing its impact on velocity encoding in this neuron.

In the third chapter I focus on stimulus-induced responses of cONv. Since stick insects continuously move their antennae during walking and most sensory system are subject to adaptation, I investigate the effect of adaptation in cONv. I took advantage of the bilateral extracellular neck-connective recordings and the bimodality of cONv to study stimulus-specific and cross-modal adaptation. I found that adaptation is cross-modal because substrate vibration pre-adapted cONv. Adaptation in cONv could improve detection of changes in antennal velocity and because antennal contact with an obstacle will produce a change in velocity, I propose that adaptation, could aid the detection of antennal contacts.

In chapter four, I combine motion capture of antennal movements with single-unit recordings of cONv in self-generated antennal movements. I reconstruct the 3D movements of the antennae during self-generated exploratory movement and during imposed deflection of the flagellum. Other than during rest, the joint-angle velocity of self-generated antennal movements were not encoded by cONv. Consistent with a context dependency of neuronal activity, antennal contacts occurring during exploratory movements were signalled by cONv. Because the putative antennal input to cONv are proprioceptive hair fields, I propose that an efference copy mechanism could take place in this system. Finally, I argue that under behaviourally relevant conditions, cONv could serve as a contact detector because it signals unintended movement.

In chapter 5, I conclude this thesis and show the limit of the extracellular recording technique to study cONv depending on the behavioural context. I further develop on the idea of an efference copy mechanism taking place in the stick insect antennal system and discuss the potential antennal inputs to cONv. Finally, I use the knowledge gathered in the previous chapters to propose potential output of cONv and its role in stick insect motor behaviour.

Acknowledgements[1]

First and foremost, I want to thank Prof. Dr. **Volker Dürr** for the opportunity to carry out this thesis work in his workgroup, for his support, thoughtful insights, and pragmatism all along these years. This thesis is one more proof, if necessary, that Germans and French can work together and accomplish nice things!

I want to thank **Josef Schmitz** for his support, discussion, and constructive feedback throughout this project. It was an honor to assist you during the various teaching modules. I am glad I met you! As a side note, I will never forget your love for chocolate in all its forms!

I want to address a big thank you to **Stephan Shuichi Haupt** for all the support you could bring me. Be it technical, personal, emotional, you were always there when I needed it. I missed you the moment you left us to go to Japan! I hope to see you again - Merci!

A special thank you goes to **Thierry Hoinville**. The person you can always talk to and expect a very frank answer, exactly the one you sometimes do not want to hear. This page is not long enough to write all the things I would like to, so I will go with a simple: Merci pour tout!

To all the **Biokyb members**, who were always there for small chats or to assist in any possible way. To **FPS Flo** for his assistance during the hard programming times and **Annelie Exter** for her wonderful technical support and compassion. To **Gitta Otte-Eusterling** for her amazing job keeping our insect colonies.

To **Holk Cruse**, your unswerving passion for science is an example for us all, it was an honor to be able to know you!

To **Malte Schilling**, I loved playing soccer with you whenever possible. It was a pleasure to exchange with you during the after-lunch coffee breaks although I will never understand your thing for sharks!

To my office mates **Chris J. Dallmann**, **Arne Gollin** and later on **Yannick Günzel**. These years in Bielefeld would not have been possible without your friendship, our not so small talks in the lab, thoughtful discussions as well as the late evening lab beers! I wish you all the careers you deserve.

To all the students I had to pleasure to supervise and help during research projects: **Andrea Gonsek**, Bachelor thesis: **Nia Papadopoulou, Marie Stelter**, or Master thesis: **Bianca Jaske** as

[1] I would like to apologize to anyone who is not listed and that deserves to be here!

well as all the “other students” who stopped by the lab at some point: **Ronja, Marco, Manon, Johannes, Miriam, Luca, Nicoletta** …..and many more.

To my friends from the German class, **Matthias Galipaud** and **Agata Grodzka**. Life in Bielefeld was never the same after you both left.

All the nice people from the **VfR Wellensiek** for providing an environment allowing me to free my mind and practice a sport that I love so much.

To all the other PhD fellows, in particular the French connection, **Charlotte Doussot, Olivier Bertrand**, I loved every single moment spent with you guys and just wish there were more!

To my friend and best man **Corentin Rendu,** for all these years of true friendship and his numerous visits in Bielefeld.

Obviously, I want to make a huge thank you to my parents **Christophe Lepreux** and **Katia Duvauchelle**. I would not be here without them. You never questioned my choice and let me study whatever and wherever I wanted to. We know each other for something like 31 years now and ever since we met you have sacrificed and provided anything I needed to succeed. For that I will be forever grateful!

I cannot finish this page without saying a few words about the person that was always in the shadow, approved my choices, and without whom the completion of this work would not have been possible. The one who kept me going on, no matter how hard things were. The one that believed in me from the first day we met. The one that, already a year ago, said “yes” to a simple question. The only one, my wife **Marine Potez.**

Contents

List of figures

Chapter 1

General Introduction

Sensing the world

Sensing the world is a shared feature throughout the animal kingdom. It provides animals vital information about their environment that will allow them to cope with various type of situations. Sensing the world is permitted by various organs probing the surrounding in far-range as well as near-range. Therefore, far-range sensing comprises everything that is not at immediate reach of the body and is outside of the personal space of the animal (Dürr and Schilling 2018). Various strategies have been deployed to sense the surrounding in far-range. Probably the most obvious sense one can think of is vision. From cyanobacteria to human, eyes have developed and allow to perceive the environment. For example, insects have compound eye that they use to avoid obstacle during flight, detect prey or food (Warrant 2017). Another important sense for survival is the capability to smell food, conspecific or even predators (Gadenne et al. 2016). Indeed, the smell of a fruit or a flower can be detected and followed by some flying insects over very large distances (Borst and Heisenberg 1982; Martin 1965; Wright 2015). Others like bats, can emit and perceive ultrasound to sense the surrounding (some aquatic species such as dolphins as well). Echolocation is a nice example of the use of ultrasound to actively perceive obstacles, prey or conspecifics and navigate in darkness (Jones 2005; Jones and Teeling 2006; Simmons et al. 1984; Simmons and Stein 1980). Some underwater species like Electric fish are capable of electrolocation using their electric organ that generates electric fields (Bennett 1971; Nelson 2011). The body of the fish is equipped with thousands of electroreceptors to perceive the change of the electric field properties such that they can detect and characterize objects in the environment (Bennett, 1971a; von der Emde et al., 2008, Chacron, 2007).

Active touch sensing

Active tactile exploration occurs everywhere, and the sense of touch is particularly important for near-range sensing. In fact, it is one of the most common sense seen throughout the animal kingdom (Prescott et al. 2011). For animal navigating in dark environments or deprived from vision, the sense of touch is vital. For instance, the whisker pads (or vibrissae) of rodents are organs that are used to detect and recognize objects (Diamond and Arabzadeh

2013; Kleinfeld et al. 2006) much like human would do with finger tips (Lederman and Klatzky 1993). The whiskers are somewhat an extension of the animal giving information about distance. Rats can also use their whiskers to find foothold position (Arkley et al. 2014) but also to hear (Neimark et al. 2003). They are actively moveable and the animal can control their range and frequency of movement (Mitchinson et al. 2007). The spatial arrangement and the projections of the whiskers in the brain is well known (Brecht et al. 1997). One whisker project in one specific region of the cortex that is distinct from the neighbouring one and forms a local map corresponding to each of them (Petersen 2007, 2019). Much like the whiskers of rodents, antennae of insects are appendages that the animals rely on to gather information about their near-range environment (Staudacher et al., 2005; Comer and Baba, 2011). In fact, they are extension of the personal space of the animals that help them to gather information of the environment over a larger volume than their body and leg cover (Dürr and Schilling 2018). The information they gather with their appendages is used to adapt their behaviour to the situations they encounter in nature. For instance, cockroaches follow walls using they antennae (Camhi and Johnson 1999). They keep their antennal flagellum in constant contact with the walls even in high speed manoeuvres. They adjust their body position according to the bending of the antenna with respect to the wall, giving them a cue of where to go (Mongeau et al. 2013). This means that the information gathered by their antennae is transferred to their locomotor system. Crickets G*ryllus bimaculatus* use their antennae to recognize their conspecifics (Comer et al. 2003). Accordingly, they are able to distinguish predators or neutral objects based on the texture of the surface that was touched (Comer et al. 2003; Okada and Akamine 2012). For instance, when a predator approaches, they respond with a fast escape response. However, if a conspecific comes to challenge their territory, they use their antennae as a fencing device for fights (Adamo and Hoy 1995; Hofmann and Schildberger 2001; Stevenson and Schildberger 2013). Cockroaches and stick insects localize objects and orientate depending on what the antennae touched (Baba et al. 2010; Comer et al. 2003; Okada and Toh 2000; Schütz and Dürr 2011). In the context of control of locomotion in insects, the antennae represent the primary source of tactile information that animal use in order to modify their behaviour according to their surroundings.

The insect antenna: "near-range sensor"

In all Ectognatha, the antennae are divided into three functional segments with two movable joints (Figure 1). The scape is the most basal segment and connects to the head, where it forms the first movable joint: the head-scape joint (HS-joint). The pedicel is the second segment and forms together with the scape the second movable joint named the scape-pedicel joint (SP-joint). Finally, the flagellum forms a long flexible structure that cannot be moved actively. In nature, the antennae come in different forms and shapes, depending on the animal species (Borror, D. J. ; Triplehorn, C. A. ; Johnson, 1989) that relate to the lifestyle and specific needs of the animals. Nevertheless, the pair of antennae are sensory organs that the animal can move actively and control allowing near-range contact (Staudacher et al. 2005). In the stick insect *Carausius morosus*, the biomechanics (Dirks and Dürr 2011), the motor system (Dürr et al. 2001) and joint-angle range (Mujagic et al. 2007) of the antennae have been well described. Muscles attached to the head cuticle can depress or elevate the scape of the antenna. There is only one degree of freedom around the head-scape joint such that the scape can move in one plane only. The plan of the scape-pedicel joint is slightly slanted with respect to that of the head-scape joint. Two muscles, the abductor and the adductor muscle, located in the scape, are required to depress and elevate the pedicel respectively. Thus, stick insects can constantly adjust the frequency and range of their antennal movements. Krause and Dürr, (2012) have shown that, as the animal step over obstacles, antennal touch with the obstacle modifies the frequency and amplitude of antennal movements.

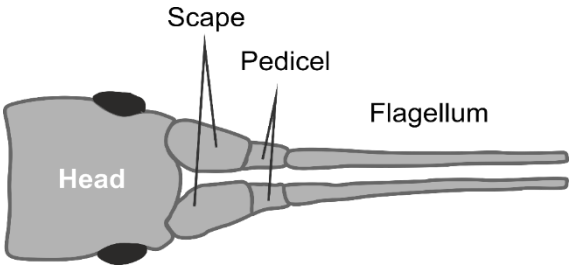


Figure 1: Drawing of the stick insect head with the two antennae.
The antenna consists of a scape, a pedicel, and a flagellum (shortened here). The antennae can be moved along two joints located between the head and the scape (Head-scape joint); and between the scape and pedicel (Scape-Pedicel joint). There is no movable joint between the pedicel and flagellum. Modified from Volker Dürr (2014).

The three segments of the antennae carry different mechanoreceptors that allow the animal to sense bending (campaniform sensilla), vibration (Johnston's organ), movement, touch and position (hairs and hair fields) on the antennal structure necessary to control and modify the animals' behaviour.

Campaniform sensilla.

Campaniform sensilla are coffee-bean-shaped structures in the cuticle (Figure 2). Much of what we know about the CS properties comes from studies carried out on CS located on the legs and wings of insects. In regions where stress is high, sensilla that have the same orientation will be grouped, especially at the joints of the legs (Pringle, 1938) and play the same role as to signal the load during resisted movement (Zill et al., 2004). They are embedded in the cuticle and innervated by one sensory neuron. Not all campaniform sensilla from the same group will present the same sensitivity to a given stimulus (Zill et al., 2004). This way, they can signal a whole range of directional forces (Zill and Moran, 1981). As the force is applied on the cuticle, the campaniform sensilla, that are oriented in the same direction as the bending, are stretched, activating their mechanoreceptive afferents as long as the cuticle is stretched (Spinola and Chapman, 1975; Zill et al., 2012). They signal both the magnitude of load and the rate of load change (Zill et al., 2012) and they serve the role of force measurement sensors. On the antennae, they are found along the flagellum and around the pedicel. In the stick insect their density appears to be decreasing towards the distal part of the flagellum (Staudacher et al., 2005, Dürr, 2014). Some insects carry a ring of CS around the distal part of the pedicel called Hicks' organ (Hicks, 1857). Since the pedicel-flagellum junction is not a moveable joint, Hicks organ will signal the load (i.e. bending) that the flagellum applies to the pedicel (Heinzel and Gewecke, 1979). Thus, they could be involved in sensing resisted flagellar contact and distance of contact thanks to their distribution.

Johnston's organ.

Located in the pedicel, this organ belongs to the group of chordotonal organs (See Field and Matheson, 1998 and Figure 2). Chordotonal organs consist of a scolopidia array which itself is composed of three main elements among which is located the sensory nerve cell (Figure 2). Flying insects rely on Johnston's organ (JO) for flight control (Gewecke, 1974; Sane et al., 2007; Dieudonné et al., 2014). An experiment in hawkmoth showed that animals with cut and reattached flagellum could fly better than hawkmoth with antenna amputated. Since the flagellar sensors were deafferented through amputation of the flagellum, this shows that the proximal segment carrying the JO was necessary for flight stability (Sane et al., 2007). Johnston's organ is stimulated by the deflection of the flagellum with high sensitivity as it is able to sense gravity and small frequency high-amplitude vibrations (Kamikouchi et al., 2009; Dieudonné et al., 2014). Thus, the vibration caused by airflow or sound can be detected. In

some insects, JO transduces near-range particle movement as occurs in sound stimuli and therefore could be used for hearing (Dreller and Kirchner, 1993). Finally, JO can sense flagellar movement generated by the animal itself but could also sense flagellar movement generated by external forces applied on the antenna.

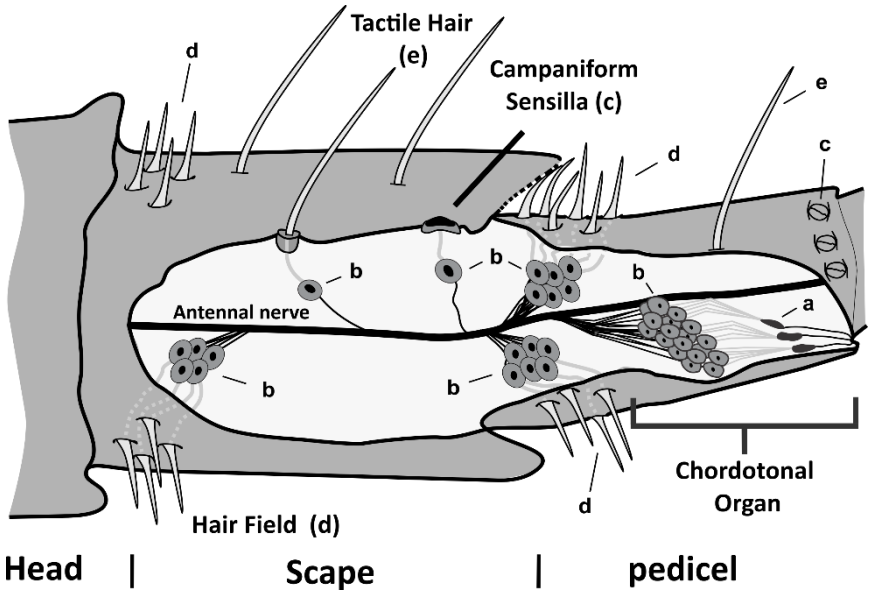


Figure 2: Schematic of mechanoreceptors found on the antennae.
Light grey area represents the inside of the antenna. a) scolopidia of the Johnston's organ, b) sensory neurons, c) campaniform sensilla, also called hicks' organ when located around the distal part of the pedicel. d) Hair fields (hair plates and/or hair rows), e) tactile hairs. At the head scape joint and scape -pedicel joint the cuticle softens to become a membrane that folds over the hairs and deflects them. The number and distribution of sensors is not representative of a real antenna but serve as a visualization of where they can be found. The joint menbranes are often represented as dotteed lines. Hey fold over the hair fields during movement of the joints.

Mechanosensory hairs.

There are several types of mechanosensory hairs with various distribution and number along the flagellum (Staudacher et al., 2005,Figure 2). There are single hairs that have different mechanosensory properties. For instance, the tactile hairs are flexible sensilla that vary in their diameter and length. They are connected to one nerve cell that is activated when the hair is bent in a specific direction. In cockroach, they can tell contact position (Okada and Akamine 2012). Another type of sensory hairs found on the antenna is hair fields. Unlike tactile hairs, they are organized in patches of several mechanosensory hairs at the base of a joint (Figure 2). The number of hairs per patch is variable. In the stick insect they appear either in the form of an array that is randomly distributed (hair plate) or as a row of hairs (Krause et al. 2013). Like the tactile hairs they are activated upon bending. The response of a single hair to deflection has been shown to be phasic-tonic in the cockroach (Okada and Toh 2001) but the population of hairs can encode joint angle velocity. In the cockroach, shaving these hairs altered the detection of obstacle and delayed climbing responses (Harley et al. 2009). In the stick insect, shaving of these hairs has been shown to increase the range of the antennal movement (Krause et al. 2013). Thus, hair plates and hair rows can signal joint angle, position and in principle also encode the velocity of antennal movements. The more and faster the joint is flexed, the more and faster hairs are deflected. A conceptual model, including the coding properties of hair fields, has shown that the information they signal is sufficient to generate spiking activity of characterised descending interneurons that encode movement, position and velocity (Ache and Dürr 2015). Since they can measure antennal deflections, antennal hair fields could measure antennal deflection generated by the animal itself or by external forces applied on the antenna.

Other type of sensors

The antennae also bear a large number of other sensory hairs on the surface. Each hair contains one or more sensory cells such as thermoreceptor (temperature), hygroreceptor (humidity) chemoreceptor (smell). Usually, these types of hairs have a hole at their tip by which molecules can pass through, allowing the animal to smell and taste. (for a review see Staudacher et al., 2005). They can all be used actively to infer information about the surroundings of the animal that could allow them to react accordingly.

Given the large number of sensilla/sensory neurons that equip the antennae, it is easy to understand that animals use their antenna for a variety of behaviours. However, in order to benefit from the tactile antennal information and exploit it for appropriate behaviours, the nervous system needs to extract temporal and spatial features from the different antennal mechanosensors. Consequently, information gathered by the antennae must be conveyed to the thoracic ganglia and transferred to the locomotor apparatus and modify the behaviour accordingly. However, one essential question remains: How is the activity of sensory neurons interpreted and converted by downstream motoneurons such that the appropriate action is executed?

Sensory neurons are activated regardless of the origin of the activation. Be it passive (from external sources) or active (generated by the animal's own movement), the mechanosensors will be stimulated. However, it is not necessarily relevant to always react to sensory activation. Interneurons that relays information from the mechanosensors to downstream regions that trigger an appropriate behaviour are at the first stage where this distinction can occur. However, several factors may impact the way the nervous system performs these tasks. The interneurons are likely embedded in a neural network that potentially receive information from more than one sensor. They may also receive feedback from motoneurons and their level of basal (spontaneous) activity can interfere with the treatment of the information. It is known that the baseline activity of the neuronal system may influence the ability of the animal to respond to cues. For instance, spontaneous neuronal spiking activity can be used as a marker of the state of the animal (Koren and Denève 2017) and thus affect the reaction of the animal. Furthermore, the response of the animal to a stimulus can depend on the level of familiarity (or the repeated exposure to) of the stimulus for the subject. For example, at the end of the day we do not perceive the flagrance of our own perfume while the others can still smell it. This means that the system has adapted and/or habituated to the smell (Pellegrino et al. 2017). Another important factor in the detection of cues is the discrimination between a movement that is self-generated (I want to move), resisted (I want to move but something is impairing that movement) or an imposed movement (I do not want to move but external elements are forcing me). There is a difference of a scene moving in front of us while being static and us looking through the window of a train. In the latter case our own movement is responsible for the change in the scene.

Insects as a study model of the control of locomotion.

Control of locomotion has been extensively studied in insects (Cruse et al. 2009; Delcomyn 1999; Dürr et al. 2018; Graham 1985; Laurent 1991; Wilson 1966). However, only few studies have focused on the neural circuitry that is involved to control the legs upon antennal stimulation yet bridging the gap between the head and the thorax. In cockroach, Ye and Comer (1996) identified a pair of giant descending mechanosensory neurons involved in escape behaviour called DMI-a1 and b1. The DM interneurons are activated upon a tap on the antenna and air by puffs. They arborize in all three thoracic ganglia in the vicinity of neurons known to be involved in the control of leg movements (Murrain and Ritzmann 1988; Ritzmann and Pollack 1986, 1990; Stubblefield and Comer 1989). The latency for transmission of antennal touch is fast enough to carry the information and trigger the escape response. In a companion paper, the authors showed that DMI activity followed by antennal touch was preceding the behavioural response. Moreover, the touch triggered more activity in the contralateral DMI than the ipsilateral counterpart, which in return determined the direction of the escape response. In crickets (*Gryllus bimaculatus*), six giant DINs responding to antennal touch have been identified (Schöneich et al. 2011). Four out of six have their soma located in the brain and descend through the connectives throughout the thoracic ganglia where they branch in the vicinity of locomotor networks. Some of these neurons (DBNc1-2/c2-2; DBNi1-2/i2-1) were previously shown to encode movement direction or antennal position during passive deflection of the antenna (Gebhardt and Honegger 2001). Two other DINs have their soma located in the Gnathal ganglion (Schöneich et al. 2011). All of them transmit short-latency action potentials following antennal contact, making these giant DINs part of a fast transmitting pathway that connects the antenna to the locomotor system of the animal. In the stick insect, Ache and Dürr (2013) characterized five groups of DINs conveying information about the direction, position, velocity of the antenna. Three velocity-sensitive DINs have been identified and characterized in more details (Ache et al., 2015) and could, in principle, be involved in the reach-to-grasp behaviour observed after antennal contact in the stick insect (Schütz and Dürr 2011).

Example of the stick insect Carausius morosus.

Stick insects *Carausius morosus* are obligatory walkers, they cannot jump or fly and their adaptiveness in locomotion has been studied extensively. They can walk on flat or uneven terrain, upside-down or on leaves (Cruse 1976). Pioneer work from Cruse (1990) defined rules necessary for inter-leg coordination during walking. Based on these rules, a computational walking model has been proposed (Cruse et al. 1998) and has been further developed since then (e.g., Schilling et al., 2013). Like many other insects, the gait of the animal changes depending on the walking speed (Cruse et al. 2009). At slow speed (10 to 20 mm/s) most insects are generally walking in tetrapod gait meaning that 4 legs are in contact with the ground while two are in swing phase. At higher speed (50 to 60 mm/s,), the gait pattern transitions to a tripod gait, consisting of three legs on the ground and three legs in swing (Wilson, 1966; Delcomyn, 1971). Given that multiple legs hit the ground at each cycle, the footfall frequency (i.e., frequency of touch-down events for all legs) for an animal walking slowly is 4 Hz and can reach 10 Hz and more during fast walking (Lepreux et al., 2019, their Fig. 12). To guarantee stability of the animal during the transition from stance (leg in contact with the ground) to swing (leg in the air), local feedback mechanisms could take place to prevent a leg to lift off. Indeed, a leg that carries the load of the animal cannot lift off before the load is transferred to another leg. This is made possible by the touch down of a neighbouring leg that is concomitant with an unloading of the leg allowing to lift off (Dallmann et al. 2017). Berg and colleagues (2015), showed that activation of a local interneuron called i4 can elicit searching movement of the leg. When the leg does not touch the ground while stepping, the leg transitions into circular movement to search for foothold. However, stick insect do not only rely on local mechanisms to control their legs and posture (for review see Dürr et al., 2018). Indeed, stick insects are nocturnal, have poor vision and live in cluttered environments. They heavily rely on tactile exploration to orient themselves and adapt their behaviour during locomotion. The front legs of the stick insect are long enough to reach the tip of the antennae when the animal is in thanatosis (Figure 3, camouflage behaviour where the front and middle legs are pointing forward while the hindlegs point backward along the body axis, the animals look like a branch, avoiding them to be eaten by predators. Bässler, 1983; Skelhorn, 2018). This anatomical peculiarity allows the animals to reach with their front legs anything that has been touched by the antennae. Schütz and Dürr (2011) showed that stick insects react with a fast and targeted reaching movement of a front leg after antennal

contact with an obstacle. This happens regardless of the position of the leg when the antennal contact occurred within 40 ms. Thus, information concerning antennal contact such as position, movement, velocity is rapidly transferred to the front legs. The information required for such behaviour could be conveyed by the previously identified descending interneurons that connect the brain (i.e. antennae) to the thoracic ganglia (*i.e.* locomotor networks, Ache et al., 2015). Thus, these neurons are good candidates for studying transfer of antennal information to the legs and allow to study control mechanisms that permit the animal to adapt its body motion according to descending antennal mechanosensory information.

Figure 3: Stick insect Carausius morosus in thanatosis.
Top: Dorsal view of the animal. Bottom: Ventral view of the animal. Note that the front and middle legs are pointing forward while the hind legs are pointing backward along the body axis. The tip of the front legs reaches the tip of the flagellum.

Encoding of antennal movement in the stick insect *Carausius morosus*

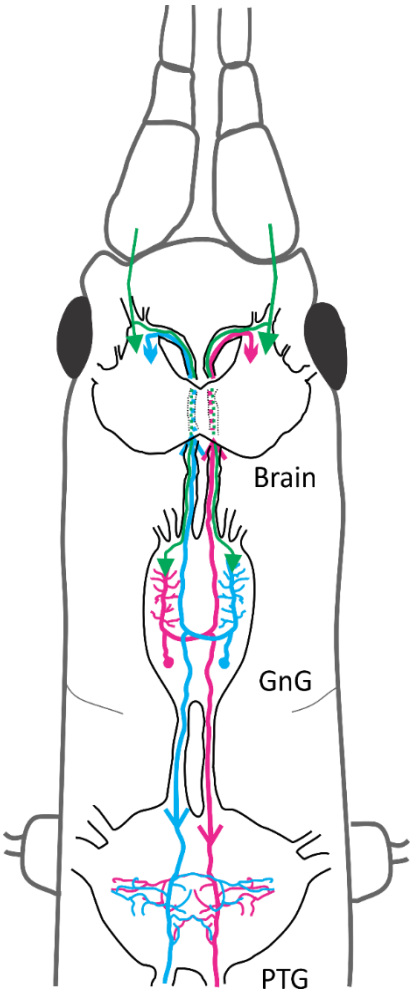


Figure 4: Drawing of stick insect and the innervation of both cONv.
The somata of left (magenta) and right (cyan) cONv are located in the Gnathal ganglion (GnG). The axons descend contralaterally in the thorax of the animal and arborize in the Prothoracic ganglia (PTG) and the meso- and meta-thoracic ganglion (not depicted here). Note that both cONvs send an ascending branch of their axon to the brain. The green arrows show the antennal input to the brain through the antennal nerve and the input to cONv via the circumesophageal connective s. Drawing not at scale. For illustration purposes, the body outlines are shown for orientation and the connectives between the brain and the GnG were elongated, and the ganglia are larger than *in-situ*.

The interneurons types characterized by Ache and colleagues (2013), convey short-latency information about contact, joint position, joint movement, and joint velocity. Three are located in the Gnathal ganglion (GnG, formerly known as Suboesophageal Ganglion (SOG) Ito et al., 2014), and descend through the neck connective until, at least, the first thoracic ganglion (Ache et al., 2015). Among them, the ipsilateral and contralateral ON-Type velocity-sensitive neurons (acronyms iONv and cONv respectively) encode the joint angle velocity of the scape-pedicel joint of the antenna by increasing their firing rate with increasing velocity. Contrary to iONv and cONv, one DIN showed a decrease in firing rate as the joint velocity increased and was named the OFF-Type velocity-sensitive neuron (OFFv). Together, they form a pathway that connects the brain and the thoracic ganglia of the stick insect. The cONv neurons are particularly interesting. They are arranged as a bilateral pair, their large axons descend contralaterally (with respect to the soma) on both sides of the ventral nerve chord and through the whole nervous system, from the gnathal ganglion to the metathorax (Figure 4, Ache et al., 2015). They receive their synaptic input from the contralateral dendrites (anterior to the soma) and they arborize where other neurons related to the leg control are located (Berg et al. 2015;

Büschges 1989; Casagrand and Ritzmann 1992; Pollack et al. 1988; Ritzmann and Pollack 1990; Stubblefield and Comer 1989). The putative synaptic input to cONv is believed to be the antennal hair fields. Ache and colleagues (2015) showed that the hair field afferents overlap with the dendrites of cONv. However, no direct and functional connection between the hair field and cONv has ever been shown.

The latency of cONv from antennal deflection to the prothoracic ganglion has been estimated to be 15 ms which is well under the 40 ms required by the animal to retarget their leg after antennal contact (Schütz and Dürr 2011). This shows that cONv could be an important element involved in the rapid transfer of sensory information necessary to tell the leg where the obstacle is. More than just encoding the antennal joint-angle velocity, cONv also encodes substrate vibration triggered by single taps on the substrate (Figure 5B and Figure 6B). Contrary to the stimulation with unilateral ramp-and-hold stimulus, responses to substrate vibration are bilateral. This means that for each tap on the recording table, both cONv fire simultaneously. These two properties make cONv a bimodal neuron with asymmetrical responses.

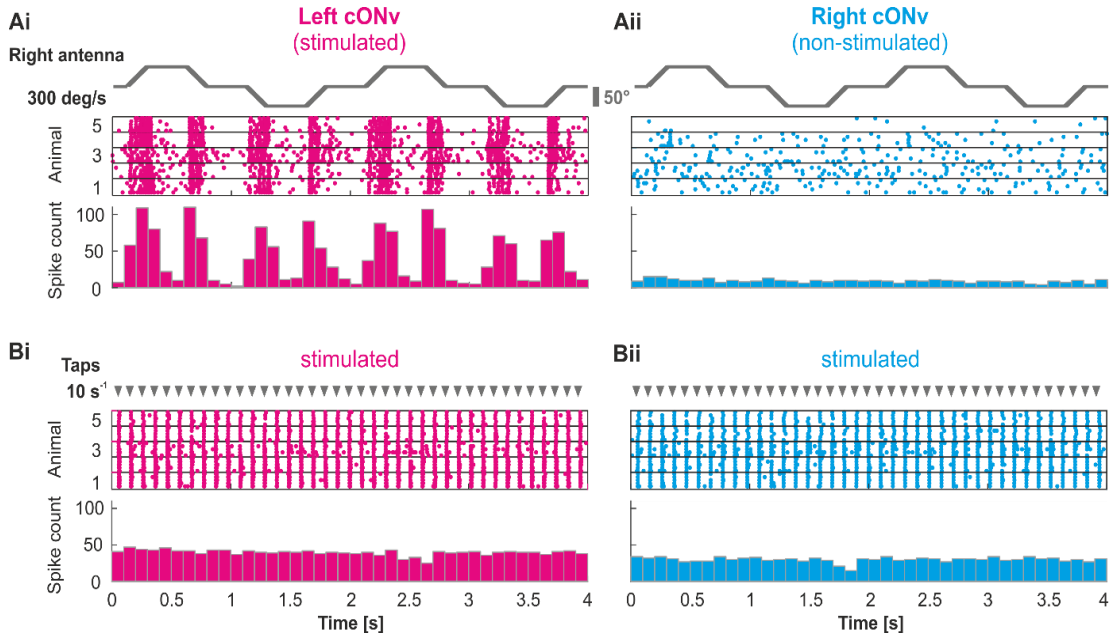


Figure 5: cONv responds reliably to antennal stimulation and substrate vibration.
The raster plots show the activity of the left (magenta) and right (cyan) cONv from five preparations with five trials per animal during passive movement of the right antenna using ramp-and-hold stimuli at 300 deg/s. The horizontal black lines in the raster plots delimit trials from different animals. Histograms show the total spike count per 100 ms bins. Left panels show recordings from the left cONv (Magenta, responds to movement of the right antenna); right panels show simultaneously recorded spikes of the right cONv (Cyan). B) As above, but during stimulation through substrate vibration with tapping frequencies of 10 s^{-1}. N = 5 animals with n = 5 trials per animal. Adapted from Lepreux et al., 2019.

Furthermore, cONv shows one particular advantage: its action potentials are of large amplitude in extracellular recordings. Indeed, Ache and colleagues (2015) carried out extracellular neck connective recordings with hook electrodes in parallel with intracellular recordings of cONv (Figure 6). They found that for every cONv spike in the intracellular recording there was a large amplitude spike in the extracellular recording (Figure 6). These spikes where easily and unambiguously identifiable as they were the largest among all units in the extracellular neck-connective recording.

Since cONv is a giant descending interneuron that encodes joint angle velocity, responds to substrate vibration and arborizes in the thoracic ganglia, it is a good candidate to be part of the pathway controlling the reach-to-grasp behaviour of the animal. Owing to its bimodality and its large action potential, it is possible to study both cONv neurons simultaneously and potentially understand their role under behaviourally relevant conditions.

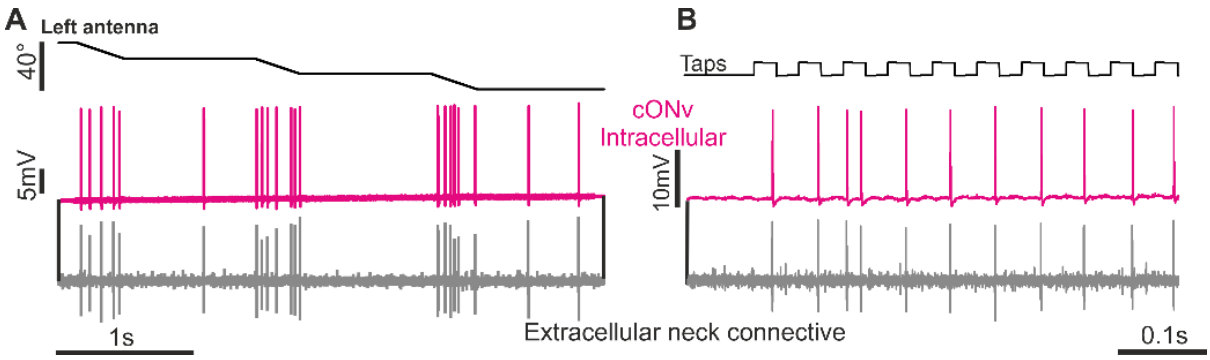


Figure 6: cONv can be identified in extracellular neck connective recordings.
A: Intracellular recording of cONv (magenta) during ramp-and-hold stimulus (top trace in black). Grey trace shows a simultaneous extracellular neck connective recording with hook electrodes. B: As in A but during substrate vibration (top squared signal). Spikes with the largest amplitude in the extracellular neck connectives recording match the cONv spikes recorded intracellularly. Modified after Ache et al., 2015.

Aim and objectives

Many animals rely on tactile cues to adapt their behaviour appropriately. The cues as well as the behavioural responses are well controlled and well known. However, between the stimulus and the behavioural response, a neuronal processing is necessary to convert the sensory activation into muscular actions. Which neurons and how they process the information to finally command the muscles to act as intended upon tactile stimulation is not well understood. In the stick insect, we have the opportunity to study an identified giant descending interneuron that possibly links the antennae to the thoracic networks which control the legs. Using this model, we may be able to identify strategies taking place in other organisms.

For instance, the capability of the system to interpret the message carried by the neurons can be an important factor in the outcome of a task. If the message is not clear and/or noisy, the system can fail to react properly. Neurons that show spiking activity when stimulated can also display spontaneous action potentials (i.e. not correlated to any obvious stimuli) which can perturb the message (Shadlen and Newsome 1994, 1995). A receiver must be able to differentiate actions potentials that occur spontaneously than those generated by a relevant stimulus. cONv encodes antennal joint-angle velocity and displays fluctuating spontaneous activity when not stimulated. Thus, I used cONv and its response properties to study how its spontaneous activity may affect its joint-angle velocity encoding (Chapter 2).

Moreover, most if not all, sensory systems show adaptation to the repetition of the same type of stimulus (Hewson and Tarrega 2017; Wark et al. 2007). Neurons respond less strongly the n^{th} time than to the first time of continuous stimulation. Adaptation plays an important role in the flexibility of neural coding (Weber and Fairhall 2019) and can be stimulus-specific (Ogawa and Oka 2015; Ringo 1996; Triblehorn and Schul 2013), be presented across stimuli (Mcburney 1972; Smith and McBurney 1969), and also be cross-modal (Wang et al. 2017). Since, cONv is a sensory neuron I investigated the adaptation to continuously moving antenna and analysed the effect of substrate vibration to study cross-modal adaptation (Chapter 3).

Finally, one of the most important aspect of the control of actions is dependent on the context. Is it relevant to react to this stimulus in this particular condition? For this, animals should be able to distinguish whether the activation of their sensory system is due to their

own action on the world or the world that is moving (Chagnaud et al. 2012; Huston and Jayaraman 2011). Is this movement self-triggered or generated by external forces? Since cONv encodes velocity of imposed antennal deflection, I asked if self-generated antennal movements trigger the same response as passive movements (Chapter 4).

In the context of tactually guided behaviour, understanding the behaviour of cONv and its role could allow us to decipher how stick insects control their body upon antennal contact. Knowing what exactly and how cONv is encoding tactile cues could then be transferred in an already existing neuronal model (Ache and Dürr 2015) and deepen the understanding of neuronal coding upon tactile stimulation and the role of other DINs in various species.

The aims of my thesis were:

- To determine whether the spontaneous activity is correlated in both cONv and whether it can be influenced by its activation through the known modalities the neurons respond to.
- To determine how stimulus encoding in cONv is possible under fluctuating spontaneous activity.
- To study how adaptation in cONv may impact the antennal velocity encoding.
- To assess whether the substrate vibration modality affects the encoding of antennal movement.
- How cONv encodes antennal movement generated by the animal vs-imposed stimulation.
- To propose a possible role for cONv in the stick insect.

References

Ache JM, Dürr V (2013) Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway. J Neurophysiol 110:2099–2112.

Ache JM, Dürr V (2015) A computational model of a descending mechanosensory pathway involved in active tactile sensing. PLoS Comput Biol 11.

Ache JM, Haupt SS, Dürr V (2015) A direct descending pathway informing locomotor networks about tactile sensor movement. J Neurosci 35:4081–4091.

Adamo SA, Hoy RR (1995) Agonistic behaviour in male and female field crickets, Gryllus bimaculatus, and how behavioural context influences its expression. Anim Behav 49:1491–1501.

Arkley K, Grant RA, Mitchinson B, Prescott TJ (2014) Strategy change in vibrissal active sensing during rat locomotion. Curr Biol 24:1507–1512.

Baba Y, Tsukada A, Comer CM (2010) Collision avoidance by running insects: Antennal guidance in cockroaches. J Exp Biol 213:2294–2302.

Bässler U (1983) Neural basis of elementary behavior in stick insects. Springer: Berlin, Heidelberg.

Bennett MVL (1971) Electric organs. Fish Physiol 5:347–491.

Berg EM, Hooper SL, Schmidt J, Büschges A (2015) A Leg-local neural mechanism mediates the decision to search in stick insects. Curr Biol 25:2012–2017.

Borror, D. J. ; Triplehorn, C. A. ; Johnson NF (1989) An introduction to the study of insects.

Borst A (2009) Drosophila's view on insect vision. Curr Biol 19:R36–R47.

Borst A, Heisenberg M (1982) Osmotropotaxis in Drosophila melanogaster. J Comp Physiol □ A 147:479–484.

Brecht M, Preilowski B, Merzenich MM (1997) Functional architecture of the mystacial vibrissae. Behav Brain Res 84:81–97.

Büschges A (1989) Processing of sensory input from the femoral chordotonal organ by spiking interneurones of stick insects. J Exp Biol 144:81–111.

Camhi JM, Johnson EN (1999) High-frequency steering maneuvers mediated by tactile cues: antennal wall-following in the cockroach. J Exp Biol:631–643.

Casagrand JL, Ritzmann RE (1992) Evidence that synaptic transmission between giant interneurons and identified thoracic interneurons in the cockroach is cholinergic. J Neurobiol 23:627–643.

Chacron M (2007) Electrolocation. Scholarpedia, 2(1):1411

Chagnaud BP, Simmers J, Straka H (2012) Predictability of visual perturbation during locomotion: implications for corrective efference copy signaling. Biol Cybern 106:669–679.

Comer CM, Baba Y (2011) Active touch in orthopteroid insects: Behaviours, multisensory substrates and evolution. Philos Trans R Soc B Biol Sci 366:3006–3015.

Comer CM, Parks L, Halvorsen MB, Breese-Terteling A (2003) The antennal system and cockroach evasive behavior. II. Stimulus identification and localization are separable antennal functions. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 189:97–103.

Cruse H (1976) The control of body position in the stick insect (Carausius morosus), when walking over uneven surfaces. Biol Cybern 24:25–33.

Cruse H (1990) What mechanisms coordinate leg movement in walking arthropods? Trends Neurosci 13:15–21.

Cruse H, Dürr V, Schilling M, Schmitz J (2009) Principles of insect locomotion. Cogn Syst Monogr 1:43–96.

Cruse H, Kindermann T, Schumm M, Dean J, Schmitz J (1998) Walknet - A biologically inspired network to control six-legged walking. Neural Networks 11:1435–1447.

Dallmann CJ, Hoinville T, Dürr V, Schmitz J (2017) A load-based mechanism for inter-leg coordination in insects. Proc R Soc B Biol Sci 284.

Delcomyn F (1971) The locomotion of the cockroach periplaneta americana. J Exp Biol 54:443–452.

Delcomyn F (1999) Walking robots and the central and peripheral control of locomotion in insects. Auton Robots 7:259–270.

Diamond ME, Arabzadeh E (2013) Whisker sensory system - From receptor to decision. Prog Neurobiol 103:28–40.

Dieudonné A, Daniel TL, Sane SP (2014) Encoding properties of the mechanosensory neurons in the Johnston's organ of the hawk moth, Manduca sexta. J Exp Biol 217:3045–3056.

Dirks JH, Dürr V (2011) Biomechanics of the stick insect antenna: Damping properties and structural correlates of the cuticle. J Mech Behav Biomed Mater 4:2031–2042.

Dreller C, Kirchner WH (1993) Hearing in honeybees: localization of the auditory sense organ. J Comp Physiol A 173:275–279.

Dürr V, König Y, Kittmann R (2001) The antennal motor system of the stick insect Carausius morosus: Anatomy and antennal movement pattern during walking. J Comp Physiol - A Sensory, Neural, Behav Physiol 187:131–144.

Dürr V, Schilling M (2018) Transfer of spatial contact information among limbs and the notion of peripersonal space in insects. Front Comput Neurosci 12:101.

Dürr V, Theunissen LM, Dallmann CJ, Hoinville T, Schmitz J (2018) Motor flexibility in insects: adaptive coordination of limbs in locomotion and near-range exploration. Behav Ecol Sociobiol 72:15.

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv In Insect Phys 27.

Gadenne C, Barrozo RB, Anton S (2016) Plasticity in insect olfaction: To smell or not to smell? Annu Rev Entomol 61:317–333.

Gebhardt M, Honegger HW (2001) Physiological characterisation of antennal mechanosensory descending interneurons in an insect (Gryllus bimaculatus, Gryllus campestris) brain. J Exp Biol 204:2265–2275.

Gehring WJ (2014) The evolution of vision. Wiley Interdiscip Rev Dev Biol 3:1–40.

Gewecke M (1974) The antennae of insects as air-current sense organs and their relationship to the control of flight. In: Experimental Analysis of Insect Behaviour, pp 100–113. Springer Berlin Heidelberg.

Graham D (1985) Pattern and control of walking in insects. Adv In Insect Phys 18:31–140.

Harley CM, English BA, Ritzmann RE (2009) Characterization of obstacle negotiation behaviors in the cockroach, Blaberus discoidalis. J Exp Biol 212:1463–1476.

Heinzel HG, Gewecke M (1979) Directional sensitivity of the antennal campaniform sensilla in locusts. Naturwissenschaften 66:212–213.

Hewson L, Tarrega A (2017) Sensory adaptation. Time-Dependent Meas Percept Sens Eval:67–87.

Hicks JB (1857) On a new structure in the antennae of insects. Trans Linn Soc London 22:147–154.

Hofmann HA, Schildberger K (2001) Assessment of strength and willingness to fight during aggressive encounters in crickets. Anim Behav 62:337–348.

Huston SJ, Jayaraman V (2011) Studying sensorimotor integration in insects. Curr Opin Neurobiol 21:527–534.

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S, Heisenberg M, Homberg U, Jenett A, Keshishian H, Restifo LL, Rössler W, Simpson JH, Strausfeld NJ, Strauss R, Vosshall LB (2014) A systematic nomenclature for the insect brain. Neuron 81:755–765.

Jones G (2005) Echolocation. Curr Biol 15:R484-8.

Jones G, Teeling EC (2006) The evolution of echolocation in bats. Trends Ecol Evol 21:149–156.

Kamikouchi A, Inagaki HK, Effertz T, Hendrich O, Fiala A, Göpfert MC, Ito K (2009) The neural basis of Drosophila gravity-sensing and hearing. Nature 458:165–171.

Kleinfeld D, Ahissar E, Diamond ME (2006) Active sensation: insights from the rodent vibrissa sensorimotor system. Curr Opin Neurobiol 16:435–444.

Koren V, Denève S (2017) Computational account of spontaneous activity as a signature of predictive coding Blackwell KT, ed. PLOS Comput Biol 13:e1005355.

Krause A, Dürr V (2012) Active tactile sampling by an insect in a step-climbing paradigm. Front Behav Neurosci 6:30.

Krause AF, Winkler A, Dürr V (2013) Central drive and proprioceptive control of antennal movements in the walking stick insect. J Physiol Paris 107:116–129.

Laurent G (1991) Sensory control of locomotion in insects. Curr Opin Neurobiol 1:601—604.

Lederman SJ, Klatzky RL (1993) Extracting object properties through haptic exploration. Acta Psychol (Amst) 84:29–40.

Lepreux G, Haupt SS, Dürr V (2019) Bimodal modulation of background activity in an identified descending interneuron. J Neurophysiol 122:2316–2330.

Martin H (1965) Osmotropotaxis in the honey-bee. Nature 208:59–63.

Mcburney DH (1972) Gustatory cross adaptation between sweet-tasting compounds. Percept Psychophys 11:225–227.

Mitchinson B, Martin CJ, Grant RA, Prescott TJ (2007) Feedback control in active sensing: Rat exploratory whisking is modulated by environmental contact. Proc R Soc B Biol Sci 274:1035–1041.

Mongeau J-M, Demir A, Lee J, Cowan NJ, Full RJ, Full RJ (2013) Locomotion- and mechanics-mediated tactile sensing: antenna reconfiguration simplifies control during high-speed navigation in cockroaches. J Exp Biol 216:4530–4541.

Mujagic S, Krause AF, Dürr V (2007) Slanted joint axes of the stick insect antenna: an adaptation to tactile acuity. Naturwissenschaften 94:313–318.

Murrain M, Ritzmann RE (1988) Analysis of proprioceptive inputs to DPG interneurons in the cockroach. J Neurobiol 19:552–570.

Neimark MA, Andermann ML, Hopfield JJ, Moore CI (2003) Vibrissa resonance as a transduction mechanism for tactile encoding. J Neurosci:6499–6509.

Nelson ME (2011) Electric fish. Curr Biol 21:R528–R529.

Ogawa H, Oka K (2015) Direction-specific adaptation in neuronal and behavioral responses of an insect mechanosensory system. J Neurosci 35:11644–11655.

Okada J, Akamine S (2012) Behavioral response to antennal tactile stimulation in the field cricket Gryllus bimaculatus. J Comp Physiol A 198:557–565.

Okada J, Toh Y (2000) The role of antennal hair plates in object-guided tactile orientation of the cockroach (Periplaneta americana). J Comp Physiol A Sensory, Neural, Behav Physiol 186:849–857.

Okada J, Toh Y (2001) Peripheral representation of antennal orientation by the scapal hair plate of the cockroach Periplaneta americana. J Exp Biol 204:4301–4309.

Pellegrino R, Sinding C, de Wijk RA, Hummel T (2017) Habituation and adaptation to odors in humans. Physiol Behav 177:13–19.

Petersen CCH (2007) The functional organization of the barrel cortex. Neuron 56:339–355.

Petersen CCH (2019) Sensorimotor processing in the rodent barrel cortex. Nat Rev Neurosci 20:533–546.

Pollack AJ, Ritzmann RE, Westin J (1988) Activation of DUM cell interneurons by ventral giant interneurons in the cockroach,periplaneta americana. J Neurobiol 19:489–497.

Prescott TJ, Diamond ME, Wing AM (2011) Active touch sensing. Philos Trans R Soc Lond B Biol Sci 366:2989–2995.

Pringle JWS (1938) Proprioception in insects II. The action of the campaniform Sensilla on the legs. J Exp Biol 15:114–131.

Ringo JL (1996) Stimulus specific adaptation in inferior temporal and medial temporal cortex of the monkey. Behav Brain Res 76:191–197.

Ritzmann RE, Pollack AJ (1986) Identification of thoracic interneurons that mediate giant interneuron-to-motor pathways in the cockroach. J Comp Physiol A 159:639–654.

Ritzmann RE, Pollack AJ (1990) Parallel motor pathways from thoracic interneurons of the ventral giant interneurons system of the cockroach,Periplaneta americana. J Neurobiol 21:1219–1235.

Sane SP, Dieudonné A, Willis MA, Daniel TL (2007) Antennal mechanosensors mediate flight control in moths. Science (80-) 315:863–866.

Schilling M, Hoinville T, Schmitz J, Cruse H (2013) Walknet, a bio-inspired controller for hexapod walking. Biol Cybern 107:397–419.

Schöneich S, Schildberger K, Stevenson PA (2011) Neuronal organization of a fast-mediating cephalothoracic pathway for antennal-tactile information in the cricket (Gryllus bimaculatus DeGeer). J Comp Neurol 519:1677–1690.

Schütz C, Dürr V (2011) Active tactile exploration for adaptive locomotion in the stick insect. Phil Trans R Soc B 366:2996–3005.

Shadlen MN, Newsome WT (1994) Noise, neural codes and cortical organization. Curr Opin Neurobiol 4:569–579.

Shadlen MN, Newsome WT (1995) Is there a signal in the noise? Curr Opin Neurobiol 5:248–250.

Simmons JA, Kick SA, Lawrence BD (1984) Echolocation and hearing in the mouse-tailed bat, Rhinopoma hardwickei: acoustic evolution of echolocation in bats. J Comp Physiol A 154:347–356.

Simmons JA, Stein RA (1980) Acoustic Imaging in Bat Sonar: Echolocation Signals and the Evolution of Echolocation. J Comp Physiol A 135:61–84.

Skelhorn J (2018) Avoiding death by feigning death. Curr Biol 28:R1135–R1136.

Smith D V., McBurney DH (1969) Gustatory cross-adaptation: Does a single mechanism code the salty taste? J Exp Psychol 80:101–105.

Spinola SM, Chapman KM (1975) Proprioceptive indentation of the campaniform sensilla of cockroach legs. J Comp Physiol A 96:257–272.

Staudacher EM, Gebhardt M, Dürr V (2005) Antennal movements and mechanoreception: neurobiology of active tactile sensors. Adv In Insect Phys 32:49–205.

Stevenson PA, Schildberger K (2013) Mechanisms of experience dependent control of aggression in crickets. Curr Opin Neurobiol 23:318–323.

Stubblefield GT, Comer CM (1989) Organization of giant interneuron projections in thoracic ganglia of the cockroach Periplaneta americana. J Morphol 200:199–213.

Triblehorn JD, Schul J (2013) Dendritic mechanisms contribute to stimulus-specific adaptation in an insect neuron. J Neurophysiol 110:2217–2226.

von der Emde G, Amey M, Engelmann J, Fetz S, Folde C, Hollmann M, Metzen M, Pusch R (2008) Active electrolocation in Gnathonemus petersii: Behaviour, sensory performance, and receptor systems. J Physiol Paris 102:279–290.

Wang X, Guo X, Chen L, Liu Y, Goldberg ME, Xu H (2017) Auditory to visual cross-modal adaptation for emotion: Psychophysical and neural correlates. Cereb Cortex 27:1337–1346.

Wark B, Lundstrom BN, Fairhall A, Mombaerts P, Zador T (2007) Sensory adaptation. Curr Opin Neurobiol 17:423–429.

Warrant EJ (2017) The remarkable visual capacities of nocturnal insects: vision at the limits with small eyes and tiny brains. Philos Trans R Soc London Ser B, Biol Sci 372.

Weber AI, Fairhall AL (2019) The role of adaptation in neural coding. Curr Opin Neurobiol 58:135–140.

Wilson DM (1966) Insect walking. Annu Rev Entomol 11:103–122.

Wright GA (2015) Olfaction: Smells like fly food. Curr Biol 25:R144–R146.

Ye S, Comer CM (1996) Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, Periplaneta americana. J Neurosci 16:5844–5853.

Zill SN, Moran DT (1981) The exoskeleton and insect proprioception. I. Responses of tibial campaniform sensilla to external and muscle-generated forces in the american cockroach, Periplaneta americana. J Exp Biol 91:1–24.

Zill SN, Schmitz J, Büschges A (2004) Load sensing and control of posture and locomotion. Arthropod Struct Dev 33:273–286.

Zill SN, Schmitz J, Chaudhry S, Büschges A (2012) Force encoding in stick insect legs delineates a reference frame for motor control. J Neurophysiol 108:1453–1472.

Chapter 2 [2]

Bimodal modulation of background activity in an identified descending interneuron

Abstract

In the absence of any obvious input, sensory neurons and interneurons can display resting or spontaneous activity. This is often regarded as noise and removed through trial averaging, although hit may reflect history-dependent modulation of tuning or fidelity and, thus, be of functional relevance to downstream interneurons. We investigated the history dependence of spontaneous activity in a pair of identified, bimodal descending interneurons of the stick insect, called contralateral ON-type velocity-sensitive interneurons (cONv). The bilateral pair of cONv conveys antennal mechanosensory information to the thoracic ganglia, where it arborizes in regions containing locomotor networks. Each cONv encodes the movement velocity of the contralateral antenna, but also substrate vibration as induced by discrete tapping events. Moreover, cONv display highly fluctuating spontaneous activity that can reach rates similar to those during antennal movement at moderate velocities. Hence, cONv offers a unique opportunity to study history-dependent effects on spontaneous activity and, thus, encoding fidelity in two modalities. In this work, we studied unimodal and cross-modal effects as well as unilateral and bilateral effects, using bilateral recordings of both cONv neurons, while moving one antenna and/or delivering taps to induce substrate vibration. Tapping could reduce spontaneous activity of both neurons, whereas antennal movement reduced spontaneous activity of the contralateral cONv neuron only. Combination of both modalities showed a cooperative effect for some parameter constellations, suggesting bimodal enhancement. Since both stimulus modalities could cause a reduction of spontaneous activity at stimulus intensities occurring during natural locomotion, we conclude that this should enhance neuronal response fidelity during locomotion.

[2] This chapter was published as: Bimodal modulation of background activity in an identified descending interneuron. Gaetan Lepreux; Stephan, S, Haupt; Volker Dürr, *J Neurophysiol* , 2019. doi:10.1152/jn.00864.2018.

New & Noteworthy**:** The spontaneous activity in a pair of identified, descending insect interneurons is reduced depending on stimulus history. At rest, spontaneous activity levels are correlated in both interneurons, indicating a common drive from background activity. Whereas taps on the substrate affect both interneurons, antennal movement affects the contralateral interneuron only. Cross-modal interaction occurs, too. Since spontaneous activity is reduced at stimulus intensities encountered during natural locomotion, the mechanism could enhance neuronal response fidelity during locomotion.

Introduction

Neuronal activity that is not correlated with sensory stimulation, motor actions, or other events is often regarded as a purely additive, signal-confounding activity component. Accordingly, removing this confounding component by trial averaging is often considered to reveal the properties of stimulus encoding. Indeed, Arieli et al. (1996) found that subtracting spontaneous activity patterns before single-trial stimulation resulted in responses approximating the trial average. More generally, spontaneous activity is usually considered as that part of the recorded neuronal activity that could not be interpreted in the scope of an experiment. As yet, given that spontaneous activity necessarily affects synaptic drive to downstream neurons, an important first step to understanding its potential function is to identify whether and how it may be modulated in a predictable manner. Indeed, it is known that spontaneous activity can be modulated pharmacologically or environmentally (Zhantiev et al. 2006), and several recent studies have tried to resolve its potential function. One suggestion is that spontaneous activity has a marker function related to the state of the animal and/or as a message (Koren and Denève 2017). Another is that spontaneous activity may extend the range of neuronal coding (Tantirigama et al. 2017). Moreover, neurons were shown to "replay spontaneously" activity patterns equivalent to encoded external stimuli, which is thought to be a key mechanism of memory consolidation (Carr et al. 2011; Karlsson and Frank 2009; Skaggs and McNaughton 1996). Thus, spontaneous activity may have various functions of its own, although it may be difficult to reach conclusions about these functions in simple neuronal systems. In the present study, we investigate factors of history-dependent modulation of spontaneous activity in an identified descending interneuron that is thought to be involved in the rapid transfer of sensory information to the thoracic ganglia where locomotor networks reside. Behavioural analysis of walking and climbing stick insects showed that these animals respond to tactile contact of an obstacle with short latency reaching or retargeting movements of a front leg (Schütz and Dürr 2011). Several descending interneurons (DINs) have been recorded in the neck connectives of stick insect that convey information about antennal posture, movement, and contact to the thoracic ganglia (Ache and Dürr 2013). Among these, three velocity-sensitive DINs were described in more detail (Ache et al. 2015), including the bilateral pair of contralateral ON- type velocity-sensitive neurons (cONv; Figure 7F). cONv responds to two kinds of mechanosensory stimulation:

substrate vibration (Figure 7, D and E) and movement of the contralateral antenna (Figure 7, A and B). The movement-induced response of cONv is velocity dependent and quickly subsides as movement is interrupted. During a ramp-and-hold deflection of the antenna (e.g., Figure 7A), cONv may reach mean spike rates beyond 100 s^{-1} during the ramps, but not during the hold phases. The vibration-induced response was described as a reliable spike response to mechanical impact events remote to the body, e.g., to gentle taps on the setup (Figure 7, D and E). The frequency tuning of this input has not been determined, but it was shown to follow substrate tapping frequencies reliably up to 20 s^{-1} with at least one spike per tap (Ache et al. 2015). Other sensory-evoked responses of cONv have not been identified. However, the neuron displays strongly fluctuating spontaneous activity in the absence of sensory stimulation (Figure 7C) that may reach instantaneous spike rates similar to those during slow antennal movement (Figure 7, B and C). As a consequence, this spontaneous activity must reduce tuning fidelity of movement-induced response. In the behavioural context of tactually induced reaching movements during locomotion, a major question concerns how this fluctuating spontaneous activity is affected by proprioception of antennal movement and exteroception of substrate vibration, both of which occur during locomotion.

Two properties of cONv render this pair of descending interneurons particularly suited for the investigation of history-dependent modulation of spontaneous activity. First, the spikes of cONv can be identified reliably from extracellular neck connective recordings, because it has the largest spike amplitude of all axons in the connective (Figure 7.). Second, there is one cONv interneuron on each side of the ventral nerve cord (Figure 7F): whereas the two of them respond jointly to a substrate vibration stimulus, each one of them responds separately to motion of the respective contralateral antenna. As a consequence, bilateral recording of both cONv interneurons allows controlled experimental separation of unimodal vs. bimodal effects, as well as unilateral (asymmetrical) vs. bilateral (symmetrical) effects, on spontaneous activity.

In this study we exploited this unique situation to test different stimulus combinations in behaviourally relevant stim-ulus ranges. In particular, we tested 1) to what extent spontaneous activity levels are correlated in both cONv, 2) to what extent sensory stimulation history affects spontaneous activity, and 3) whether (or not) the effect of bimodal stimulation equals the summed effects of unimodal stimulation.

To do so, we distinguished two activity states of cONv: a state of spontaneous activity in the absence of a stimulus and a state of stimulus-induced activity (Figure 7G). The state of stimulus-induced activity is driven by either 1) antennal movement (see shaded areas below the ramps of the ramp-and-hold stimulus in Figure 7, with corresponding dot raster plots in Figure 8) or 2) substrate vibration. In the first case, the stimulus-induced response consists of several spikes per ramps; in the second case, it consists of one or two spikes per tap (see Figure 7, D and E, Figure 8, D and E, and Figure 9). The state of spontaneous activity comprises randomly fluctuating responses during either 1) long episodes between stimuli, when the neuron can be considered to be at rest (resting activity), or 2) short periods between stimuli, when there is no evidence for stimulus- induced drive to the neuron (intermittent spontaneous activity, Figure 7G). In the case of cONv, peristimulus time histograms (PSTH) reveal that intermittent spontaneous activity occurs during the hold phases of the ramp-and-hold stimulus (Figure 8, Ai and Bi) and during a brief "sensory response window" between subsequent taps (Figure 7D; for definition of the sensory response window, see material and methods, Figure 9). Thus, we use the term "spontaneous activity" as any random spike activity for which repeated responses to identical stimuli do not show reliable enhancement of the mean spike rate.

Irrespective of what actually causes spontaneous activity in cONv neurons, we assumed that intrinsic factors as well as background activity of the nervous system may have contributed (Figure 7G). If so, we reasoned that correlation of spontaneous activity between the two cONv neurons per animal should be caused by background activity. Moreover, we differentiate "resting activity" from "intermittent spontaneous activity" to estimate history-dependent effects on spontaneous activity by relating the two. To do so, we used bilateral extracellular hook electrode recordings (Figure 7F) in quiescent, immobilized animals and determined the spontaneous activity in both cONv interneurons per animal before (resting), during (intermittent), and after (resting) various combinations of antennal movement and substrate vibration stimuli. With regard to the questions stated above, we show that 1) ~80% of the variance in resting activity can be explained through a linear bilateral correlation, 2) intermittent spontaneous activity is reduced during episodes of sensory stimulation of both modalities tested, although for limited parameter ranges, and 3) the effect of stimulus history may be stronger for bimodal stimulation than expected for stochastically independent sources.

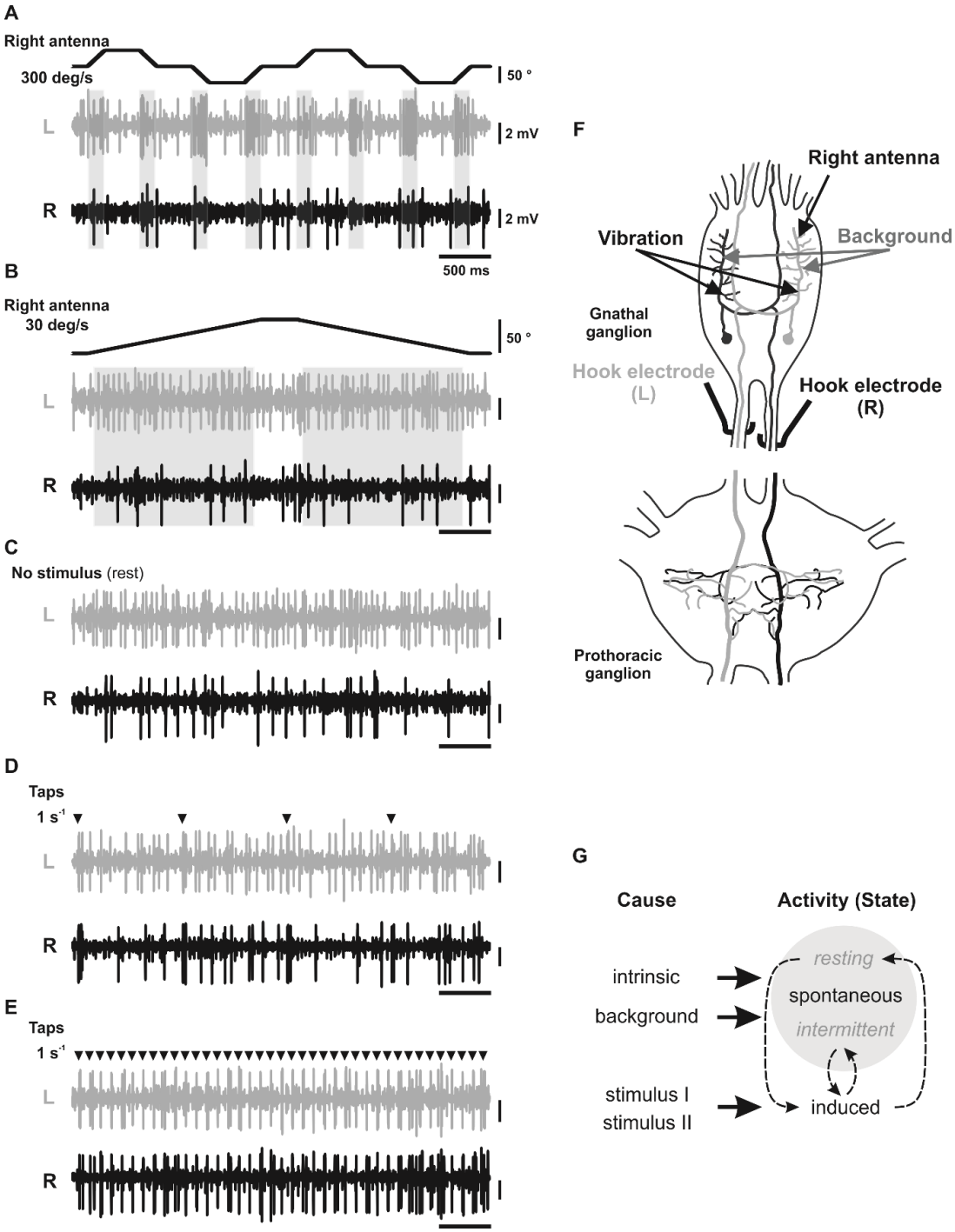


Figure 7: Examples of bilateral extracellular neck-connective recordings at rest and during stimulation with two modalities.

Black traces show contralateral ON-type velocity-sensitive interneuron (cONv) spike activity in the right (R) neck connective, whereas gray traces show simultaneous recordings of the left (L) neck connective. Spikes with the biggest amplitudes in the connectives belong to the corresponding cONv. A and B: extracellular bilateral neck connective recordings during passive deflection of the right antenna at a velocity of 300°/s (A) or 30°/s (B). Note that only the cONv in the left connective responded to the antennal movements (see gray-shaded areas below ramps). C: recording of the same preparation, but in absence of a stimulus. Both cONv neurons display randomly fluctuating spontaneous activity (resting activity). D and E: same preparation, but during stimulation through substrate vibration, i.e., by tapping onto the setup at 1 (D) or 10 s^{-1}(E). Note that both cONv neurons respond to each tap (arrowheads) due to substrate vibration (compare with Figure 8, D and E). (continue next page).

***Figure 7**: (caption resume). F: schematic of the gnathal ganglion (GnG; top) and prothoracic ganglion (bottom) indicating the branching pattern of cONv and its known inputs (arrows). Only the input of the right antenna (activating the left cONv) is indicated. The cONv soma is located in the GnG, and the axons descend contralaterally along the neck connective where hook electrodes were recording. G: schematic view of the possible causes and activity states of cONv. Throughout this study we distinguish two kinds of randomly fluctuating spontaneous activity: resting activity before and after stimulus presentation, and intermittent spontaneous activity during brief periods between stimulus-induced synaptic drive to the neuron. Stimulus I and stimulus II refer to the vibration and antennal movement stimuli used in this study.*

Methods

Animals

Adult female stick insects (*Carausius morosus*), bred at Bielefeld University were used. All legs were removed, and the animals were fixed ventral side down on a balsa wood platform using dental glue (Protemp; 3M EPSE, Neuss, Germany). The body cuticle was cut open along the dorsal midline axis, from the neck to the metanotum, and kept open with 0.2-mm steel minutien pins. To access the neck connectives, the head capsule was opened along its posterior third, and the underlying muscles were ablated while the gut was kept intact but lifted out and fixed aside of the body with minutien pins. Special care was taken to remove as few tracheae as possible and not to sever any nerves of the gnathal ganglion (GnG; formerly called the subesophageal ganglion; Ito et al. 2014) or prothoracic ganglion. Finally, the body was filled with stick insect saline solution (180 mM NaCl, 4 mM KCl, 5 mM CaCl2·2H2O, 1 mM MgCl2·6H2O, 10 mM HEPES, and 10 mM sucrose).

Stimulation and recording

Throughout this study, two types of stimuli were used, corresponding to the known sensory input modalities that cONv neurons respond to.

Substrate vibration. To generate substrate vibration, a solenoid (model ITS-LZ 1335; Intertec) was placed directly on the recording table, 40 cm away from the animal. The solenoid delivered taps on the recording table at frequencies of 1, 3, 6, or 10 s^{-1} for a duration of 20 s. The tapping frequency and duration were controlled through the digital output of a CED Micro1401 laboratory interface (Cambridge Electronic Design, Cambridge, UK) and triggered in randomized order via a custom-written script.

Scape-pedicel joint deflection. For details about the passive movement of the scape-pedicel (SP) joint, see Ache and Dürr (2013). In brief, we simulated antennal movement

around the SP joint of the antenna, deflecting the flagellum by rotation about a slanted joint axis (Mujagic et al. 2007). For contactless deflection of the antenna, a minutien pin was inserted into the flagellum previously cut around the 4th and the 6th proximal annulus and moved by a magnet mounted on a motor potentiometer (12-V direct current; Inelta, Munich, Ger-many). The position of the antenna was altered in a ramp-and-hold sequence with 50° dorsal and 50° ventral deflection from the resting posture (e.g., see .Figure 7A and Figure 8A). The velocity and angular range of the movement, as well as the number of ramp-and-hold phases, were controlled via the CED Micro1401 through a custom-written script. The angular range of stimulation for the SP joint was 100°, corresponding to the range of active antennal movement about the SP joint in the *C. morosus* (Mujagic et al. 2007). The ramp-and-hold stimulus consisted of 20 staircases, where each staircase was a sequence of displacement from rest to the dorsal limit, from the dorsal limit back to rest, then from rest to the ventral limit, and from the ventral limit back to rest (e.g., 2 staircases in Figure 7A and Figure 8A). The hold phases (no movement) always had a duration of 335 ms, whereas the ramps (movement) had a duration of 1.667 and 0.167 s for velocities of 30 and 300°/s, respectively.

Extracellular recording. Tungsten hook electrodes (Figure 7F) for en passant whole nerve connective recordings were used (see Schmitz et al. 1988), and voltages were amplified 1,000 times (MA-101, MA-102; Electronics Workshop, Dept. of Animal Physiology, University of Cologne, Germany). Reference electrodes were placed in the saline solution, posterior to the recording sites. The neck connectives were recorded at a sampling rate of 10 kHz with an analog-to-digital converter (CED Micro1401) and Spike2 software (both from Cam-bridge Electronic Design, Cambridge, UK).

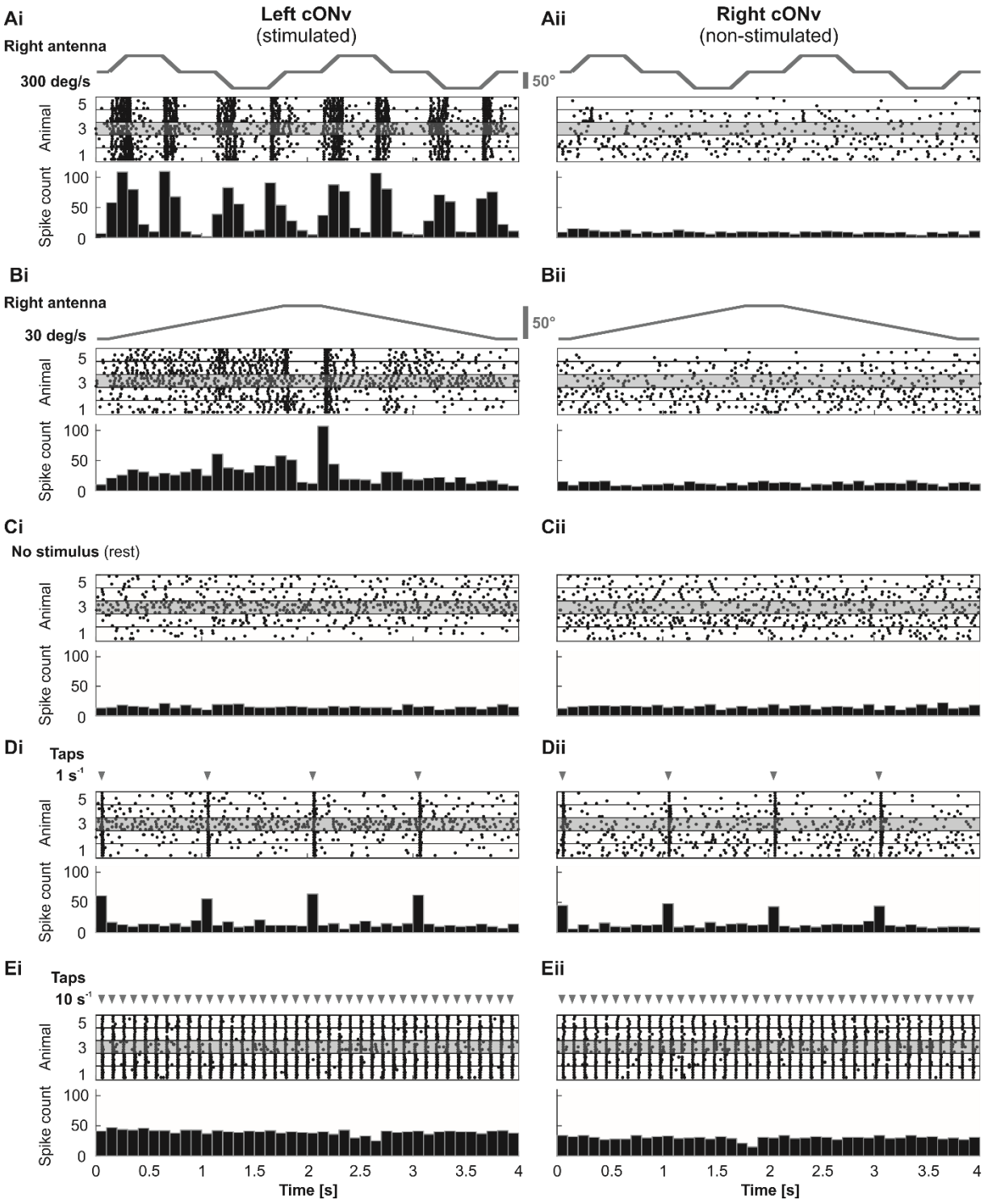


Figure 8: cONv shows intermittent spontaneous activity between sensory-induced activity.

Other than spontaneous activity, stimulus-induced activity is characterized by reliably enhanced activity during repeated trials. A and B: raster plots show the activity of the left (i) and right cONv (ii) from 5 preparations with 5 trials per animal during passive antennal movement stimuli with 300 (A) or 30°/s ramps (B). Horizontal black lines in raster plots delimit trials from different animals. Shaded horizontal area shows the animal used for Figure 7; N = 5 animals with n = 5 trials per animal. Histograms show the total spike count per 100-ms bins. Ai and Bi show recordings from the left cONv (responds to movement of the right antenna); Aii and Bii show simultaneously recorded spikes of the right cONv. C: same preparations, but in absence of stimulus. D and E: same preparations, but during stimulation through substrate vibration with tapping frequencies of 1 (D) or 10s^{-1} (E).

Data analysis and statistics

***Spike detection*.** As described by Ache et al. (2015), cONv can be identified in extracellular hook electrodes recordings according to three criteria: First, cONv displays the largest amplitude spikes in quiescent animals (Figure 7). Second, cONv respond to both substrate vibration (Figure 7, D and E, and Figure 8, D and E) and passive deflection of the flagellum of the contralateral antenna (left connective; i.e., left cONv in Figure 7, A and B, and Figure 8, Ai and Bi). Finally, cONv neurons do not respond to the deflection of the ipsilateral antenna (right connective, i.e., right cONv in Figure 7 A and B, and Figure 8, Aii and Bii). In our recordings, a detection threshold was set offline in Spike2 such that only the largest unit was selected. It was then verified that both other criteria were met. This was done for both connectives, because each hemiganglion of the GnG contains one cONv soma (see Figure 7F). Since only one antenna was moved throughout the main experiment, only one of the two recorded cONv interneurons was stimulated by antennal movement (compare Figure 8 Ai and Bi, with Figure 8Aii and Bii), whereas both interneurons were stimulated by substrate vibration (Figure 7F; compare Figure 8Di and Ei, with Figure 8Dii and Eii). Data were then saved as MATLAB files and analyzed with custom-written MATLAB scripts (version 9.2; MathWorks R2017a).

Substrate vibration. Intermittent spontaneous activity of cONv during substrate vibration was calculated outside the sensory response window that was defined as the window with reliable stimulus-induced activity per tap. For this, we created peristimulus time histograms (PSTH) and raster plots for all animals and tapping frequencies (Figure 9; note that raster plots show only a subset of all responses). The mean cumulative sum of spikes per 1-ms bin and the PSTH for a window of +/-50 ms before and after the onset of each tap (Figure 9) show that cONv responded with a latency of 10–25 ms to individual taps, irrespective of tapping frequency. All cONv spikes falling in the sensory response window from onset of each tap to 30 ms post tap (Figure 9, shaded area) were considered to be stimulus induced and were ignored for the calculation of intermittent spontaneous activity during stimulation (Levakova et al. 2015). The median number of spikes per tap within the stimulus response window was one. In 23.55% of all sensory response windows, more than one spike was detected. The likelihood of a false positive in a randomly placed window of the same size as the stimulus response window was 4.42%. Resting activity before and after substrate

vibration was calculated as the mean spike rate during 20s intervals before and after each vibration stimulus.

Antennal movement. The intermittent spontaneous activity during ramp-and-hold changes in antennal position was calculated as the mean spike rate during the hold phases only. These occurred at three different positions, i.e., at rest (0°), at the dorsal limit (+50°), and at the ventral limit (-50°). A total of 80 hold phases (20 staircases comprising 4 hold phases each) were considered per trial. Due to the response latency of cONv, stimulus-induced activity outlasted the ramp phases. To exclude this delayed activity, all spikes that occurred during the first 150 ms of the hold phases were excluded. We then counted the spikes occurring in the next 100 ms, amounting to an 8-s interval (80 * 100 ms) per trial.

Resting activity before and after stimulation was calculated by considering 80 sham hold phases of 100-ms duration. The sham hold phases were separated by 200 ms such that we could sample the spontaneous activity before and after antennal stimulation over a longer period. Although always only one of the two cONv responded to antennal movement, we ran the same analysis for both the stimulated and the non-stimulated cONv.

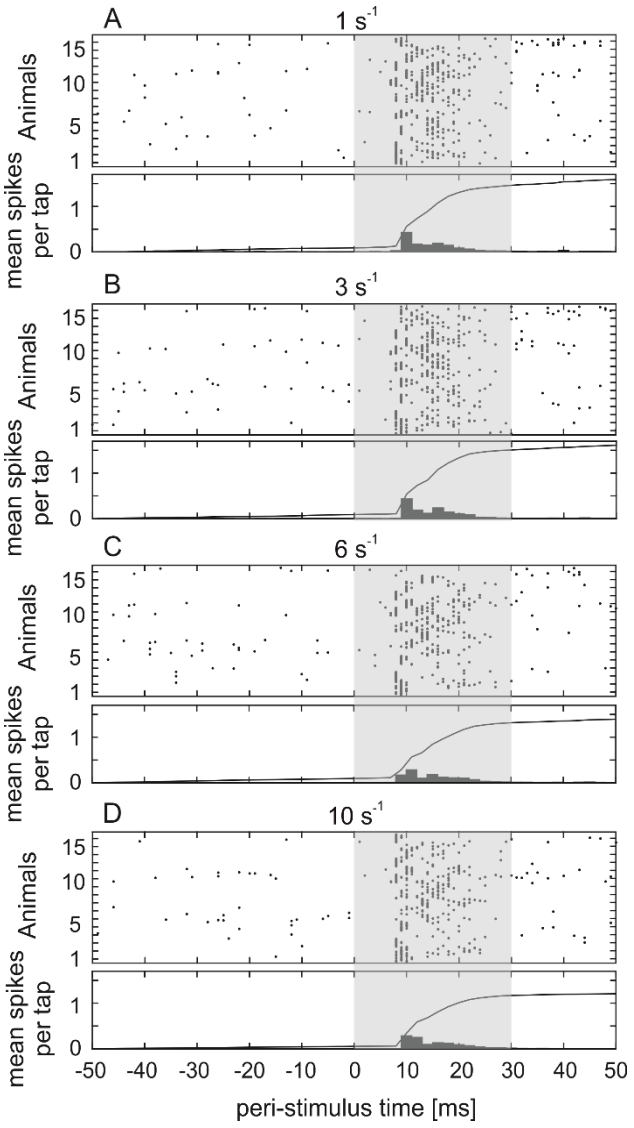


Figure 9: cONv responds reliably to individual taps on the substrate with a delay of 10 to 25 ms.
Raster plots and peristimulus time histograms of cONv activity aligned to the onset of individual taps (t = 0 s) for all 4 tapping frequencies: 1 (A), 3 (B), 6 (C), and 10 s^{-1} (D). Raster plots show only the first 20 taps per animal, but histograms include all data from N = 16 animals. Black line shows the mean cumulative sum of cONv spikes per tap occurring within 50 ms before and after a tap. Histograms show mean spike number per 1-ms bin. Intermittent spontaneous activity during substrate vibration was thus calculated as the sum of cONv spikes occurring outside the "sensory response window" (gray-shaded area, i.e., from tap onset to 30 ms post tap).

Bimodal stimulation. Spontaneous activity during bimodal stimulation (antennal movement + substrate vibration) was calculated by combining both methods explained above. Thus, the spike counts during the hold phases of the ramp-and-hold stimulus were considered. The exception was that whenever a tap occurred during a hold phase, cONv spikes occurring during the sensory response window were ignored. Spontaneous activity before and after stimulation was calculated as for antennal stimulation alone, using the same kind of sham hold episodes as described above. We combined two velocities (30 and 300°/s) with two tapping frequencies (1 and 10 s^{-1}). For each of these four stimulus combinations, we analyzed the activity of both the bimodally stimulated cONv (responding to antenna substrate vibration) and the unimodally stimulated cONv (responding to substrate vibration only).

Relative change of spontaneous activity. The relative changes of spontaneous activity during and after stimulation were defined as follows*:*

$$\Delta \mathrm{R}_{During} = \frac{\mathrm{R}_{during}}{\mathrm{R}_{before}} - 1\ ,\ \Delta \mathrm{R}_{After} = \frac{\mathrm{R}_{after}}{\mathrm{R}_{before}} - 1,$$

where R is the estimate of the spontaneous spike rate and ΔR is the normalized change in spontaneous activity.

Baseline activity. The baseline activity of a neuron was defined as the mean spontaneous activity before each single one of N stimulus presentations, such that

$$R_{Base} = \frac{1}{N}\sum_{i=1}^{N} R_{before}^{i}$$

Box-and-whisker plots. In figures, the limits of the boxes are drawn at the first (Q1) and third (Q3) quartiles of the distribution, whereas whiskers show the minimum and the maximum values of the distribution. Data points below Q1 - 1.5·IQR (interquartile range) or above Q3 + 1.5·IQR are plotted as outliers of data (crosses), whereas the mean is indicated by white bars.

Absolute change of spontaneous activity. Data were separated into 15 consecutive bins of spontaneous activity, with 5 bins before, 5 bins during, and 5 bins after stimulation. cONv activity per bin (b) was then compared with the baseline activity $\mathrm{R}_{\mathrm{Base}}$ and averaged *across N animals as follows:*

$$\Delta R(b) = \frac{1}{N} * \sum\nolimits_{k=1}^{N} \left(\frac{\mathrm{R(b)}_k}{\mathrm{R}_{Base}} - 1 \right).$$

Bootstrap. Data were bootstrapped 1,000 times using the "bstrp" function of MATLAB as follows: given the sample size N of the distribution P, N values were randomly drawn from P such that a new distribution, P1, of size N was obtained. The mean of the distribution P1 was then calculated, and this process was repeated 1,000 times. The 2.5% and 97.5% quantiles of the resulting distribution of means yielded the bootstrapped 95% confidence interval (CI) of the mean.

Statistical analysis. To assess the statistical significance for paired distributions, Wilcoxon's signed-rank tests for matched pairs were calculated in MATLAB. Significance of the comparisons between spontaneous activity before and during as well as before and after stimulation is shown as asterisks above the corresponding box-and-whisker plots. The 95% confidence intervals (CI) of the mean baseline activity were calculated from the spontaneous activity before stimulation of all animals.

Results

Spontaneous versus stimulus-evoked activity in cONv

As found by Ache et al. (2015), only one of the two cONv interneurons responded to antennal movement (Figure 7A and B, and Figure 8A and B), but both cONv interneurons reliably responded to substrate vibration with at least one spike per tap (Figure 7D and E, and Figure 8D and E; see schematic in Figure 7F). In the absence of substrate vibration or antennal movement, both cONv display spontaneous activity that fluctuates throughout the recording (Figure 7C and Figure 8C). Given that spontaneous activity can be observed in periods of no sensory input, we wondered to what extent this activity was affected by sensory induced changes in spike rate. This was tested first separately for each one of the two input modalities, and then for combined stimulation of both modalities.

Spontaneous activity is reduced during substrate vibration

To test to what extent substrate vibration can influence spontaneous activity of cONv, we stimulated the neuron with tapping frequencies ranging from 1 to 10 taps per second. Both cONv neurons displayed fluctuating spontaneous activity be-fore stimulation began. During stimulation, the neurons responded reliably to each tap (Figure 7D and E, and Figure 8D and E) within 30 ms of tap onset (Figure 9). Once the stimulation stopped, cONv neurons continued to fire spontaneously after a short period of silence.

To relate the effect of sensory stimulation on spontaneous activity, we first determined the level of resting activity of both cONv neurons in each animal. For this, the baseline of resting activity was calculated for a 20s interval preceding the substrate vibration stimulus. *Figure 10*A shows that this baseline activity varied considerably across animals. The lowest activity recorded was 0.25 and 0.68 s^{-1} for the left and right cONv, respectively, whereas the highest activity was 11.57 and 15.01 s^{-1}, respectively (*Figure 10*A, top and bottom, respectively). Over-all, the variability between animals was greater than the variability within an animal (*Figure 10*A, compare gray and black box-and-whisker plots).

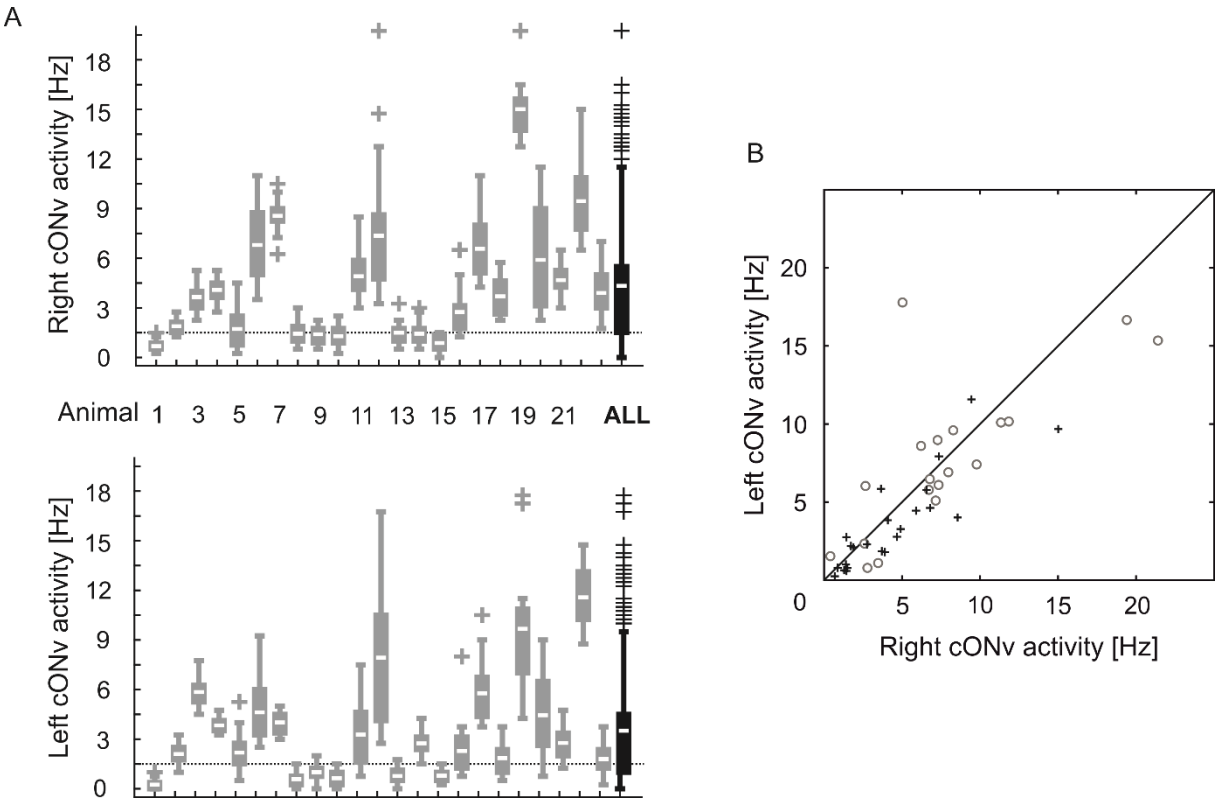


Figure 10: Spontaneous activity varies considerably among preparations.
Gray box-and-whisker plots show data for individual animals, whereas black box-and-whisker plot shows pooled data across animals. Crosses show outliers and white bars in boxplots show the mean resting spontaneous activity of 20 bouts of 5s of recording before stimulation (baseline). A) Top: right connective; bottom: left connective. Dashed black line shows a threshold set at 1.5 s^{-1}, below which spontaneous activity was too low to be considered in the further analysis; these preparations were excluded from further analysis. Overall, the variability is larger between animals than within animals. B) Correlation of left and right contralateral ON-type velocity-sensitive interneuron (cONv) activity. Crosses are data from animals used in substrate vibration experiments (N = 23; same animals as used in A and Figure 11 and Figure 12). Circles are data from animals used for antennal stimulation (N = 19; same animals as used in Figure 13 and Figure 14).

All cONv spikes falling into the sensory response window (shaded area in Figure 9) were considered to be stimulus-induced spikes and consequently removed to calculate the intermittent spontaneous activity of cONv during stimulation. The resulting activity was compared with the resting activity before and after stimulation. For tapping frequencies higher than 3 s^{-1}, the intermittent spontaneous activity during substrate vibration was significantly lower than the resting activity before stimulation (Figure 11). The left cONv showed a median reduction of spontaneous activity by 1.79 s^{-1}, corresponding to a reduction by -50% at tapping frequency of 6 s^{-1} (P = 0.017, mean = -30%) and no significant difference for the other tapping frequencies. The right cONv showed a median reduction of spontaneous activity by -1.86 s^{-1}, corresponding to a reduction by 41% (P = 0.017, mean -18%) at a tapping frequency of 6 s^{-1}, and by -2.23 s^{-1} or -55% at a tapping frequency of 10 s^{-1} (P = 0.038, mean = -35%).

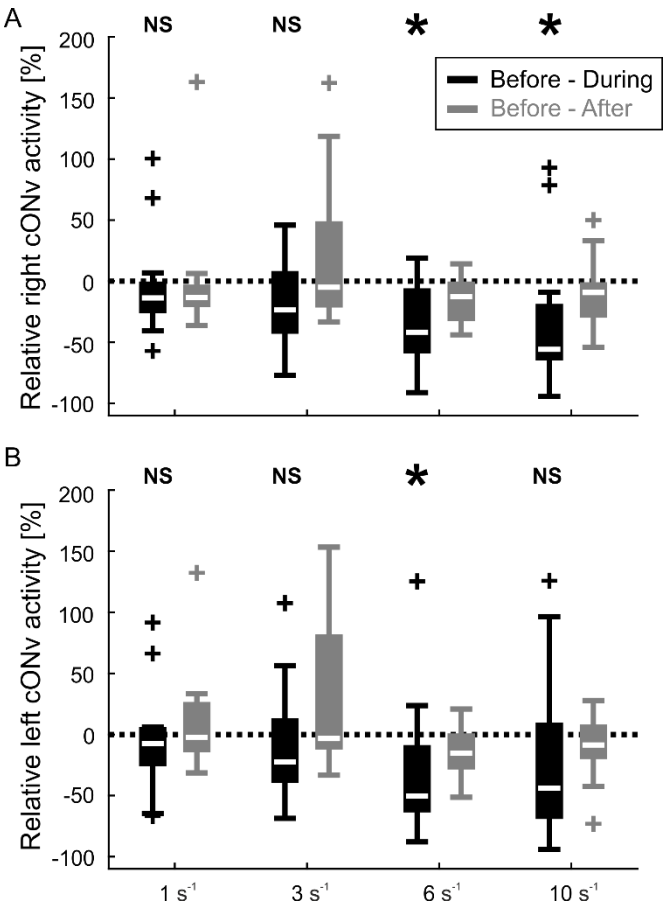


Figure 11: Mean relative change of spontaneous activity as a consequence of substrate vibration.
*Data show the ratio of the change in spontaneous activity divided by the spontaneous activity before stimulation. Box-and-whisker plots relate the intermittent spontaneous activity (during; black boxes) and subsequent resting spontaneous activity (after; gray boxes) to resting activity before stimulation in right (A) and left (B) contralateral ON-type velocity-sensitive interneurons (cONv). Negative values indicate that spontaneous activity de-creased. White bars in boxplots show the median activity and crosses are outliers. At a tapping frequency of 6 s-1, intermittent spontaneous activity of both cONv was significantly reduced. Only the right cONv showed reduction of intermittent spontaneous activity for a tapping frequency of 10 s-1. After stimulus offset, resting spontaneous activity of both cONv was transiently reduced for tapping frequencies ≥ 6s-1 (N = 16 preparations). *Statistical significance of at least P 0.05 (for exact P values, see text). NS, not significant.*

Reduction of spontaneous activity is immediate and outlasts the vibration stimulus

Next, we investigated whether the history-dependent effect on spontaneous activity has to build up over time or, rather, occurs immediately upon stimulus onset. Similarly, we wanted to know how long the reduction persisted after the stimulation stopped. To investigate this, we divided the spontaneous activity before, during, and after substrate vibration stimulation into five bins each and compared them with the baseline activity (Figure 12, left). The 95% confidence intervals of the mean (error bars in Figure 12) indicate a significant reduction of intermittent spontaneous activity after 4s of stimulation with a tapping frequency of 10 s^{-1} (Figure 12D; confidence intervals do not overlap). For all lower tapping frequencies (Figure 12, A–C), 95% confidence intervals overlap. We conclude that the reduction of spontaneous activity was statistically significant for tapping frequencies below 10 s^{-1} only when the mean effect across the 20s stimulation period is considered (e.g., see 6 s^{-1} in Figure 11), but not in the time-resolved analysis of Figure 12.

After stimulus offset, cONv was often almost silent for a few seconds. There was a significant post stimulus reduction of resting activity only for tapping frequency 10 s^{-1} (Figure 12D, left; 95% confidence intervals do not overlap for 3 consecutive bins). We conclude that history-dependent reduction of spontaneous activity persisted for 12 s.

Since it could have been possible that the observed reduction of intermittent spontaneous activity was immediately correlated with stimulus-induced activity, we related the analysis of spontaneous activity (Figure 12, left) to an equivalent analysis of stimulus-induced increase of spike activity Figure 12, right). Given that most taps triggered only a single spike (Figure 9) and that mean resting activity of cONv neurons was in the range of 3 s^{-1}, stimulus-induced activity at a tapping frequency of 1 s^{-1} is more than 50% lower than resting activity (Figure 12A, right). Accordingly, stimulus-induced activity approximately equals resting activity for tapping frequencies of 3 s^{-1} and is 70% and 200% higher at tapping frequencies of 6 and 10 s^{-1}, respectively.

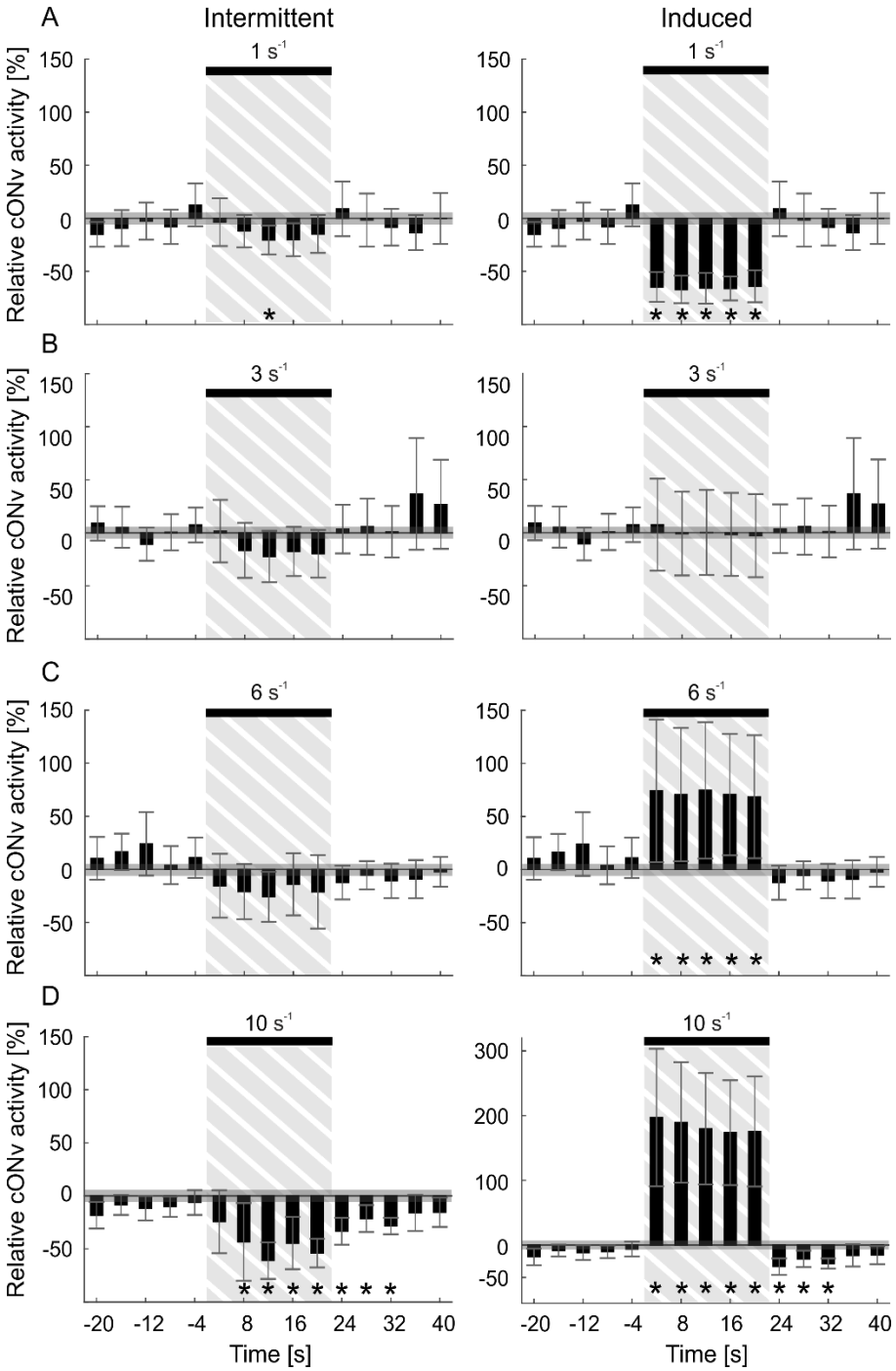


***Figure 12*: Relative change in spike activity over time for episodes of substrate vibration stimulation.**

*Time-resolved activity during substrate vibration with tapping frequencies at 1 (A), 3 (B), 6 (C), and 10 s-1 (D). Stimulation episodes are marked by a horizontal black bar and gray hatched area. Vertical black bars show mean relative change of activity per 4-s bin before, during (gray hatched area), and after stimulation, compared with base-line activity (gray bar: 95% confidence interval of the baseline activity fluctuation). Note that mean baseline activity was ~ 3 s-1 (N = 16 preparations). Error bars show 95% confidence intervals per bin. Left: relative change of intermittent spontaneous activity of contralateral ON-type velocity-sensitive interneurons (cONv); right: stimulus-induced change in activity during the same period. *Bins for which the confidence intervals do not overlap, i.e., activity significantly differs from baseline.*

Antennal movement reduces spontaneous activity

Having found history-dependent reduction of spontaneous activity in cONv following stimulation with substrate vibration, we wondered whether the second sensory input modality of cONv, antennal movement, had the same effect. To test this, we passively rotated one antenna about its scape-pedicel (SP) joint at two different velocities (30 and 300°/s). Note that, contrary to substrate vibration, only the contralaterally descending cONv responds to this kind of stimulus (compare bimodally stimulated cONv in Figure 8Ai and Bi, with unimodally stimulated cONv in Figure 8Aii and Bii). Nevertheless, the activity of the ipsilaterally descending cONv was analyzed in the same manner (subsequently referred to as "non-stimulated cONv", as opposed to "stimulated cONv"). Thus, in our bilateral recordings, we expected history-dependent reduction of intermittent spontaneous activity in the stimulated cONv only.

As for the vibration stimulus, we set out by assessing the preparation-specific baseline, i.e., the mean resting activity, of both cONv without stimulation (*Figure 10*B, circles). As in the previous sample of preparations (*Figure 10*B, crosses), baseline activity varied strongly among preparations. The minimum baseline activity was 0.44 s^{-1} and 0.75 s^{-1} for left and right neurons, respectively, whereas the maximum baseline activity was 17.5 s^{-1} and 21.4 s^{-1}, respectively. In general, variation among preparations (spread of symbols along the diagonal in *Figure 10*B) was considerably larger than variation between the two neurons of the same preparation (spread of symbols perpendicular to the diagonal in *Figure 10*B). As described in Effect on the stimulated cONv, the intermittent spontaneous activity during stimulation was calculated by averaging the activity of cONv during the hold phases, i.e., when no antennal movement occurred.

Effect on the stimulated cONv. We found a significant reduction of intermittent spontaneous activity for both stimulus types (Figure 13A). The effect reached a median reduction by -2.50 s^{-1} corresponding to -33 % at a velocity of 30 deg/s ($P_{30\ deg/s} = 4.7 * 10^{-4}$, mean = -34%) and by -1.50 s^{-1} at 300 deg/s, equivalent to nearly -30 % ($P_{300\ deg/s} = 6.78 * 10^{-4}$, mean = -25%). After stimulation terminated, the effect persisted with -1.25 s^{-1} and -1.30 s^{-1} following the 30 deg/s and 300 deg/s stimuli, respectively, corresponding to median reductions by approximately -20 % ($P_{30\ deg/s}$= 0.0018, mean = -23%, $P_{300\ deg/s}$ = 0.0042, mean =

-20%). Note that this effect was determined for the exact same distribution of windows ("sham hold phases") as for the intermittent activity.

Effect on the non-stimulated cONv. As expected from the fact that cONv does not respond to movement of the ipsilateral antenna, there was no effect on spontaneous activity of the non-stimulated cONv neuron, regardless of the movement velocity applied. In other words, spontaneous activity remained statistically indistinguishable between the resting activity before stimulation and intermittent activity during stimulation (Figure 13B, gray box plots; $P_{30\ deg/s} = 0.086$, median = - 0.250 s^{-1}; $P_{300\ deg/s} = 0.058$, median = - 0.875 s^{-1}), as well as for resting activity after stimulation (Figure 13B, black box plots; $P_{30\ deg/s} = 0.76$, median = -0.12 s^{-1}, $P_{300\ deg/s} = 0.48$, median = -0.62 s^{-1}). This indicated that the history-dependent effect of antennal movement on cONv did not cross the midline to affect spontaneous activity of the other cONv neuron.

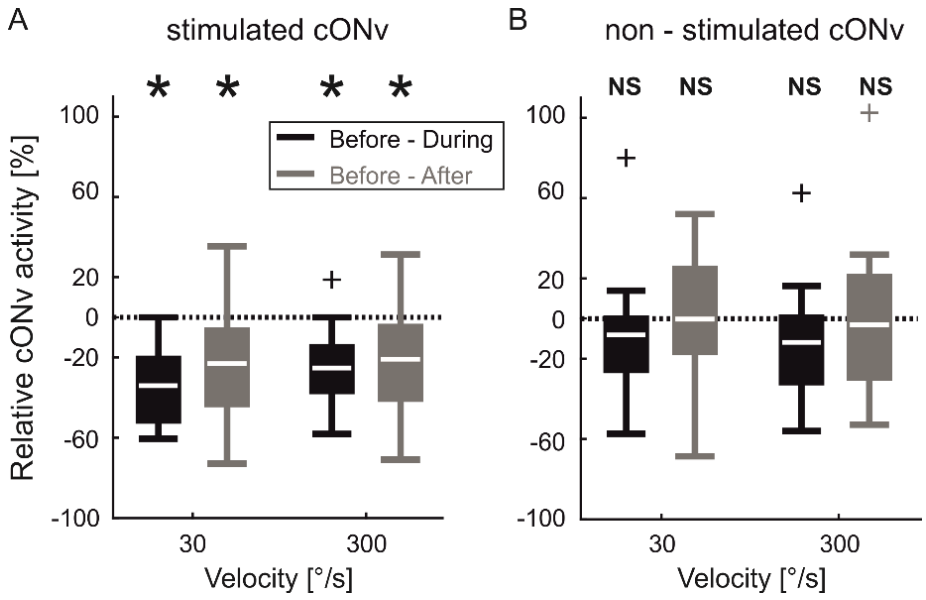


Figure 13: Mean relative change of cONv spontaneous activity during antennal movement.
*Normalized change of intermittent activity during (black boxes) and resting activity after (gray boxes) antennal movement for the stimulated (A) and non-stimulated cONv (B). Negative values mean that spontaneous activity decreased. Crosses show outliers and white bars in boxplots show the median (N = 16 preparations). Only the stimulated cONv shows reduction of spontaneous activity. *Statistical significance of at least P < 0.05 (for exact P values, see text). NS, not significant.*

*R*eduction of spontaneous activity is immediate and outlasts antennal stimulation

Given the fast and persistent effect of substrate vibration on spontaneous activity, we wanted to know whether the movement-induced reduction had similar temporal proper-ties. To investigate this, we again divided the two cONv recordings per preparation into 15 bins per 60-s trial, cor-responding to 5 bins before, during, and after the antennal movement stimulus. As in Figure 12, the 95% confidence interval of the mean was then compared with the 95% confidence interval of the baseline activity of the neuron (Figure 14, left). For both movement velocities, intermittent activity of the stimulated neuron was significantly lower than the resting activity no later than 5 s after stimulus onset (Figure 14A, left; from second bin onward). Spontaneous activity was still reduced for the first 10 s (2 bins) after the stimulus had stopped. As before, we then tested whether the history-dependent effects on spontaneous activity could be related to the level of stimulus-induced activity and conducted the same analysis for the stimulus-induced activity during antennal movement (Figure 13, right). The stimulated neuron showed in-creased activity during antennal movement, i.e., during the ramps of the ramp-and-hold stimulus (Figure 14A, right, shaded area) at both velocities. Stimulus-induced activity was 70% higher than the resting activity when stimulated at 30°/s, and 500% higher when stimulated at 300°/s. Moreover, for both velocities the stimulus-induced activity was higher at the onset of the stimulus (bin 6) compared with the end (bin 10). As for the mean relative change of spontaneous activity (Figure 13), the time-resolved analysis of the nonstimulated cONv showed no significant reduction of spontaneous activity (Figure 14B, right), except for a single bin (bins 9 and 7 at 30 and 300°/s, respectively). We conclude that there was no consistent history-dependent decrease of spontaneous activity during movement of the ipsilateral antenna.

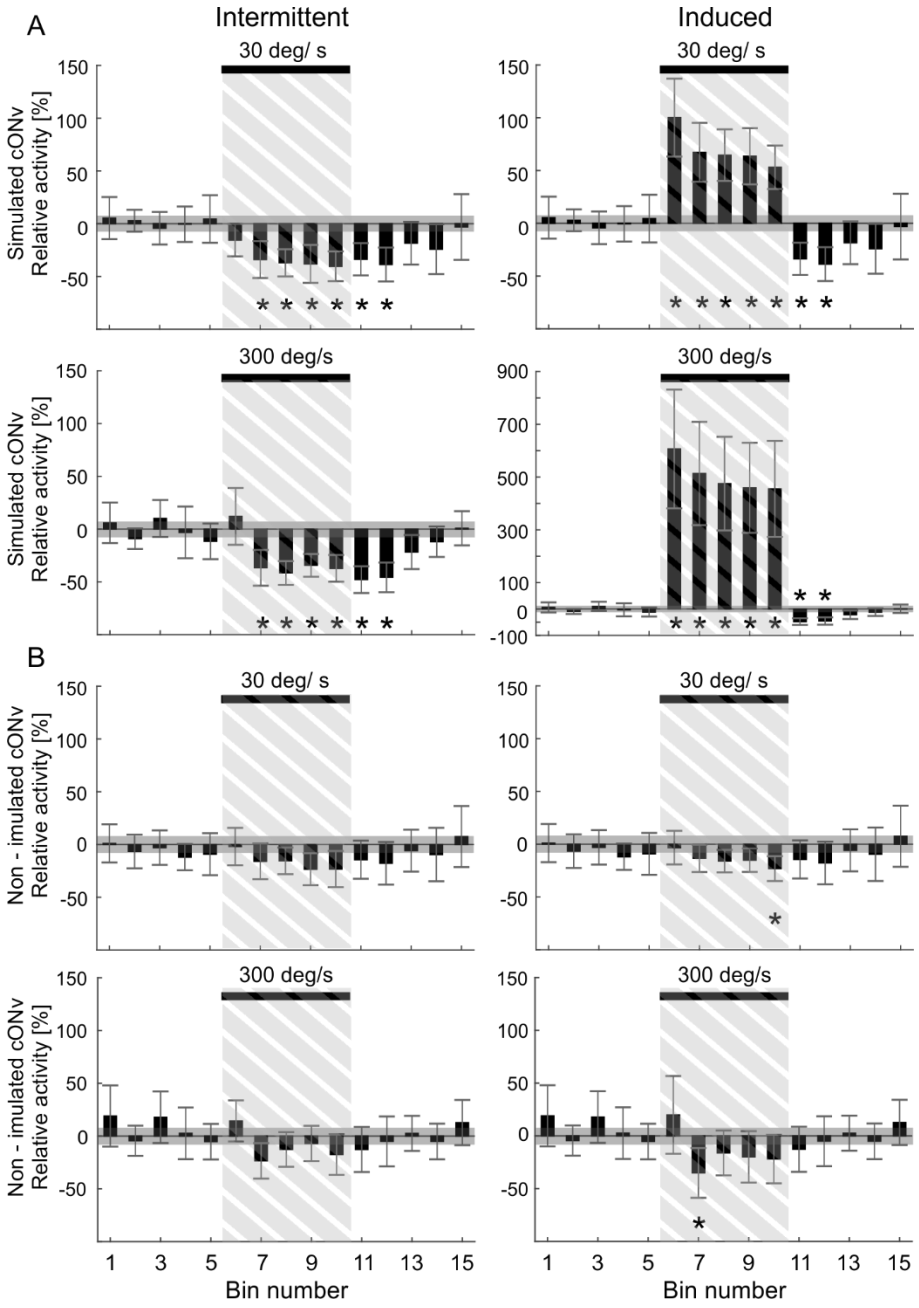


Figure 14: Time-resolved spike activity for episodes of antennal movement stimulation.
*A: stimulated contralateral ON-type velocity-sensitive interneuron (cONv) activity. B: non-stimulated cONv activity. Stimulation episodes are marked by a horizontal black bar and gray hatched area. Black bars show mean change in 5 staircase bins comprising 20 hold periods of 500-ms duration or equivalent resting periods before, during, and after antennal movement. Left: intermittent spontaneous activity (hatched gray area) of cONv. Error bars show 95%confidence intervals per bin. Gray horizontal bar shows 95% confidence interval of the baseline. Each bin was compared with the baseline activity per animal (N = 16 preparations). Right: stimulus-induced changes in spike activity during antennal stimulation as 5 bins comprising the mean activity over 16 consecutive ramps each. *Bins for which the confidence intervals do not overlap, i.e., activity significantly differs from baseline.*

Figure 15 summarizes the comparison of stimulus-induced activity and corresponding effect on intermittent spontane-ous activity. For substrate vibration (Figure 15, open symbols), the median reduction of intermittent spontaneous activity increased as the level of stimulus-induced activation of the neuron increased, as revealed by the negative slopes of the connecting lines. This was different for the antennal movement stimuli (Figure 15, filled symbols): whereas the results for the nonstimulated cONv neuron fitted well to those of the vibration stimuli, the reduction of intermittent spontaneous activity of the stimulated neuron hardly depended on the level of stimulus-induced activity. As a consequence, antennal movement at 30°/s was accompanied by much stronger reduction of spontaneous activity than a vibration stim-ulus with a tapping frequency of 6 s^{-1}, despite the fact that the level of stimulus-induced activity was vastly different in both cases. As a consequence, we conclude that the history-dependent reduction of spontaneous activity is not immedi-ately related to the preceding stimulus-induced activation of the neuron.

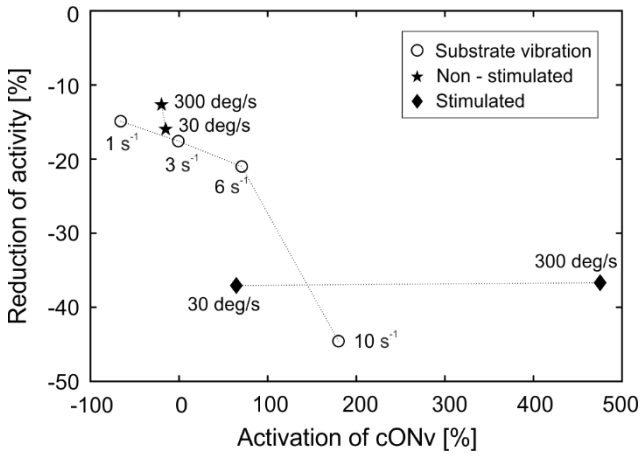


Figure 15: There is no simple dependence of the reduction of intermittent spontaneous activity on the level of stimulus-induced activation.
Summary of data in Figure 12 and Figure 14. Closed symbols represent the median reduction of intermittent spontaneous activity as a function of the level of activation during antennal stimulation for the stimulated (diamonds) and non-stimulated (stars) contralateral ON-type velocity-sensitive interneurons (cONv; N = 16 preparations). Open circles represent the median reduction of intermittent spontaneous activation as a function of the level of activation of the right cONv during the substrate vibration stimulus (N =16 preparations). Note that similar levels of stimulus-induced activity may be accompanied by vastly different reduction of spontaneous activity.

Bimodal reduction of spontaneous activity

Because the spontaneous activity of the neuron decreased when either one of the stimulus modalities were presented, we asked whether presenting both of them simultaneously had a greater effect on the neuron that received both inputs. To investigate this, we combined the two antennal movement velocities used in the previous set of measurements with one of two tapping frequencies. For this, we chose 1 s^{-1}, i.e., a tapping frequency that had no significant effect on spontaneous activity (Figure 11), and 10 s^{-1}, i.e., a

frequency that did have a significant effect. Figure 16 shows the difference of spontaneous activity when antennal movement and substrate vibration were applied simultaneously.

Effect on the bimodally stimulated cONv. On average there was a reduction of spontaneous activity by -1.50 s^{-1} (median = -31 %; mean = 25 %) and -2.12 s^{-1} (median = -41%; mean = -35 %) at 30 deg/s combined with 1 s^{-1} tapping and 300 deg/s also combined with 1 s^{-1} tapping, respectively. However, this mean reduction of intermittent spontaneous activity was not statistically significant (Figure 16A; $P_{30\ deg/s + 1\ s^{-1}} = 0.31$, $P_{300\ deg/s + 1\ s^{-1}} = 0.051$). When the 10 s^{-1} tapping frequency was combined with either antennal movement velocity, there was always a significant reduction of spontaneous activity (Figure 16A, black box plots). In this case, the median reduction was -2.5 s^{-1} (-76 %) for 30 deg/s movements ($p_{30\ deg/s + 10\ s^{-1}} = 9.76 * 10^{-4}$, mean = -69 %) and -3.12 s^{-1} (-76 %) for 300 deg/s movements ($p_{300\ deg/s + 10\ s^{-1}} = 0.002$, mean= -73 %).

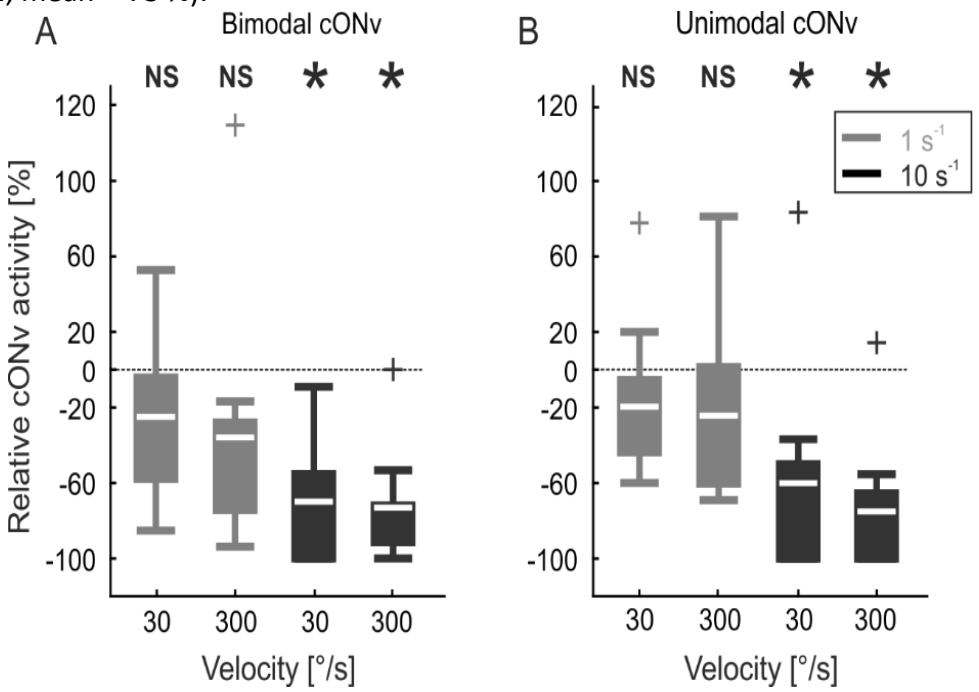


Figure 16: Mean relative change of spontaneous activity during simultaneous antennal movement and substrate vibration.

*A: reduction of spontaneous activity for the contralateral ON-type velocity-sensitive interneuron responding to both stimuli (bimodal cONv). B: spontaneous activity change for the neuron that was stimulated by substrate vibration only (unimodal cONv; N = 11 preparations). Crosses are outliers and white bars in boxplots represent the median. Negative values mean that spontaneous activity is reduced. Bimodally stimulated cONv shows reduction of spontaneous activity for all combinations except 30°/s with tapping frequency of 1 s^{-1}. Unimodally stimulated cONv shows reduction of spontaneous activity only when stimulated at a tapping frequency of 10 s^{-1}. *Statistical significance of at least P < 0.05 (for exact P values, see text). NS, not significant.*

Effect on the unimodally stimulated cONv. Similar to the bimodally stimulated neuron, the cONv that received only the substrate vibration input (Figure 16B) showed no significant reduction of activity during substrate vibration 1 s^{-1} combined with movement of the ipsilateral antenna at either velocity (Figure 16B, gray box plots; -0.75 s^{-1}, $P_{30\ deg/s\ +\ 1\ s^{-1}} = 0.25$; - 0.1 s^{-1}, $P_{300\ deg/s\ +\ 1\ s^{-1}} = 0.18$). However, it showed a significant reduction of intermittent spontaneous activity by -1.5 s^{-1} (-61%) and -2.65 s^{-1} (-82%) when the 10 s^{-1} tapping frequency was presented together with an antennal movement of the ipsilateral antenna at 30 deg/s and 300 deg/s, respectively (Figure 16B, black box plots; $P_{30\ deg/s\ +\ 10\ s^{-1}} = 0.018$, mean = -60 %, $P_{300\ deg/s\ +\ 10\ s^{-1}} = 0.002$, mean = -75 %).

To assess whether the effect of bimodal stimulation was equivalent to the summed effects of the single modalities, we combined the results of all three experimental series. This was done by random selection of three single-trial results: 1) spontaneous activity during a vibration stimulus (Figure 11 and Figure 12), 2) spontaneous activity with antennal movement (Figure 13and Figure 14), and 3) spontaneous activity following simultaneous presentation of both stimuli (Figure 16). Figure 17 shows the corresponding 1,000 comparisons of the effect on spontaneous activity during bimodal stimulation (3) against the predicted effect on spontaneous activity, assuming linear summation of the effects of each modality alone (1 + 2).

Effect on the bimodally stimulated cONv. The measured reduction of spontaneous activity of the bimodally stimulated neuron was smaller than predicted for the combination of 1 s^{-1} tapping and 30°/s movement (Figure 17A, black dots: 80% above diagonal and 20% below), indicating a sublinear summation effect. For the combinations of 1 s^{-1} tapping with 300°/s movement (Figure 17A, red dots: 39.8% above and 60.2% below) and 10 s^{-1} tapping with 30°/s movement (Figure 17A, green dots: 49.5% above and 50.6% below), the reduction of spontaneous activity largely corresponded with the linear prediction. Only the combination of the higher velocity (300°/s) and the higher tapping frequency (10 s^{-1}) led to a stronger reduction of spontaneous activity than predicted (Figure 17A, blue dots: 20.6% above and 79.6% below), indicating supralinear summation.

Effect on the unimodally stimulated cONv. The effect of bimodal stimulation on the cONv interneuron that descended ipsilaterally to the moved antenna (and hence received only the vibration stimulus) depended only on the tapping frequency, irrespective of the

antennal movement velocity applied (Figure 17B). In all cases of 1 s^{-1} tapping, reduction of spontaneous activity during bimodal stimulation was similar to the prediction (Figure 17B, black dots: 37.1% above diagonal and 62.9% below; red dots: 33.4% above and 66.6% below). In case of 10 s^{-1} tapping, the reduction of spontaneous activity was stronger than predicted, indicating a supralinear summation effect (Figure 17B, green dots: 10.2% above and 89.8% below; blue dots: 1.1% above and 98.9% below).

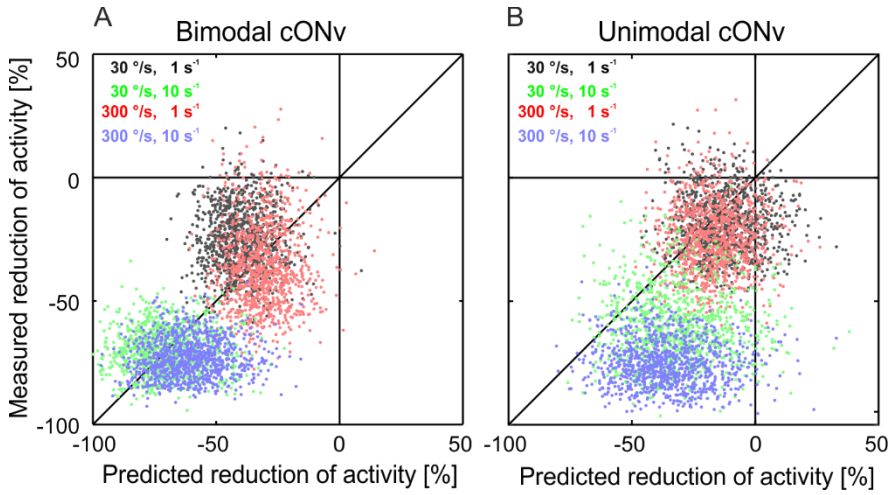


Figure 17: Effect of bimodal stimulation relative to summed effects of unimodal stimulation.
A: bimodally stimulated contralateral ON-type velocity-sensitive interneuron (cONv). B: neuron that was stimulated by vibration only (unimodal). Measured changes in intermittent spontaneous activity of cONv from animals where both stimuli were presented simultaneously (N = 11 preparations) are plotted against bootstrapped predictions of the reduction, assuming linear additive effect of both modalities (N = 16 preparations for each modality; n = 1,000 boots; see METHODS). Different colors represents different combinations of antennal movement velocity and tapping frequency: black, 30°/s and 1 s^{-1}; green, 30°/s and 10 s^{-1}; red, 300°/s and 1 s^{-1}; blue, 300°/s and 10 s^{-1}. Values lying along the diagonal mean that the measured reduction equals the prediction. Values below the diagonal mean that the reduction measured is greater than the prediction, whereas values above the diagonal mean that the reduction measured is smaller than the prediction.

Discussion

Relevance to other descending neurons

cONv is a spiking descending interneuron in the ventral nerve chord of stick insects. It responds to passive movement of the contralateral antenna with an increase in mean firing rate (Ache et al. 2015). Given the short latency of this response and the vicinity of proprioceptive afferent terminals and cONv dendrite branches, Ache et al. (2015) suggested that cONv could be involved in mediating fast, tactually induced reach-to-grasp responses of the front legs, as described by Schütz and Dürr (2011). As yet, the putative function of cONv

in tactually induced behaviour should critically depend on the degree to which its activity fluctuates without correlation with the afferent input. Given the fact that cONv also responds to substrate-borne vibration and reliably follows tapping frequencies up to 20 s^{-1} (Ache et al. 2015), this second type of afferent input should confound the reliability of the response to antennal contact events.

Although the exact role of cONv remains elusive, related descending interneurons of other species have been demonstrated to play an important role in the regulation of locomotory actions. The escape response of cockroach is an example where the giant descending interneuron DMI b-1 is involved (Ye and Comer 1996). Like cONv, DMI b-1 has its soma in the GnG, and contralateral to the descending neurite. Other than cONv, DMI b-1 was found to respond bilaterally to antennal touch (Burdohan and Comer 1996), and its activity correlated with the direction of the escape response (Ye and Comer 1996). Published recordings of DMI b-1 suggest that its resting activity is negligible (Burdohan and Comer 1996) such that even single-spike events could serve as a reliable indicator for antennal deflection and thus induce escape behaviour. Similarly, descending brain neurons in crickets that respond to antennal touch and/or movement were reported to have very low or absent spontaneous activity (Gebhardt and Honegger 2001; Schöneich et al. 2011). Fluctuating spontaneous activity as found in the present study for cONv would be problematic for reliable detection of an external stimulus unless stimulus-induced responses were very strong. Conversely, in the cockroach, Schaefer and Ritzmann (2001) showed that descending drive is important to maintain the system at a level of activity sufficient to elicit the appropriate action from motoneurons. Indeed, they reversibly removed all descending input from the connectives, resulting in reversible extinction of the escape behaviour. In this circuit, tactile stimulation of the abdomen can only elicit an escape run that includes front leg movement if the spontaneous activity in the prothoracic ganglion is already sufficiently high to trigger a fast motor neuron response. However, in our study we found a reduction of spontaneous activity following sensory activation of the neuron, which would indicate that this neuron is not involved in a similar mechanism in the stick insect.

Interanimal variation and potential origins of spontaneous activity

The results of Ache et al. (2015) already suggested that the linear velocity tuning of cONv may differ among animals in both slope and intercept (their Fig. 4E), including non-zero spontaneous activity without antennal movement. The spontaneous (resting) activity of cONv

found in our present study (Figure 7C, Figure 8C, and Figure 10) appeared to be higher than that described by Ache et al. (2015). The mean spontaneous activity per preparation ranged between 0.4 and 21 s^{-1} (Figure 10). We attribute this apparent discrepancy to differences in animal preparation. Generally, the access to the neck connective for recording entails ablation of muscles and, potentially, some smaller nerves that could alter the state of the animal and/or affect spontaneous neural activity. This is consistent with the fact that spontaneous activity was lower in the more invasive preparations for intracellular recordings of Ache et al. (2015) than in our preparations. Our finding of strong inter-animal (or inter-preparation) variation could be explained by the general state of the animal (Koren and Denève 2017) or variation of the temperature (Zhantiev et al. 2006) given the background input to the neuron may change in intensity (Figure 7G). Figure 10 shows that there was considerably more variability of cONv spontaneous activity across animals than among recordings from an individual preparation. Moreover, mean spontaneous activity in the right and left cONv of the same preparation were strongly correlated (Figure 10C), with the linear coefficient of determination being $r^2 = 0.8$ (for pooled data in Figure 10C), i.e., explaining 80% of the variance between preparations. This strongly suggests the existence of a common presynaptic drive to both cONv neurons per animal. The source of this common drive remains enigmatic, except that it is clear that its synaptic efficacy is not high enough to induce 1:1 spike coupling between the two neurons. Regarding the potential causes of spontaneous activity as listed in Figure 7G, we attribute the correlated activity of both cONv neurons to background activity within the GnG. Other possibilities include that the synaptic input driving spontaneous activity in cONv is fluctuating (Fellous et al., 2003) or that the gain of the neuron is changing in absence of sensory activation (Higgs et al. 2006).

History-dependent reduction of spontaneous activity

During stimulation by substrate vibration, only the highest tapping frequencies significantly reduced the intermittent spontaneous activity (Figure 11). The reduction occurred in both cONv interneurons, set in almost immediately after stimulus onset, reached a magnitude of almost 30%, and outlasted the end of the stimulation episode by up to 12 s (Figure 12). Although it is clear that the vibration sensor must be located in the head of the stick insect (Ache et al. 2015), its identity is still unknown. Given that in our experimental situation the head was fixed to a holder, substrate vibration may have been conveyed from the ground to the head either directly or via the body. During walking, however, vibration

may be conveyed to the sensor via the legs. Indeed, mean instantaneous frequencies of footfall during walking at slow to medium speeds range between 4 and 10 s^{-1} (Figure 18) and should exceed 10 s^{-1} for walking speeds beyond 50 mm/s. Dürr et al. (2018) reported instantaneous walking speeds of free walking stick insects up to 100 mm/s. Thus, natural footfall frequencies are well within or even above the range of tapping frequencies that resulted in significant suppression of the spontaneous activity of cONv. Assuming that the impacts generated by footfall cause similar vibrations in walking animals, we propose that walking leads to a reduction of spontaneous activity in both cONv interneurons.

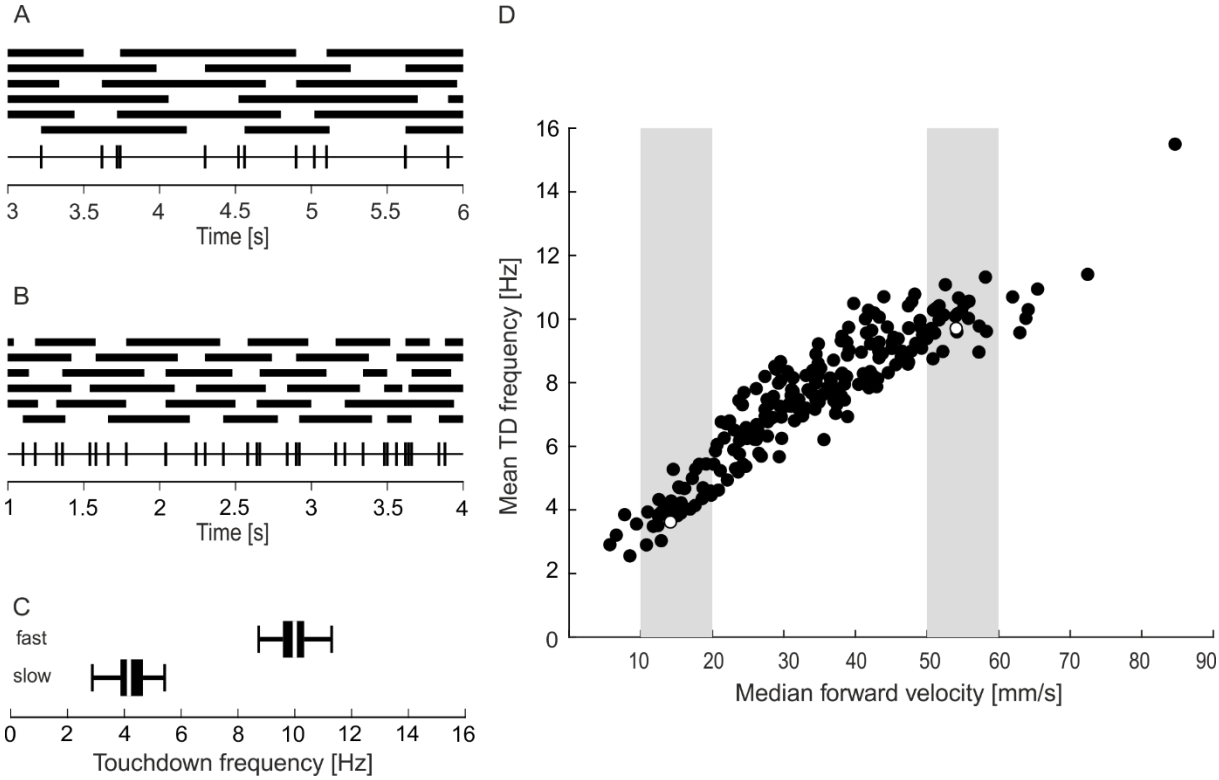


Figure 18: Footfall frequency in freely walking stick insects.
A: example podogram of a slow walking stick insect (velocity = 14.25 mm/s), measured during unrestrained walking on a straight walkway. Black bars show stance movements (retraction of leg with ground contact) for left front, middle, and hind legs (top 3 rows) and right front, middle, and hind legs (bottom 3 rows). B: same as A, but for a faster walking stick insect (velocity = 54.17 mm/s). C: footfall frequency, i.e., frequency of touch-down events for all legs of slow and fast walking stick insects. Box-and-whisker plots show data from gray-shaded areas in D (n = 35 trials from N = 7 animals). D: overall dependency of footfall frequency on average walking speed per trial (n = 231 trials from N = 12 animals). TD, touchdown. Open circles depict animals used for examples in A and B.

Similar to the effect of substrate vibration, passive movement of the antenna lead to a reduction of spontaneous activity shortly after stimulus onset, with magnitudes of 30% to40% for both velocities tested (Figure 13) and outlasting the stimulus by several seconds

(Figure 14). Given that freely walking stick insects rhythmically move both antennal joints over a range of about +/- 45° with 1–2 cycles/s (Krause et al. 2013), antennal joint angle velocities approximately vary sinusoidally according to 90F·sin (2F·t), with F being the frequency in cycles per second. Accordingly, antennal movements applied in this study fall into the range of natural antennal movement velocities, with 30°/s being in the lower range and 300°/s being close to peak velocities, assuming F = 1 cycle/s. Thus, we propose that reduction of spontaneous activity should occur during active antennal movement during walking. Contrary to substrate vibration, where both cONv interneurons showed a decrease of spontaneous activity, antennal movement caused a decrease of spontaneous activity in the contralateral interneuron only.

The spontaneous activity of the non-stimulated cONv appeared to be slightly lower during antennal movement than before the movement; however, this decrement was not significant (Figure 13). As a consequence, history-dependent reduction of spontaneous activity is a lateralized effect that must be linked to sensory-driven synaptic input. Whether this could be a postsynaptic effect occurring in the stimulated cONv only or a presynaptic effect at the afferent synapses cannot be decided on the basis of the present experiments. In any case, it cannot act on the putative common drive to both cONv interneurons that we postulate to explain strongly correlated spontaneous activity before stimulation. cONv responded to passive deflection of the antenna with several spikes per ramp and mean instantaneous spike rates up to 100 spikes/s. Therefore, movement-induced responses were generally much stronger than vibration-induced responses that comprised a single spike per tap (Figure 15). As yet, the overall effect on the spontaneous activity from both modalities was similar, despite widely different stimulus-induced spike rates.

The reduction of spontaneous activity outlasted the duration of the stimulus episodes for both modalities, whereas reduction was already effective briefly after the start of a stimulus episode (Figure 12 and Figure 14). This could be explained by hyperpolarization following strong activation (Hotson and Prince 1980; Sanchez-Vives et al. 2000a, 2000b) or presynaptic depression (Trimmer and Weeks 1991). Similarly, Zorovic´ and Hedwig (2011) showed that B-CL2 activity (a local brain neuron in cricket) was correlated with contralateral turning behaviour in response to chirps. Like cONv, B-LC2 displayed fluctuating spontaneous activity that was reduced between stimulation (i.e., chirps) in the presence of hyperpolarizing current.

In principle, both the reduction of intermittent spontaneous activity during stimulation and the aftereffect may be linked to an adaptation-like mechanism. Adaptation is defined as the reduction of activity to repeated stimulation at the same intensity (Carandini and Ferster 1997; Descalzo et al. 2005; Harris et al. 2000). Figure 14 shows that such a mechanism is likely to be present in cONv given that the stimulus-induced response to ramp-and-hold stimulus were decreasing over time. Similarly, DiCaprio et al. (2002) showed that most of the sensory neurons of the femoral chordotonal organ (fCO) of the stick insect undergo adaptation. They showed that the fCO afferents could no longer display adaptation when calcium was replaced by barium in the saline solution. Despite the fact that we focused on history-dependent reduction of spontaneous activity and not on the stimulus-induced activity itself, we believe that a similar mechanism could take place in our system. Indeed, the intra-cellular free calcium concentration could modulate the ability of the neuron to fire both in response to a stimulus (DiCaprio et al. 2002) and in the absence of a stimulus.

Cooperative effect during bimodal stimulation

Having established that both modalities can cause a history-dependent reduction of spontaneous activity, the effect of stimulus combinations could tell us about the existence of a cooperative effect. Indeed, bimodal activation of cONv through substrate vibration and antennal movement led to a supralinear reduction of spontaneous activity for one parameter constellation (Figure 16). The estimated reduction of spontaneous activity in case of bimodal stimulation was bootstrapped from independent experiments with unimodal stimulation, assuming additive, linear summation (Figure 17). When the neuron was stimulated in the multimodal regime using the low antennal movement velocity (30°/s) and the lowest tapping frequency (1 s^{-1}), the observed reduction of spontaneous activity was smaller than predicted for linear summation. However, whenever a stronger stimulus of one modality was combined with the weaker stimulus of the second modality, results showed an additive effect in the reduction of spontaneous activity. Only the stimulation with the high antennal movement velocity (300°/s) and the highest tapping frequency (10 s^{-1}) resulted in a reduction of spontaneous activity that was larger than predicted for linear summation (Figure 17). This cooperative effect of both stimulus modalities is reminiscent of a mechanism of multisensory enhancement where bimodal activation of the neuron leads to a greater reduction of spontaneous activity than the most effective modality (Stein and Stanford 2008).

Unexpectedly, the same cooperative effect was observed in the unimodally stimulated cONv (Figure 16) whenever the high tapping frequency was presented (Figure 16, unimodal cONv). This occurred despite the fact that antennal movement alone had no effect on the spontaneous activity of the ipsilateral cONv neuron. We conclude that antennal movement by itself is insufficient to drive neural activity in the ipsilateral cONv but nevertheless exerts a modulatory effect on the vibratory input. For example, descending afferents could modulate vibratory afferents presynaptically, thereby raising their effect on spontaneous activity without affecting the number of spikes generated per tap. Given the considerations on walking behaviour above, we expect that footfall must activate the vibration-sensitive input of both cONv neurons. Since we found that natural footfall frequencies cover a range that includes those tapping frequencies that strongly reduce spontaneous activity in cONv (Figure 18), spontaneous activity in both cONv neurons should be reduced whenever the animal walks. Considering further that vibration-induced activity in cONv was typically a single spike per tap (rarely two), one has to wonder whether this sensory stimulus can be relayed in a multimodal neuron that responds much more strongly to antennal movement. Instead, we pro-pose that the vibration-induced, bilateral low-frequency activity in both cONv neurons may be considered as a biasing device to increase fidelity to information about antennal movement by suppressing confounding spontaneous activity in cONv during walking.

References

Ache JM, Dürr V. Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway. *J Neurophysiol* 110: 2099–2112, 2013. doi:10.1152/jn.00281.2013.

Ache JM, Haupt SS, Dürr V. A direct descending pathway informing locomotor networks about tactile sensor movement. *J Neurosci* 35: 4081-4091, 2015. doi:10.1523/JNEUROSCI.3350-14.2015.

Arieli A, Sterkin A, Grinvald A, Aertsen A. Dynamics of ongoing activity: explanation of the large variability in evoked cortical responses. *Science* 273: 1868–1871, 1996. doi:10.1126/science.273.5283.1868.

Burdohan JA, Comer CM. Cellular organization of an antennal mechanosensory pathway in the cockroach, *Periplaneta americana*. *J Neurosci* 16: 5830–5843, 1996. doi:10.1523/JNEUROSCI.16-18-05830.1996.

Carandini M, Ferster D. A tonic hyperpolarization underlying contrast adaptation in cat visual cortex. *Science* 276: 949–952, 1997. doi:10.1126/science.276.5314.949.

Carr MF, Jadhav SP, Frank LM. Hippocampal replay in the awake state: a potential substrate for memory consolidation and retrieval. *Nat Neurosci* 14:147–153, 2011. doi:10.1038/nn.2732.

Descalzo VF, Nowak LG, Brumberg JC, McCormick DA, Sanchez-Vives MV. Slow adaptation in fast-spiking neurons of visual cortex. *J Neurophysiol* 93: 1111–1118, 2005. doi:10.1152/jn.00658.2004.

DiCaprio RA, Wolf H, Büschges A. Activity-dependent sensitivity of proprioceptive sensory neurons in the stick insect femoral chordotonal organ. *J Neurophysiol* 88: 2387–2398, 2002. doi:10.1152/jn.00339.2002.

Dürr V, Theunissen LM, Dallmann CJ, Hoinville T, Schmitz J. Motor flexibility in insects: adaptive coordination of limbs in locomotion and near-range exploration. *Behav Ecol Sociobiol* 72: 15, 2018. doi:10.1007/s00265-017-2412-3.

Fellous JM, Rudolph M, Destexhe A, Sejnowski TJ. Synaptic background noise controls the input/output characteristics of single cells in an in vitromodel of in vivo activity. *Neuroscience* 122: 811–829, 2003. doi:10.1016/j.neuroscience.2003.08.027.

Gebhardt M, Honegger HW. Physiological characterisation of antennal mechanosensory descending interneurons in an insect (*Gryllus bimaculatus*,*Gryllus campestris*) brain. *J Exp Biol* 204: 2265–2275, 2001.

Harris RA, O'Carroll DC, Laughlin SB. Contrast gain reduction in fly motion adaptation. *Neuron* 28: 595–606, 2000. doi:10.1016/S0896-6273(00)00136-7.

Higgs MH, Slee SJ, Spain WJ. Diversity of gain modulation by noise in neocortical neurons: regulation by the slow afterhyperpolarization conductance. *J Neurosci* 26: 8787–8799, 2006. doi:10.1523/JNEUROSCI.1792-06.2006.

Hotson JR, Prince DA. A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. *J Neurophysiol* 43: 409–419, 1980. doi:10.1152/jn.1980.43.2.409.

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S, Heisenberg M, Homberg U, Jenett A, Keshishian H, Restifo LL, Rössler W, Simpson JH, Strausfeld NJ, Strauss R, Vosshall LB; Insect Brain Name Working Group. A systematic nomenclature for the insect brain. *Neuron* 81: 755–765, 2014. doi:10.1016/j.neuron.2013.12.017.

Karlsson MP, Frank LM. Awake replay of remote experiences in the hippocampus. *Nat Neurosci* 12: 913–918, 2009. doi:10.1038/nn.2344.

Koren V, Denève S. Computational account of spontaneous activity as a signature of predictive coding. *PLoS Comput Biol* 13: e1005355, 2017. doi:10.1371/journal.pcbi.1005355.

Krause AF, Winkler A, Dürr V. Central drive and proprioceptive control of antennal movements in the walking stick insect. *J Physiol Paris* 107: 116–129, 2013. doi:10.1016/j.jphysparis.2012.06.001.

Levakova M, Tamborrino M, Ditlevsen S, Lansky P. A review of the ethods for neuronal response latency estimation. *Biosystems* 136: 23–34, 2015. doi:10.1016/j.biosystems.2015.04.008.

Mujagic S, Krause AF, Dürr V. Slanted joint axes of the stick insect antenna: an adaptation to tactile acuity. *Naturwissenschaften* 94: 313–318, 2007.doi:10.1007/s00114-006-0191-1.

Sanchez-Vives MV, Nowak LG, McCormick DA. Cellular mechanisms of long-lasting adaptation in visual cortical neurons in vitro. *J Neurosci* 20:4286–4299, 2000a. doi:10.1523/JNEUROSCI.20-11-04286.2000.

Sanchez-Vives MV, Nowak LG, McCormick DA. Membrane mechanisms underlying contrast adaptation in cat area 17 in vivo. *J Neurosci* 20:4267–4285, 2000b. doi:10.1523/JNEUROSCI.20-11-04267.2000.

Schaefer PL, Ritzmann RE. Descending influences on escape behavior and motor pattern in the cockroach. *J Neurobiol* 49: 9–28, 2001. doi:10.1002/neu.1062.

Schmitz J, Büschges A, Delcomyn F. An improved electrode design for en passant recording from small nerves. *Comp Biochem Physiol A Comp Physiol* 91: 769–772, 1988. doi:10.1016/0300-9629(88)90963-2.

Schöneich S, Schildberger K, Stevenson PA. Neuronal organization of a fast-mediating cephalothoracic pathway for antennal-tactile information in the cricket (*Gryllus bimaculatus* DeGeer). *J Comp Neurol* 519: 1677–1690, 2011. doi:10.1002/cne.22594.

Schütz C, Dürr V. Active tactile exploration for adaptive locomotion in the stick insect. *Philos Trans R Soc Lond B Biol Sci* 366: 2996–3005, 2011. doi:10.1098/rstb.2011.0126.

Skaggs WE, McNaughton BL. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* 271: 1870 1873, 1996. doi:10.1126/science.271.5257.1870.

Stein BE, Stanford TR. Multisensory integration: current issues from the perspective of the single neuron. *Nat Rev Neurosci* 9: 255–266, 2008. [Erratum in *Nat Rev Neurosci* 9: 406, 2008.] doi:10.1038/nrn2331.

Tantirigama ML, Huang HH, Bekkers JM. Spontaneous activity in the piriform cortex extends the dynamic range of cortical odor coding. *Proc Nat Acad Sci USA* 114: 2407–2412, 2017. doi:10.1073/pnas.1620939114.

Trimmer BA, Weeks JC. Activity-dependent induction of facilitation, depression, and post-tetanic potentiation at an insect central synapse. *J Comp hysiol A Neuroethol Sens Neural Behav Physiol* 168: 27–43, 1991. doi:10.1007/BF00217101.

Ye S, Comer CM. Correspondence of escape-turning behavior with activity ofdescending mechanosensory interneurons in the cockroach, *Periplaneta americana*. *J Neurosci* 16: 5844–5853, 1996. doi:10.1523/JNEUROSCI.16-18-05844.1996.

Zhantiev RD, Chukanov VS, Korsunovskaya OS. Effect of temperature on spontaneous interneuron activity in grasshoppers *Tettigonia cantans* and *Metrioptera roeselii* (Orthoptera, Tettigoniidae). *J Evol Biochem Physiol* 42:686–690, 2006. doi:10.1134/S0022093006060044.

Zorovic´ M, Hedwig B. Processing of species-specific auditory patterns in the cricket brain by ascending, local, and descending neurons during standing and walking. *J Neurophysiol* 105: 2181–2194, 2011. doi:10.1152/jn.00416.2010

Chapter 3

One-way cross-modal adaptation in a descending interneuron of the Indian stick insect

Abstract

Sensory systems adjust their sensitivity through adaptation, i.e., by a history-dependent shift of the stimulus-response characteristic of their sensory neurons. This mechanism is found in many, if not all, neural sensory systems as it can improve coding efficiency in terms of discriminability of stimulus intensities. For example, in motion-encoding proprioceptors, adaptation to constant stimulation during continuous active movement could ensure high responsiveness to changes in movement speed. In the stick insect *Carausius morosus*, continuous active antennal movement occurs during locomotion such that an obstacle on the path can be detected. Therefore, contact of the antennae with an obstacle would lead to a reduction of antennal velocity. If adaptation could ensure high responsiveness of neurons that encodes the antennal velocity during locomotion, these neurons could also act as contact detectors. For animal foraging in the dark the detection of these contacts is necessary for their orientation. Here, we measure adaptation in an identified descending interneuron of the stick insect that conveys antennal proprioceptive information to the thoracic ganglia: The contralateral ON-type velocity-sensitive (cONv) interneuron. The soma of this interneuron is located in the gnathal ganglion (GnG) where it receives afferent input about antennal movement velocity as well as substrate vibration. Because of the two sensory input modalities of cONv, we asked whether cONv adapts independently to both modalities or rather shows cross-modal adaptation. We found that cONv adapted in the same manner regardless of the velocity applied and that adaptation was not specific to a particular velocity but was dependent on the stimulus intensity. Moreover, substrate vibration delivered prior to antennal movement could set cONv closer to adaptation, suggesting cross-modal adaptation.

Introduction

Adaptation is defined by a reduction of neuronal response to repeated stimulus presentation. In contrast, a reduction of the behavioural response to pertaining stimuli is called habituation (Hewson and Tarrega 2017; for a thorough definition of habituation see Rankin et al. 2009). One of the best-known examples of adaptation is given by the visual system. During the transition from a dark room to a bright exterior occurs a very large change of stimulus intensity such that local differences in image intensity cannot be encoded for across the entire, physiologically relevant intensity range. Accordingly, the systems rapidly adapt to the prevalent intensity range such that the local differences are detectable. Adaptation occurs in most if not all sensory system, avoids a system saturation, permits novelty detection and efficient coding (Wark et al., 2007; Triblehorn and Schul, 2013; Prešern et al., 2015). By shifting the stimulus-response characteristic from low to high intensities or vice versa, adaptation can increase the range of stimulus detection (Whitmire and Stanley, 2016). Adaptation is the underlying neuronal basis of habituation and habituation is thought to be simple for a learning. Both are present in different types of organism going from single cell organisms to humans (Rose and Rankin, 2001; Haupt and Klemt, 2005; Prešern et al., 2015; Castaldi et al., 2016). Reviews covering adaptation mostly focus on the visual system (Shapley and Enroth-Cugell, 1984; Clifford et al., 2007; Kohn, 2007; Wark et al., 2007; Weigelt et al., 2008; Rieke and Rudd, 2009) or on audition (Eggermont, 1985; Wark et al., 2007; Solomon and Kohn, 2014; Pellegrino et al., 2017) and others focus on different sensory systems (Dalton, 2000; Wilson, 2009). Computational models of adaptation show that its general mechanism is similar across sensory systems (Weber and Fairhall, 2019; Weber et al., 2019). Nevertheless, adaptation occurs in a variety of form. For instance Harris et al. (2000) identified two mechanisms explaining the sensory adaptation of wide field motion sensitive cells of flies to motion stimuli. On the one hand there is a subtractive process that is activated by stimuli exciting the neurons and on the other hand, there is a reduction in contrast gain that occurs even if the neurons are not excited by the adapting stimulus. Descalzo et al. (2005) showed that fast spiking neuron displayed spike frequency adaptation in the visual cortex of ferret if they are stimulated over longer periods of time. The spiking activity is decreasing with persisting activation without relationship with the stimulus intensity. Other than neurons of the visual system, mechanoreceptors can also show signs of adaptation (French and Torkkeli,

1994). For instance, two types of mechanosensory neurons innervating the slit sense organ of spiders were shown to adapt to cuticle deformation(Juusola and French, 1998). The adaptation of each type differed in their time course of recovery where one type recovered two times faster than the other (Torkkeli et al., 2001). Furthermore, when adaptation occurs as a response to stimulus from a class of compound and it is referred to as cross adaptation. In the gustatory system, responses to some types of sugars can adapt if the subject repeatedly tastes sucrose beforehand and similar effects have been observed for salts (Mcburney, 1972; Lawless and Stevens, 1983; Hewson and Tarrega, 2017). In these cases, the stimuli involved the same receptor mechanism across compound types that are responsible for adaptation. On the other hand, when adaptation occurs between different kinds of modalities, for instance between audition and vision, it is referred to as cross-modal adaptation. Cross-modal adaptation occurs in multisensory neurons that integrate information from more than one sensory afferent (Elliott et al., 2009; Wang et al., 2017). However, studies on cross-modal adaptation of multisensory neurons remains scarce.

In the stick insect, a pair of descending interneurons called cONv (Ache et al., 2015) is known to show signs of adaptation to repeated episodes of antennal movement (Lepreux et al., 2019). Since cONv is a bimodal descending interneuron that not only encodes antennal joint velocity but also responds to substrate vibration (Ache et al., 2015; Lepreux et al., 2019) we asked whether cross-modal adaptation occurs in this system. Indeed, contrary to the antennal input that descends from the brain, substrate vibration input reaches cONv in the Gnathal Ganglion (GnG, formerly known as Suboesophageal ganglion, Ito et al. 2014). Recently, we found that cONv reduced its spontaneous activity between episodes of sensory stimulation through antennal movements and/or substrate vibration (Lepreux et al., 2019). If the spontaneous activity was the result of fluctuating presynaptic drive, adaptation could reduce the overall sensitivity of the neuron and the presynaptic input would thus be less efficient to drive cONv spiking. Moreover, the pair of giant descending cONv interneurons are part of a pathway that connects the brain to the thorax. This pathway is hypothesized to play a role in the reach to grasp behaviour of the animal upon antennal contact described by Schütz and Dürr (2011). As stick insects continuously move their antennae while walking, adaptation could affect the ability of the animal to detect obstacles. Here we study history-dependent effects on cONv activity by repeated stimulation with antennal movement and substrate vibration, using bilateral extracellular neck connective recordings in quiescent

animals. In particular, we asked how adaptation depends on stimulus intensity and how it is affected by various kinds of deviant stimuli, including i) a change in antennal movement velocity, ii) movement of the other (ipsilateral) antenna, or iii) substrate vibration. We found that adaptation is a robust effect that could be induced by both modalities tested. While adaptation to antennal movement did not affect the response to our substrate vibration stimuli, cross-modal adaptation occurred the other way round.

Methods

Animals

Adult female stick insects (*Carausius morosus),* bred at Bielefeld University were used. The legs of the animal were removed, and the animal was immobilized on a balsa wood platform and both the head and the head-scape joints of both antennae were fixed using dental glue (Protemp, 3M EPSE, Neuss, Germany). Incision along the dorsal midline was made with razor blade and the neck connectives was accessed after removal of 1/3 of the head capsule and ablation of the underlying muscles. The oesophagus and gut were lifted out and gently placed aside of the body cavity, which was then filled with stick insect saline (NaCl: 180 mM, KCl: 4 mM, $CaCl_2$ * $2H_2O$: 5 mM, $MgCl_2$ * $6H_2O$: 1 mM, HEPES: 10 mM, Sucrose: 10mM).

Recording and stimulation

Extracellular recording. Tungsten hook electrodes for en-passant whole-nerve connective recordings (Schmitz et al., 1988) were amplified and filtered (MA-101, MA-102, Electronics workshop, University of Cologne, Germany) and sampled at 10 kHz with an A/D converter (CED Micro1401) by use of Spike2 software (both by Cambridge Electronic Design, Cambridge, UK). Reference electrodes were placed in the saline-filled body cavity of the animal.

Scape-pedicel (SP) joint movement. Antennae were moved in their natural joint angle and velocity range. Antennal movement was induced passively and contact-free by means of a magnet that was mounted on a motor potentiometer (Inelta, 12V DC, Megatron, Munich, Germany). This system moved a minutien pin that had been inserted into the stump of the flagellum. The deflection of the SP-joint (velocity and angular range) was controlled by a custom written script in Spike2 through D/A converter channel of the Micro1401 system. The antennal movement time course followed a staircase of four consecutive ramp-and-hold deflections, i.e., from rest to dorsal, dorsal to rest, rest to ventral and ventral to rest (Figure

19). In the bilateral movement paradigm (Figure 19C), due to the configuration of the two motor potentiometers and the juxtaposition of the two antennae, the movement range of each antenna was halved to either the dorsal or the ventral part of their natural action range. Although the movement of the antenna was halved, a staircase still consisted of four consecutives ramp-and-hold displacements.

*Substrate vibration. S*ubstrate vibration was induced by taps onto the recording table using a solenoid (Intertec, ITS-LZ 1335) at frequencies of 1 Hz, and 10 Hz. Tapping frequency was controlled through a digital output channel of the CED Micro1401 system (for details see Lepreux et al., 2019).

Experimental design.

Throughout this study we used a total of five experimental paradigms that differed in the treatment between blocks of antennal movement (Figure 19): treatments comprised either ramp-and-hold movement of the antenna at one of two velocities, a period of rest, or substrate vibration. Each paradigm was tested with a separate group of animals. In total, 78 animals were used. Where stimulus parameters were varied, this was done in a pseudorandom order.

“Pause paradigm”: A pause of 60 seconds without stimulation was inserted between two blocks of antennal movement at the same velocity (Figure 19A).

“Deviant velocity paradigm”: A treatment block with antennal movement at a different velocity was inserted between two blocks of antennal movement at the same velocity (Figure 19B).

“Ipsilateral antenna paradigm”: A treatment block with movement of the ipsilateral antenna with a ramp-and-hold stimulus at 300 deg/s. Although the cONv neurons sometimes look like they respond to movement of their ipsilateral antenna, Fig *S1 and S2* show that only the cONv contralateral to the antenna responds reliably (e.g., see Figure 20). Since the adapting stimulus of blocks 1 and 2 were always applied to this contralateral antenna, the treatment of the “ipsilateral antenna paradigm” involved movement of the opposite antenna, i.e., ipsilateral to the recorded cONv axon (Figure 19C). Figure 20 shows an example of activity of both cONv during the first staircase of each block of stimulation (i.e.: block 1: contralateral antenna, treatment: ipsilateral antenna, block 2: contralateral antenna).

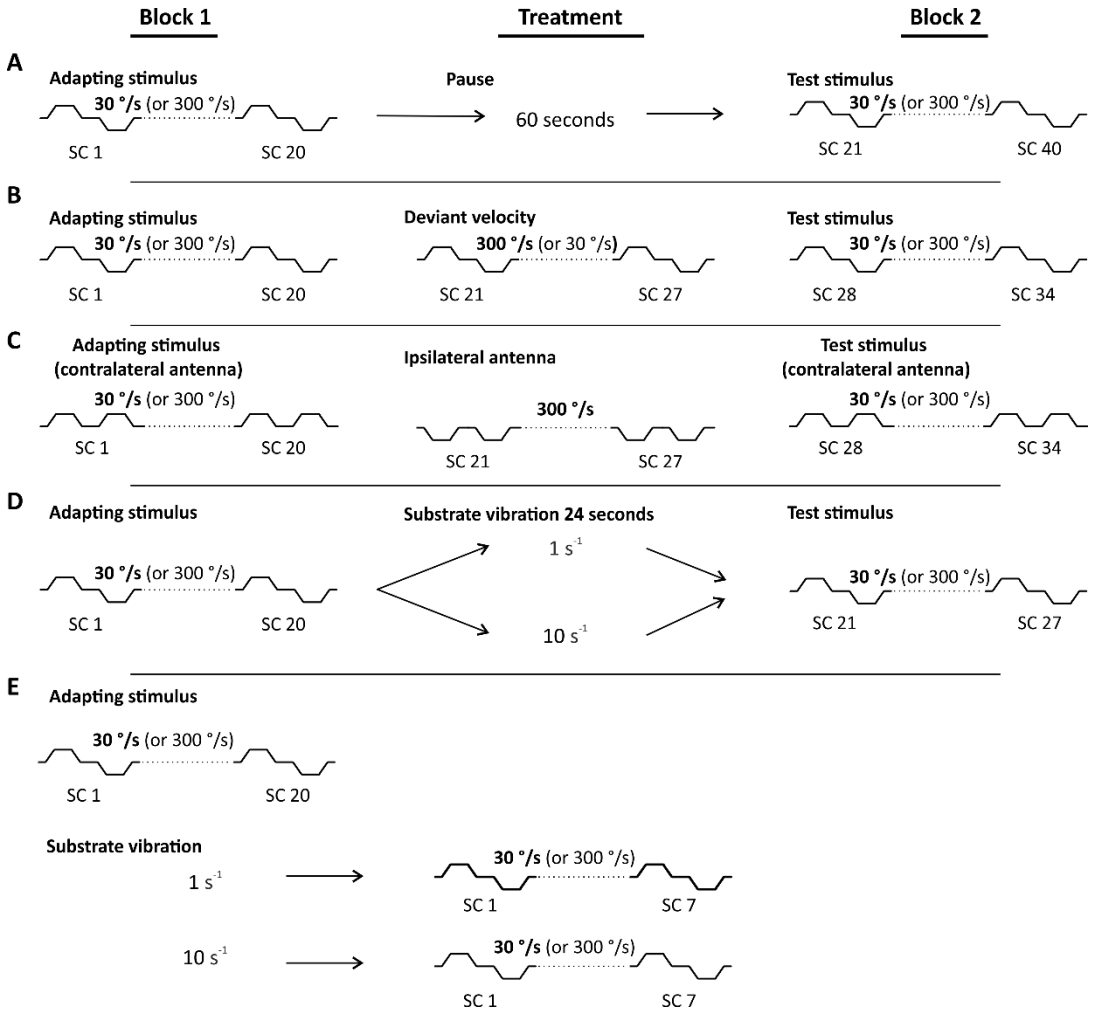


Figure 19: Experimental design.

The experiment consists of 2 blocks of antennal movement stimulation that are separated by a treatment (except E where only the trailing block was used).

A) Pause paradigm: Block 1: The antenna was moved for 20 staircases (SC 1 to SC 20) with ramp-and-hold deflections at a velocity of either 30 deg/s or 300 deg/s. Treatment: The antenna was held at its anatomical resting position for 60 s. Block 2: As block 1 (SC 21 to SC 40).

B) Deviant velocity paradigm: Block 1 as in "pause paradigm" (A). Treatment: Without delay, the same (contralateral) antenna was moved for 7 SC at a higher or lower velocity than in block 1 (SC 21 to SC 28; 300 deg/s after adaptation to 30 deg/s; 30 deg/s after adaptation to 300 deg/s). This velocity is referred to as "deviant velocity" as it differed from the velocity of the adapting stimulus in block 1. Block 2: As Block 1 except that only 7 SC were used (SC 28 to SC 34).

C) Ipsilateral antenna paradigm: Block 1: As in A and B except that the movement range was limited to the dorsal half (from + 10° to + 50°). Treatment: The opposite antenna (ipsilateral to the cONv adapted during block 1) was moved at 300 deg/s. The movement was limited to the ventral half (-50° to -10°). Block 2: As Block 1, except that the antenna was moved for 7 staircases (SC 28 to SC 34).

D) Vibration paradigm 1: Block 1: Same as in A and B. Treatment: Immediately following the last staircase of block 1, substrate vibration was induced by tapping frequencies of either $1s^{-1}$ or $10\ s^{-1}$ for 24 s. Block 2: As block 1 but only 7 SC (SC 21 to SC 28).

E) Vibration paradigm 2: Control: As block 1 in A and B (SC 1 to SC 20). Treatment: After pause of at least 180 s, the same substrate vibration treatment as in D was applied. Block 2: As control, but with only 7 SC (SC 21 to SC 28).

For illustration purposes the dashed lines represent the same ramp-and-hold stimulus repeated until the last staircase of the same sequence (for example SC 2 to SC 19 in block 1 of A).

"Vibration paradigm 1": A treatment of substrate vibration for 24 s with a tapping frequency of either 1 s^{-1} or 10 s^{-1} was inserted between the two blocks of antennal movement at the same velocity (Figure 19D).

"Vibration paradigm 2": The treatment consisted of a long pause (3 minutes) followed by substrate vibration with tapping frequencies of 1 s^{-1} or 10 s^{-1} for a period of 24 seconds. After that a test block of antennal movement at the same velocity as before was applied (Figure 19E).

Note that for each paradigm, the delay before the first set of stimulation and between a new set of stimulation was 180 seconds in order to allow full recovery to rest and to avoid any history-dependent effects on cONv activity. Within each trial, there were no delays between the blocks 1 and 2 and the treatment.

Data analysis

Spike detection. For a detailed description of the data analysis see Lepreux et al, 2019. In brief, cONv spike detection was done offline in Spike2 by applying a threshold that was decided by the experimenter individually for each neuron (two neurons per preparation), based on the quality of the signal-to-noise ratio and on the spike amplitude, the bilateral response of cONv to substrate vibration and unilateral response to deflection of the contralateral antenna. Data were then saved as MATLAB files and analysed with custom-written MATLAB scripts (version 9.2, MathWorks R2017a).

Spike rate. The spiking activity of cONv was described by a mean spike rate per staircase. It was calculated by averaging the total spike count during the four consecutive ramps per staircase for both cONv.

Box–whisker plots. The limits of the boxes were drawn at the first and third quartiles of the distribution (Q1 and Q3 respectively), thus comprising the inter-quartile range (IQR), while whiskers show the minimum and the maximum values of the distribution. Data points beyond 1.5*IQR below or above the IQR box were plotted as outliers (crosses). The median was plotted as a white bar.

Confidence interval of the median. The variation of the recorded responses is shown as a shaded area. Its limits were calculated according to the following formulae:

The 95 % upper limit: $m + 1 + \frac{n}{2} + \frac{1.96 * \sqrt{n}}{2}$ *; The 95% lower limit:* $m - \frac{n}{2} - \frac{1.96 * \sqrt{n}}{2}$*;*

where, 'm' is the median of the data set and 'n' the sample size.

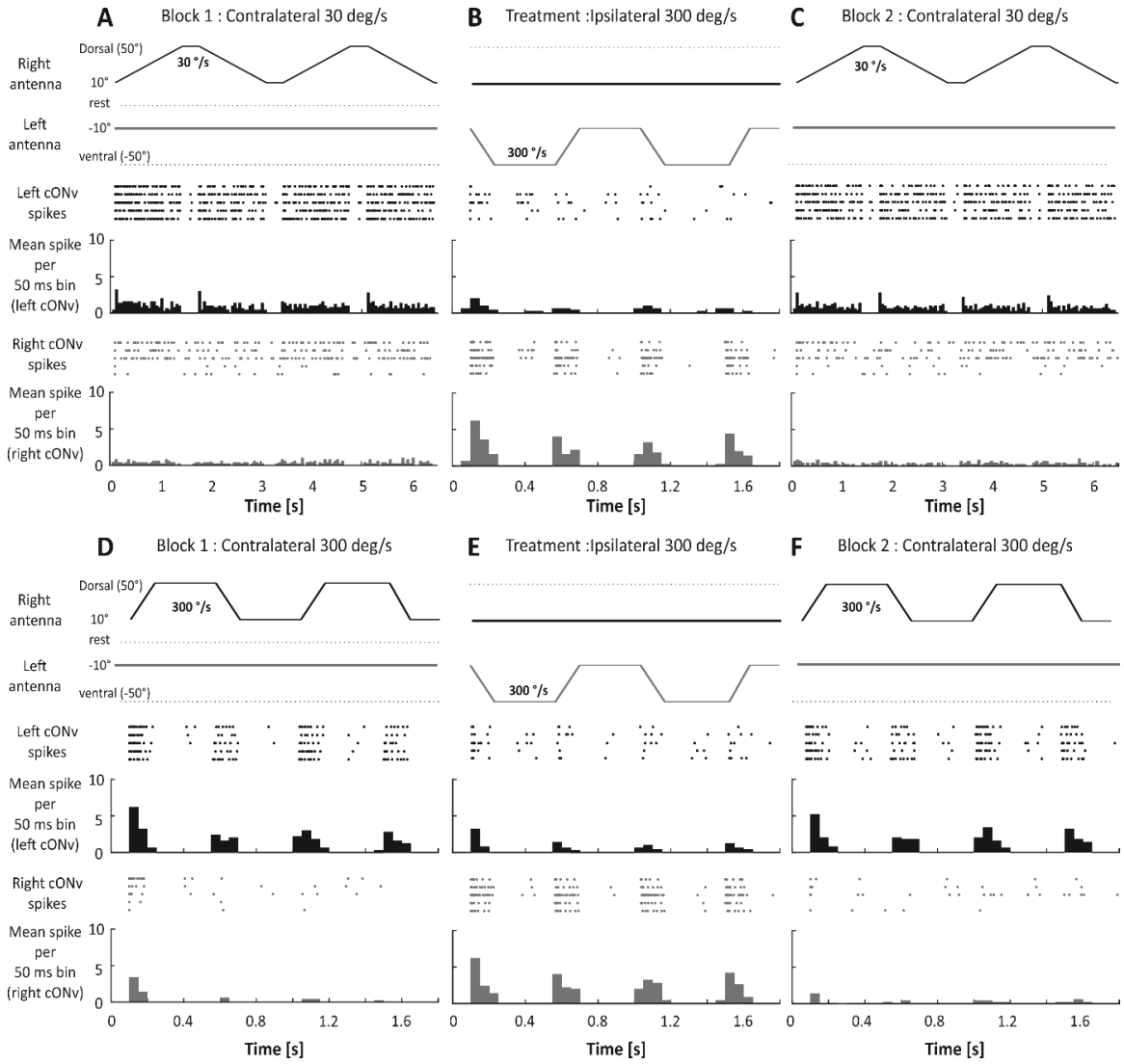


Figure 20: Example data from 5 animals of the "ipsilateral antenna paradigm"

The top rows show the ramp-and-hold stimulus for both the right and left antenna (black and grey lines respectively). The anatomical resting position of the antennae (rest) and the extreme dorsal and ventral position are shown as a dashed line where necessary. The velocity of the ramp-and-hold stimulus is noted next to the ramps. The raster plots show the spiking activity of the right and left cONv (black and grey dots, respectively) in 5 animals (one line per animal) to: i) staircase SC 1 of the right (contralateral) antenna at 30 deg/s (A) and at 300 deg/s (D), ii) staircase SC 21 of the left (ipsilateral) antenna at 300 deg/s (B, E), and iii) staircase SC 28 of the right (contralateral) antenna at 30 deg/s (C) or 300 deg/s (F). Note that, for illustration purposes, only 1 staircase of block 1 (A and C), treatment (B and E) and block 2 (C and F) are depicted here, despite the fact that the trial consisted of 34 staircases, as described in Figure 19C. Histograms show the mean number of spike from the right and left cONv (black and grey bars respectively) per 50 ms bins. The treatment (B and E) was movement of the left (ipsilateral) antenna using ramp-and-hold stimulus at 300 deg/s. N = 5 animals with n = 1 trial per animal.

S*tatistical analysis.* To assess the statistical significance of the results, Wilcoxon's signed rank test for matched pairs was calculated using MATLAB. Throughout this study, we compared the activity of cONv during the first staircase and the last staircase of the block 1 to determine the degree of adaptation. The activity during these two staircases were then compared to the activity during the first staircase of block 2, so as to quantify the effect of the treatment. In the fifth paradigm, the activity of the staircase that followed substrate vibration (SC 1) was compared to the activity of the first and last staircases of the control. For clarity, the staircase numbers and their corresponding antennal movement velocities will be mentioned as follows: SC_{30} 21 = Staircase number 21 at a velocity of 30 deg/s.

Results:

cONv responses to ramp-and-hold attenuate over time.

To determine whether cONv firing rate adapted to repeated antennal movement we started by presenting a succession of ramp-and-hold stimuli at a velocity of 30 deg/s or 300 deg/s. We calculated cONv activity as the mean firing rate per staircase (SC) where each staircase consists of 4 consecutive ramps of a ramp-and-hold stimulus sequence (see methods).

Figure 21 shows that regardless of the velocity applied, cONv activity decreased with repeated antennal movement (Figure 21A, Figure 21B). When the antenna was moved at 30 deg/s, there was a significant reduction of the median activity between the first and the last staircase (-32%, SC_{30} 1 = 16.5 s^{-1}; SC_{30} 20 = 13.5 s^{-1}; P = 0.0058, N = 19 animals).

At a velocity of 300 deg/s cONv activity for the last staircase was lower than for the first staircase (-35 %, SC_{300} 1 = 57 s^{-1}; SC_{300} 20 = 37.5 s^{-1}; P = 1.29*10^{-4}, N = 19 animals). We conclude that cONv showed adaptation at both high and low antennal velocities.

Is cONv recovering from adaptation?

Having found that cONv adapted during both 30 deg/s and 300 deg/s antennal movement, we needed to determine the time it takes for recovery. To test if the response intensities of the neuron could come back to its initial level, we introduced a resting period (no movement) of 60 s between two blocks of 20 staircases (Figure 19A, Figure 21). If the responses to the first staircase from each block before the pause (block 1, SC 1) and after the pause (block 2, SC 21) were not significantly different, we would call it "complete recovery". However, if the response to the first staircase of block 2 (*i.e.* after the pause) was lower than

for the first staircase though still higher than for the last staircase of block 1, we would call it “incomplete recovery”. Finally, if the response to the first staircase of block 2 was not significantly different from that to the last staircase of block 1, there would be “no recovery” at all.

After a break of 60 s without stimulation, the spike rate of cONv at 30 deg/s to the first staircase (SC 21) was significantly higher than that to the last staircase of block 1 (SC 20; +26%; P = 0,0017; SC_{30} 20 = 13.5 s^{-1} ; SC_{30} 21 = 19.5 s^{-1}, Figure 21A). Since there was no significant difference of activity between the first staircase of each block of stimulation (SC 1, SC 21; P = 0.79) we conclude that there was complete recovery after a 60 s pause. At a velocity of 300 deg/s, the response of cONv to the first staircase following the pause was significantly higher than the last response before the pause (+ 27 %, P = 1.30*10^{-4}, SC_{300} 21 = 54 s^{-1}; SC_{300} 20 = 37.5 s^{-1}, Figure 21B), thus indicating recovery. However, since the response of cONv to SC_{300} 21 was significantly lower than that to SC_{300} 1 (-9 %; P = 0,016), the recovery was incomplete. To allow for more time for full recovery and to avoid stimulus history-dependent modulation of cONv in the subsequent experiments, we tripled the duration of the pause to 180 s before starting a new block of stimulation.

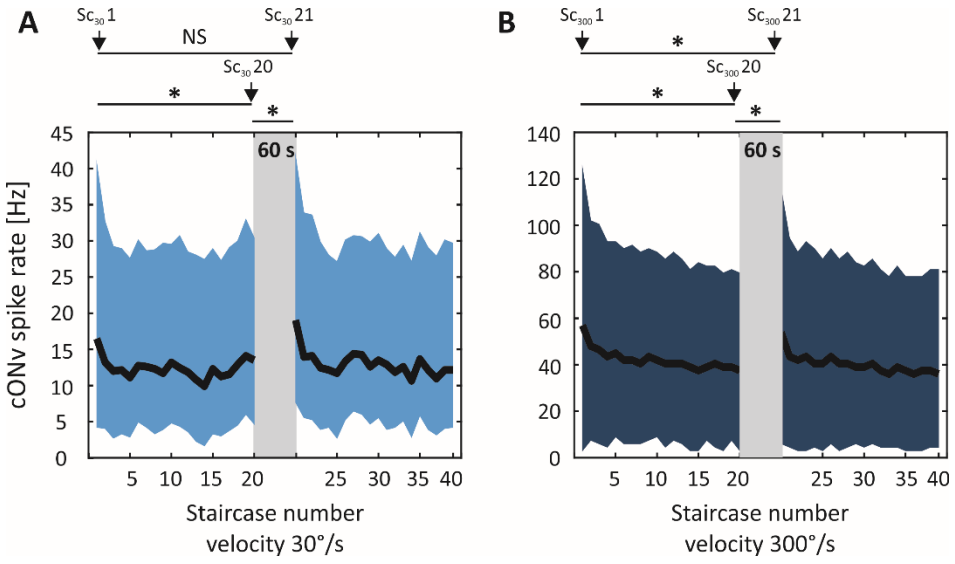


Figure 21 : Recovery from adaptation after a 60 s pause.

A: Response of cONv to repeated antennal movement (staircase, ‘SC’) at velocities of 30 deg/s (light blue) and B: 300 deg/s (dark blue). The grey bars represent a pause of 60 s where no stimulus was given. Shaded areas show the 95 % confidence interval of the median. The black lines show the median cONv activity for each staircase. Arrows show the staircases we used for statistical comparison in the following figures. SC_{30} 1 means: first staircase at a velocity at 30 deg/s while SC_{300} 30 means: Staircase number 20 at a velocity of 300 deg/s. Statistically significant results (P> 0.05) are represented by a star. N = 19 animals.

Effect of deviant velocity on adaptation.

Since cONv showed adaptation to repeated stimulation and recovered after a sufficiently long pause of stimulation, we asked whether cONv responses would recover faster when a deviant, new velocity is introduced after the adaptation block 1. We used a paradigm of standard and deviant velocity as shown in Figure 19B.

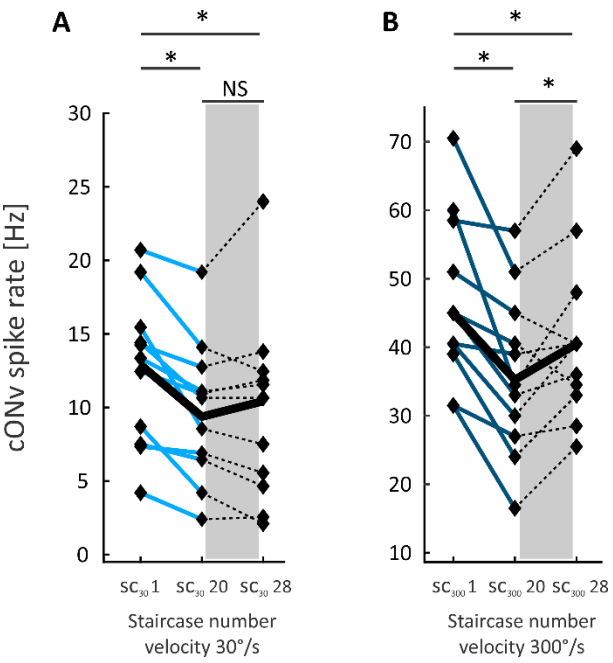


Figure 22: cONv may recover from adaptation despite stimulation with a deviant velocity. *Symbols show the spike rate of cONv during the corresponding staircases at velocities of 30 deg/s (light blue, A) and 300 deg/s (dark blue, B). Lines connect the data points belonging to the same animal. The dotted lines connect data points before (SC 20) and after (SC 28) deviant velocity stimulus (shown as grey area). Responses of the neuron to the deviant stimulation are shown in Fig. S1. The black lines are the median activity during each staircase. N= 16 animals.*

As before, cONv adapted as it responded more strongly to the first staircase, irrespective of the adapting velocity (SC_{30} 1 = 12.9 s^{-1}, SC_{30} 20 = 9.22 s^{-1}, P = $4.88*10^{-4}$; SC_{300} 1 = 45 s^{-1}, SC_{300} 20 = 33.75 s^{-1}, P= $4.88*10^{-4}$, Figure 22A). cONv did not recover from adaptation if the deviant velocity was faster than the adapting velocity (adapt: 30 deg/s, deviant: 300 deg/s). As Figure 22A shows, the response of cONv to the first staircase after the deviant velocity of 300 deg/s was not significantly different from its response to the last staircase of block 1 at 30 deg/s (SC_{30} 28 = 10.42 s^{-1}, P = 0.913; see supplementary Fig. S1A for responses to deviant stimuli). Conversely, after adapting to 300 deg/s, there was incomplete recovery from adaptation during stimulation with a lower deviant velocity (SC_{300} 28 = 35.25 s^{-1}; SC_{300} 20 = 33.75 s^{-1}; P = $9.765*10^{-4}$ Figure 22B). Thus, cONv may recover from adaptation at least partially even during persistent stimulation with antennal movement, provided that the deviant velocity was slower and, that, the induced spike rate was lower than for the adapting stimulus.

Velocity-specific adaptation.

Next, we were interested to know if the response of cONv to the deviant velocity was different to its naïve response or to its adapted response level for the same velocity (i.e. first and last staircase of a block 1). Thus, we compared the activity of cONv during the first staircase of the deviant velocity (SC_{300} 21, Figure 22A) to its activity during the first and last staircase of block 1 for the same velocity (SC_{300} 1 and SC_{300} 20, Figure 22B; and corresponding staircases for 30 deg/s). We reasoned that, if adaptation was velocity-specific, cONv should respond to the deviant velocity with the same intensity as if it would be stimulated at this velocity for the first time. The response to the deviant stimulus was the same as after adaptation (deviant SC_{30} 21 = 9.75 s^{-1}; naïve SC_{30} 1 = 12.9 s^{-1}, P= 0.0025; adapted SC_{30} 20 = 9.22 s^{-1}, P = 0.5, Figure 22A). Thus, after antennal movement at 300 deg/s, the response of cONv to a staircase at 30 deg/s was adapted. This was different for the higher deviant velocity as the response to the deviant velocity of 300 deg/s (deviant SC_{300} 21= 38.25 s^{-1}) was significantly lower than its non-adapted response (naive SC_{300} 1 = 45 s^{-1}, P = 0.0039) and significantly higher than its adapted response (adapted SC_{300} 20 = 33.75 s^{-1}, P = 0.0068). Thus, antennal movement at 30 deg/s before movement at 300 deg/s partially adapted cONv to movement at 300 deg/s. Altogether these results show that the neuron does not specifically adapt to one velocity. Rather, the observed effects are consistent with the idea that strength of adaptation depends in stimulus intensity, such that movement at 30 °/s causes weaker adaptation than movement at 300 °/s.

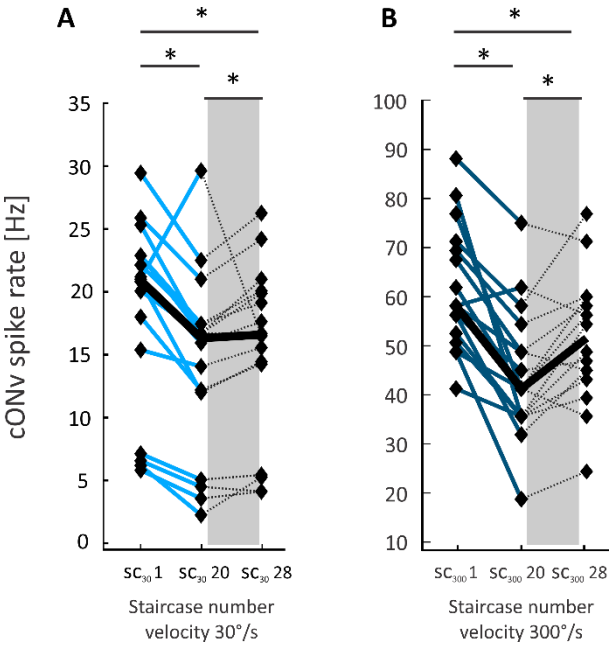


Figure 23: cONv partially recovers from adaptation during movement of the ipsilateral antenna.

Symbols show the spike rate of cONv during ramp-and-hold stimuli at A) 30 deg/s (light blue) and B) 300 deg/s (dark blue). Lines connect data points belonging to the same animal. The dotted lines connect data points before (SC 20) and after (SC 28) movement of the ipsilateral antenna (shown as grey area). Responses to ipsilateral antennal movement are shown in Fig.2 and suppl. Fig. S2). The black lines show the median activity during each staircase. N = 16 animals.

Movement of the ipsilateral antenna

Although there is no evidence of interconnection between the two cONv with respect to their antennal input, we showed in a previous study (Lepreux et al., 2019) that the resting activities of both cONv are highly correlated (although fluctuating) and that sensory activation can reduce spontaneous activity. Note that there is one cONv neuron per hemiganglion (Ache et al., 2015). Thus, we asked if stimulation of the ipsilateral antenna, i.e. the antenna cONv does not respond to, can modulate the adaptation of cONv. Similar to the paradigm used in the previous section we inserted a movement stimulus between blocks 1 and 2, except that ipsilateral antenna was moved (Figure 19C, Figure 20). In order to avoid confusion, we will refer only to the cONv stimulated in blocks 1 and 2 (for activity of the other, ipsilateral cONv neuron see supplementary Fig. S2 and Figure 20B, Figure 20E).

Similar to. Figure 21A, Figure 23A confirms that movement at 30 deg/s adapts cONv activity (-25.91 %, SC_{30} 1 = 20.90 s^{-1}, SC_{30} 20 = 16.31 s^{-1} P = 5.27*10^{-4}). After movement of the ipsilateral antenna for 7 SC, the response to the first staircase of block 2 was significantly lower than for the first staircase of block 1 (-16%, SC_{30} 1 = 20.90 s^{-1}; SC_{30} 28 = 16.59 s^{-1}; P= 5.26*10^{-4} , Figure 23A) and significantly higher than the response to the last staircase of block 1 (+10 %, P = 0.01, Figure 23A), indicating incomplete recovery from adaptation. The results were similar for antennal movement at 300 deg/s. cONv adapted during block 1 (-24.7 %, SC_{300} 1 = 58.12 s^{-1}, SC_{300} 20 = 41.25 s^{-1} P = 5.28*10^{-4}, Figure 23B) and after moving the ipsilateral antenna, the response to the first staircase of block 2 was significantly stronger than for the last staircase of block 1 (SC_{300} 20 = 41.25 s^{-1}, SC_{300} 28 = 51.56 s^{-1}, P = 0.01, Figure 23B), but still lower than for the first staircase of block 1 (-18%, SC_{300} 1 = 58.12 s^{-1}; SC_{300} 28 = 51.56 s^{-1}; P = 0.0021,Figure 23B). Since this was the same as observed in Figure 21, we conclude that movement of the ipsilateral antenna may have a weak effect on the state of adaptation of the contralateral cONv as it may slow or even prevent full recovery from adaptation.

Cross-modal adaptation through substrate vibration

As cONv not only responds to antennal movement but also to substrate vibration (as induced by tapping on the recording table; Ache et al., 2015; see also Fig. S3) and we previously found a weak cross-modal effect on background activity of cONv (Lepreux et al., 2019), we tested whether substrate vibration could also affect the recovery of the neuron from adaptation. To assess this, we used a paradigm in which we inserted an episode of

substrate vibration between blocks 1 and 2 (24 s, at a tapping frequency of 1 s^{-1} or 10 s^{-1}, Figure 19D). We reasoned that an effect of substrate vibration on adaptation of cONv should result in a deviation from the partial or full recovery seen in Figure 21. As before, the neuron adapted when the antenna was moved at 30 deg/s (- 26%, SC_{30} 1 = 12.37 s^{-1}, SC_{30} 20 =7.8 s^{-1}, P = 0.002) or at 300 deg/s (- 27%, SC_{300} 1= 46.5 s^{-1}, SC_{300} 20= 30.75 s^{-1}, P = 0.002;Figure 24).

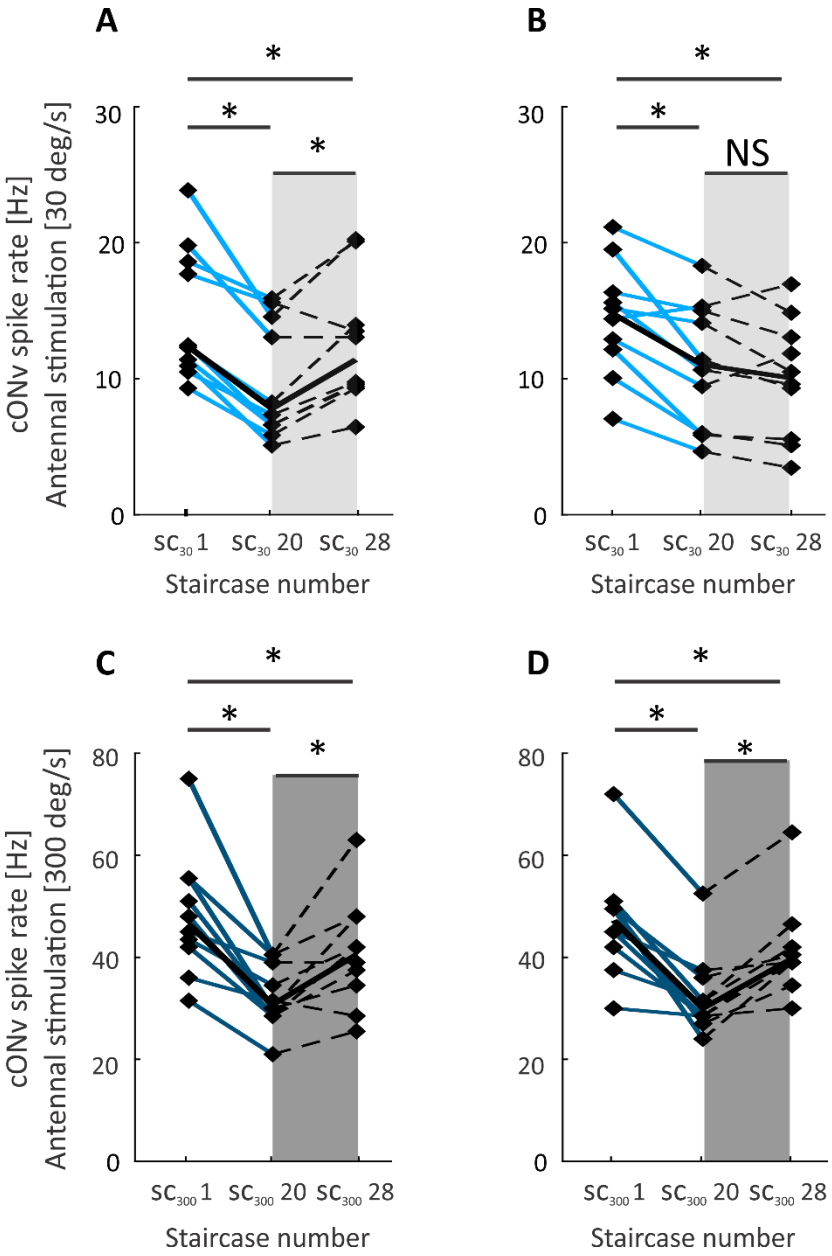


Figure 24: Substrate vibration may interfere with recovery from adaptation.

A: Response of cONv to antennal movement at 30 deg/s (light blue) before (SC_{30} 1 and SC_{30} 20) and after (SC_{30} 21) substrate vibration at a tapping frequency of 1 s^{-1} (light grey). B as in A, but the tapping frequency was 10 s^{-1} (dark grey). C and D: as in A and B respectively but the antenna was moved at a velocity of 300 deg/s (dark blue). Lines are connecting the data points belonging to the same animals. Symbols show the firing rate during the staircases number 1, 20 and 21. Staircase 21 corresponds to the first antennal movement after substrate vibration (grey boxes). N = 12 animals.

After substrate vibration at 1 s^{-1}, the response to the first staircase of block 2 was significantly higher than that to the last staircase of block 1 (SC_{30} 21 = 11.4 s^{-1}, SC_{30} 20 =7.8 s^{-1} P =0.017, Figure 24A) and significantly lower than that to the first staircase of block 1 (SC_{30} 1 = 12.37 s^{-1}, P = 0.0059; Figure 24A), thus indicating partial recovery from adaptation. We observed the same effect when substrate vibration at 1 s^{-1} was presented between antennal movement at 300 deg/s (Figure 24C). Again, the first staircase of block 1 caused the highest spike rate, the adapted response was lowest and the response after substrate vibration was in between (SC_{300} 1= 46.5 s^{-1}, SC_{300} 20= 30.75 s^{-1}, SC_{300} 21 = 40.5 s^{-1}, Figure 24C). Thus, the effect of a tapping frequency of 1 s^{-1} was the same as that for movement of the ipsilateral antenna: it affected recovery from adaptation in case of the 30 °/s movement.

These results were different for substrate vibration induced by a tapping frequency of 10 s^{-1}. In this case, the response to the first staircase of block 2 was not significantly different than the adapted response to the last staircase of block 1 (SC_{30} 20 = 11.02 s^{-1}, SC_{30} 21 = 10.05 s^{-1}, P =0,1602, Figure 24B).

If the adapting velocity was 300 deg/s, the results were similar to what we observed for the lower tapping frequency: the response to the first staircase of block 2 was 15 % higher than it was just before substrate vibration (SC_{300} 20 = 30 s^{-1}, SC_{300} 21 = 39.75 s^{-1}, P = 9.7656*10^{-4}, Figure 24D) but still significantly lower than for the first staircase of block 1 (-10%, SC_{300} 1 = 47.25 s^{-1}, SC_{300} 21 = 39.75 s^{-1}, P = 0.017, Figure 24D). Thus, at that velocity, the neuron partially recovered during the substrate vibration stimulus. Altogether, these results indicate that substrate vibration may interfere with the recovery of cONv from adaptation. This, however, raised the question as to whether cONv would adapt to the presentation of the substrate vibration stimulus alone.

Can substrate vibration alone pre-adapt cONv?

To test whether substrate vibration alone could adapt cONv we applied the same substrate vibration stimuli as before, but without prior antennal movement (Figure 19E). As a control, block 1 with a set of 20 staircases was presented once, followed by a long break of 180s to ensure full recovery from adaptation (Figure 19E). Thus, we compared the spike rate of cONv after substrate vibration to its response to an isolated stimulus block of antennal movement.

As usual cONv adapted to antennal movement at either velocity during the control block (Figure 25A, D). There was a decrease of -35.2 % of activity at 30 deg/s and a decrease of -39.4% at 300 deg/s.

After tapping at $1s^{-1}$ the response to antennal movement at 30 deg/s was not significantly different from the response to the first staircase of the control (SC_{30} 1 test = 12.75 s^{-1}, SC_{30} 1 control = 13.2 s^{-1} , P = 0.21, Figure 25A, B) but significantly higher than the response to the last staircase of the control condition (SC_{30} 20 control = 8.55 s^{-1}, P = $1.22*10^{-4}$; Figure 25A, B). The results were similar for antennal movement at 300 deg/s (SC_{300} 1 test = 48 s^{-1}, SC_{300} 1 control = 49.50 s^{-1}, P = 0.2146, SC_{300} 20 control = 30 s^{-1}, P = $6.135*10^{-5}$; Figure 25D,E).

Since the activity of cONv after substrate vibration at $1s^{-1}$ was not different from the naïve response of the control condition, we conclude that a tapping frequency of $1s^{-1}$ does not change the adaptation rate of cONv. However, after tapping at 10 s^{-1}, the response to antennal movement at 30 deg/s was significantly lower than during the first staircase of the control (SC_{30} 1 test = 11.2 s^{-1}, SC_{30} 1 control = 12.75 s^{-1} P = 0,0104; Figure 25A, C) and significantly higher than the adapted response at 30 deg/s (SC_{30} 20 control = 8.55 s^{-1}, P = 0.0202, Figure 25A, C). The same effect was observed when the antenna was moved at 300 deg/s (SC_{300} 1 test = 39 s^{-1}, SC_{300} 1 control = 49.5 s^{-1}; P = 0.0015; SC_{300} 20 control = 30 s^{-1}, P = $6.1035*10^{-5}$, Figure 25D, F). Moreover, cONv underwent adaptation during the 7 staircases of movement stimuli in block 2. Since the response of cONv immediately after the substrate vibration stimulus in Figure 25D to F fell between the first, naive response of the control block and the last, adapted response of the control block we conclude that substrate vibration may indeed pre-adapt cONv, though only for sufficiently high stimulus intensities (i.e. tapping frequency).

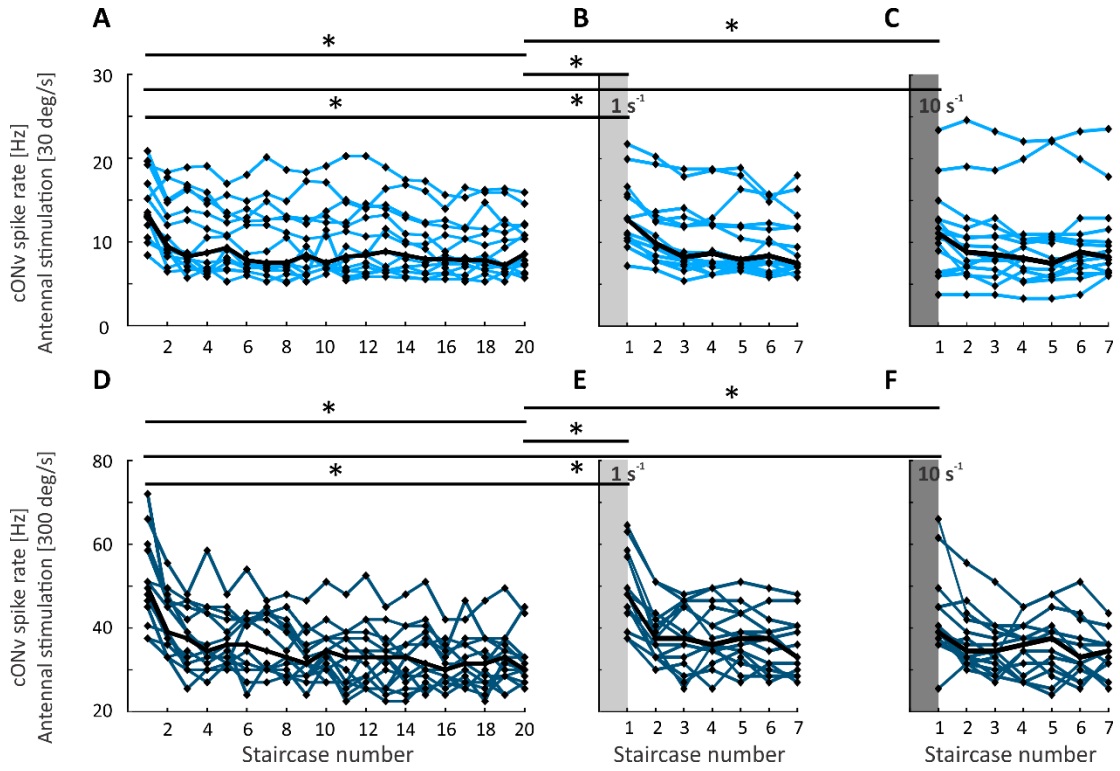


Figure 25: Substrate vibration may pre-adapt cONv.

Top row: Response of cONv to antennal movement at 30 deg/s (Light blue). Bottom row: Response of cONv to antennal movement at 300 deg/s (dark blue). A: Response of cONv to 20 staircases at 30 deg/s. B: cONv activity during 7 staircases at 30 deg/s following 24 s of substrate vibration at a tapping frequency of 1 s^{-1} (light grey area). As in B, but after 24 s of substrate vibration at a tapping frequency of 10 s^{-1} (dark grey area). D, E, and F: As in A, B and C respectively, except that the antennal stimulus was 300 deg/s. Black lines show the median response for each staircase. Diamond symbols depict the spike rate per staircase. N= 15 animals.

Discussion

Adaptation is not complete

cONv responses to the staircase composing the ramp-and-hold stimulus significantly decreased by - 32 % between the first staircase (SC 1) and the last staircase (SC 20) at a velocity of 30 deg/s. At 300 deg/s the responses intensity of cONv decreased by -35 %. The absolute decrease at both velocities is in the same range however the mean spike rate during the movement at 300 deg/s is higher than 30 deg/s (Fig. S1A and S1B). Thus, the reduction of activity in term of number of action potentials is greater during the adaptation at 300 deg/s. This shows that the decrement of activity depends on the stimulus intensity. However, even under high intensity stimulation, cONv did not show complete adaptation (e.g. no spiking activity to the stimulus) but reached a steady state. Conversely, French and Torkkeli (1994)

showed that a sensory neuron of the cockroach fully adapts. The receptor neuron decreased its firing rate until no action potentials were detectable. Contrary to the sensory neuron in the cockroach, cONv is located is at least one synapse away from the antennal mechanoreceptor (Ache et al., 2015) and in our study the stimulator alternates between ramps (movement) and holds (no movement). One explanation could be that the alternation between activation (movement) and rest (no movement) is sufficient to prevent the complete adaptation of the sensory cell. However, Ache and colleagues (2015) used sinusoidal waveform stimulation and complete adaptation did not occur (see their figure 6). Nonetheless, the adaption measured in our study is a very robust effect. For each paradigm, a new group of animals was used, and a significant decrement of cONv activity at both velocities was measured (Figure 21 to 25).

cONv recovers spontaneously from adaptation

After 60 s of rest the neuron showed a recovery of its response to the 30 deg/s stimulus (Figure 21A). A recovery of activity after 60 s of rest has also been reported by Trimmer and Weeks (1991) for hair sensory neurons of the larval *Manduca sexta* and in other sensory neurons of the drosophila (Corfas and Dudai, 1990). However, at a velocity of 300 deg/s the recovery of the neuron was only partial (Figure 21B) meaning that for a higher intensity stimulus the recovery of the response to its initial level takes more time than when the stimulus intensity is lower.

After movement of the ipsilateral antenna the neuron also partially recovered from adaptation. One possibility is that a full recovery could have been prevented during movement of the ipsilateral antenna. However, the duration of the ipsilateral movement is 15 s which is under the 60 s required for full recovery after 30 deg/s. It is thus possible that the timing for recovery is the same for both the 60 s pause and the ipsilateral movement with the difference being that the ipsilateral movement duration was shorter.

Adaptation is cross modal

cONv does not only respond to antennal movement but also to substrate vibration (Ache et al., 2015, Lepreux et al., 2019, Figure 20; Fig. S3). We found that substrate vibration given at a high tapping frequency could influence the timing of adaptation in cONv. After substrate vibration at a tapping frequency of 10 s^{-1} cONv encoded the joint velocity with a similar rate than an adapted neuron for antennal movement of 30 deg/s (Figure 25). However,

when the movement velocity was 300 deg/s following the substrate vibration at 10 s^{-1}, the response of the neuron was higher than the adapted response but lower than the non-adapted response for the same velocity suggesting pre-adaptation (Figure 24 and Figure 25). Since substrate vibration reaches cONv through a different channel than the putative antennal hair field afferents (Ache et al., 2015) we conclude that the adaptation in cONv is cross-modal.

Adaptation likely occurs though intracellular modifications of cONv

Adaptation has been reported to be specific to a particular stimulus in different organism (Ringo, 1996; Triblehorn and Schul, 2013; Ogawa and Oka, 2015). Stimulus specific adaptation reduces the neuronal response to a recurring stimulus while a response to a new stimulus should be enhanced (Triblehorn and Schul, 2013). Once cONv has adapted to a stimulus, its response to a new velocity are significantly weaker than if the neuron would be stimulated by this velocity for the first time (Figure 23). The response level of the neuron to the new velocity is almost as low as if the neuron had already adapted to this velocity. Depending on the stimulus intensity the neuron will either partially recover from adaptation or will stay adapted (Figure 22). Indeed, when the deviant velocity was faster than the adapting velocity (Deviant velocity paradigm in Figure 19) the neuron was activated more strongly during the deviant stimulus than during the adapting stimulus (Fig. S1). Accordingly, when the adapting and deviant velocity were swapped the level of activation of cONv during the deviant velocity was lower than during the adapting velocity (Fig. S1). Moreover, during the ramp-and-hold stimulus at 30 deg/s, the level of activation of cONv is in the range of the spontaneous activity of cONv (Lepreux et al., 2019) and a period of 60 s without antennal movement (where only spontaneous activity is recorded) allowed the neuron to at least partially recover from adaptation. It is consistent with the fact that the presence or absence of fluctuating inputs to the neurons can impact the timing of adaptation (Wark et al., 2007) and the mechanisms of adaptation can dynamically modify the intrinsic time constant of a neuron depending on stimulation history Gilboa et al. (2005). Accordingly, substrate vibration triggered one spike per tap (Fig. S3). Thus, cONv fired less spikes during the substrate vibration at 1 s^{-1} than at 10 s^{-1} and when it was presented between two set of antennal movement (Substrate vibration paradigm I, Figure 24) the neuron either partially recovered from adaptation or stayed adapted (Figure 22B).

However, adaptation can be due to several factors, intrinsic or extrinsic to the neuron. For instance, the structure attached to the mechanosensor and its own properties can affect signal transduction and adaptation. (French, 1988, 1992; French and Torkkeli, 1994). Although we did not measure the properties of the antennal structure and its mechanosensors, this is unlikely to influence adaptation here. Indeed, adaptation is not specific to a stimulus and is cross-modal meaning that adaptation is most likely occurring through intracellular modification in cONv rather than presynaptically.

Mechanism of adaptation

Adaptation is cross-modal and is likely to occur at the intracellular level of cONv. This is consistent with what has been shown in other systems. For instance, an interneuron of the auditory pathway of the Neoconocephalus katydids (TN-1) was shown to display adaptation (Schul et al., 2012) and that the mechanisms of adaptation cannot take place in the receptor neurons and should be regulated at the level of the interneuron TN-1 dendrites (Schul et al., 2012; Prešern et al., 2015). This phenomenon in the TN-1 is likely regulated through Sodium and calcium gated potassium current (Triblehorn and Schul, 2013). In Drosophila, It has been shown that blockade of the intracellular calcium release from the endoplasmic reticulum lead to an impairment of the adaptation in neurons of the olfactory system (Murmu et al., 2011). In crickets, adaptation in the lobular giant movement detector (LGMD) is mediated by calcium dependent potassium conductance (Peron and Gabbiani, 2009). The generation of action potential and its recovery requires activity of the cellular ion pumps to provide the necessary ion equilibrium of a cell. Thus, the internal ability for a neuron to fire an action potential will depend on the timing of the previous action potential, on its ion balance and its ability to reverse the change of ion concentration after an action potential was fired. For instance, initiation of action potential and its propagation requires high concentration of sodium channels (Kole et al., 2008) while calcium signals were shown to be involve in the modification of synapses regulated by post-synaptic reactions (Augustine et al., 2003). Neurons of the rats hippocampus were found to dynamically change their threshold depending on the input activity suggesting that adaptation can be regulated locally through history dependent changes (Scott et al., 2014). In the stick insect, DiCaprio et al., (2002) showed that replacement of Ca^{2+} by Ba^{2+} drastically or completely reduced adaptation of the femoral chordotonal organ sensory neuron while increase of Ca^{2+} enhanced adaptation. They hypothesized that Ca^{2+} and K_{Ca} channels could be involved in this phenomenon. Whether

these intracellular dynamics occur in cONv is beyond the scope of this study and will require further investigations to decipher the cellular mechanisms by which cONv adaptation takes place. However, given the change of activity occurring here it is very likely that some of these mechanisms are shared in our system and could impact the behaviour of the stick insect. Indeed, other giant neurons of insects have been found to undergo adaptation and these neurons often play a key role in behavioural task crucial for survival. The direction the cricket takes during escape response is modulated by adaptation occurring to the GI fibre when air puffed are delivered on the body of the animal.(Ogawa and Oka, 2015). In locust, LGMD undergoes adaptation that permits to discriminate looming stimuli over translating stimuli such that the animal does not react to translating stimuli (Peron and Gabbiani, 2009). cONv is likely to convey information from the antenna to the locomotor networks (Ache et al., 2015). In a previous study we hypothesized that substrate vibration could be triggered by footfall during walking (Lepreux et al., 2019, the Fig. 12). Since adaptation has been shown to increase the range of coding, it is possible that cONv adapts during walking. Moreover, if adaptation also ensures high responsiveness of cONv to changes of velocity that could be encountered, for instance, when the antenna hits an obstacle, cONv could in principle reliably detect when the contact occurs which is a prerequisite for front the leg (re)targeting described by Schütz and Dürr (2011) in the stick insect.

References

Ache JM, Haupt SS, Dürr V (2015) A direct descending pathway informing locomotor networks about tactile sensor movement. J Neurosci 35:4081–4091.

Augustine GJ, Santamaria F, Tanaka K (2003) Local calcium signaling in neurons. Neuron 40:331–346.

Castaldi E, Aagten-Murphy D, Tosetti M, Burr D, Morrone MC (2016) Effects of adaptation on numerosity decoding in the human brain. Neuroimage 143:364–377.

Clifford CWG, Webster MA, Stanley GB, Stocker AA, Kohn A, Sharpee TO, Schwartz O (2007) Visual adaptation: Neural, psychological and computational aspects. Vision Res 47:3125–3131.

Corfas G, Dudai Y (1990) Adaptation and fatigue of a mechanosensory neuron in wild-type drosophila and in memory mutants. J Neurosci 10:491–499.

Dalton P (2000) Psychophysical and behavioral characteristics of olfactory adaptation. Chem Senses 25:487–492.

Descalzo VF, Nowak LG, Brumberg JC, McCormick DA, Sanchez-Vives M V. (2005) Slow adaptation in fast-spiking neurons of visual cortex. J Neurophysiol 93:1111–1118.

DiCaprio RA, Wolf H, Büschges A (2002) Activity-dependent sensitivity of proprioceptive sensory neurons in the stick insect femoral chordotonal organ. J Neurophysiol 88:2387–2398.

Eggermont JJ (1985) Peripheral auditory adaptation and fatigue: A model oriented review. Hear Res 18:57–71.

Elliott T, Kuang X, Shadbolt NR, Zauner K-P (2009) Adaptation in multisensory neurons: impact on cross-modal enhancement. Network 20:1–31.

French A (1988) Transduction mechanisms of mechanosensilla. Annu Rev Entomol 33:39–58.

French A, Torkkeli P (1994) The basis of rapid adaptation in mechanoreceptors. Physiology 9:158–161.

French AS (1992) Mechanotransduction. Annu Rev Physiol 54.

Gilboa G, Chen R, Brenner N (2005) History-dependent multiple-time-scale dynamics in a single-neuron model. J Neurosci 25:6479–6489.

Harris RA, O'Carroll DC, Laughlin SB (2000) Contrast gain reduction in fly motion adaptation. Neuron 28:595–606.

Haupt SS, Klemt W (2005) Habituation and dishabituation of exploratory and appetitive responses in the honey bee (Apis mellifera L.). Behav Brain Res 165:12–17.

Hewson L, Tarrega A (2017) Sensory adaptation. Time-Dependent Meas Percept Sens Eval:67–87.

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S, Heisenberg M, Homberg U, Jenett A, Keshishian H, Restifo LL, Rössler W, Simpson JH, Strausfeld NJ, Strauss R, Vosshall LB (2014) A systematic nomenclature for the insect brain. Neuron 81:755–765.

Juusola M, French AS (1998) Adaptation properties of two types of sensory neurons in a spider mechanoreceptor organ. J Neurophysiol 80:2781–2784.

Kohn A (2007) Visual adaptation: Physiology, mechanisms, and functional benefits. J Neurophysiol 97:3155–3164.

Kole MHP, Ilschner SU, Kampa BM, Williams SR, Ruben PC, Stuart GJ (2008) Action potential generation requires a high sodium channel density in the axon initial segment. Nat Neurosci 11:178–186.

Lawless HT, Stevens DA (1983) Cross adaptation of sucrose and intensive sweeteners. Chem Senses 7:309–315.

Lepreux G, Haupt SS, Dürr V (2019) Bimodal modulation of background activity in an identified descending interneuron. J Neurophysiol 122:2316–2330.

Mcburney DH (1972) Gustatory cross adaptation between sweet-tasting compounds. Percept Psychophys 11:225–227.

Murmu MS, Stinnakre J, Réal E, Martin JR (2011) Calcium-stores mediate adaptation in axon terminals of Olfactory Receptor Neurons in Drosophila. BMC Neurosci 12.

Ogawa H, Oka K (2015) Direction-specific adaptation in neuronal and behavioral responses of an insect mechanosensory system. J Neurosci 35:11644–11655.

Pellegrino R, Sinding C, de Wijk RA, Hummel T (2017) Habituation and adaptation to odors in humans. Physiol Behav 177:13–19.

Peron S, Gabbiani F (2009) Spike frequency adaptation mediates looming stimulus selectivity in a collision-detecting neuron. Nat Neurosci 12:318–326.

Prešern J, Triblehorn JD, Schul J (2015) Dynamic dendritic compartmentalization underlies stimulus-specific adaptation in an insect neuron. J Neurophysiol 113:3787–3797.

Rankin CH, Abrams T, Barry RJ, Bhatnagar S, Clayton DF, Colombo J, Coppola G, Geyer MA, Glanzman DL, Marsland S, McSweeney FK, Wilson DA, Wu CF, Thompson RF (2009) Habituation revisited: An updated and revised description of the behavioral characteristics of habituation. Neurobiol Learn Mem 92:135–138.

Rieke F, Rudd ME (2009) The challenges natural images pose for visual adaptation. Neuron 64:605–616.

Ringo JL (1996) Stimulus specific adaptation in inferior temporal and medial temporal cortex of the monkey. Behav Brain Res 76:191–197.

Rose JK, Rankin CH (2001) Analyses of habituation in Caenorhabditis elegans. Learn Mem 8:63–69.

Schmitz J, Büschges A, Delcomyn F (1988) An improved electrode design for en passant recording from small nerves. Comp Biochem Physiol Part A Physiol 91:769–772.

Schul J, Mayo AM, Triblehorn JD (2012) Auditory change detection by a single neuron in an insect. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 198:695–704.

Schütz C, Dürr V (2011) Active tactile exploration for adaptive locomotion in the stick insect. Phil Trans R Soc B 366:2996–3005.

Scott RS, Henneberger C, Padmashri R, Anders S, Jensen TP, Rusakov DA (2014) Neuronal adaptation involves rapid expansion of the action potential initiation site. Nat Commun 5.

Shapley R, Enroth-Cugell C (1984) Visual adaptation and retinal gain controls. Prog Retin Res 3:263–346.

Solomon SG, Kohn A (2014) Moving sensory adaptation beyond suppressive effects in single neurons. Curr Biol 24:R1012–R1022.

Torkkeli PH, Sekizawa SI, French AS (2001) Inactivation of voltage-activated Na+currents contributes to different adaptation properties of paired mechanosensory neurons. J Neurophysiol 85:1595–1602.

Triblehorn JD, Schul J (2013) Dendritic mechanisms contribute to stimulus-specific adaptation in an insect neuron. J Neurophysiol 110:2217–2226.

Trimmer BA, Weeks JC (1991) Activity-dependent induction of facilitation, depression, and post-tetanic potentiation at an insect central synapse. J Comp Physiol A 168:27–43.

Wang X, Guo X, Chen L, Liu Y, Goldberg ME, Xu H (2017) Auditory to visual cross-modal adaptation for emotion: Psychophysical and neural correlates. Cereb Cortex 27:1337–1346.

Wark B, Lundstrom BN, Fairhall A, Mombaerts P, Zador T (2007) Sensory adaptation. Curr Opin Neurobiol 17:423–429.

Weber AI, Fairhall AL (2019) The role of adaptation in neural coding. Curr Opin Neurobiol 58:135–140.

Weber AI, Krishnamurthy K, Fairhall AL (2019) Coding Principles in Adaptation. Annu Rev Vis Sci 5:427–449.

Weigelt S, Muckli L, Kohler A (2008) Functional magnetic resonance adaptation in visual neuroscience. Rev Neurosci 19:363–380.

Whitmire CJ, Stanley GB (2016) Rapid sensory adaptation redux: A circuit perspective. Neuron 92:298–315.

Wilson DA (2009) Olfaction as a model system for the neurobiology of mammalian short-term habituation. Neurobiol Learn Mem 92:199–205.

Woods EJ, Frost BJ (1977) Adaptation and habituation characteristics of tectal neurons in the pigeon. Exp Brain Res 27:347–354.

Supplementary figures

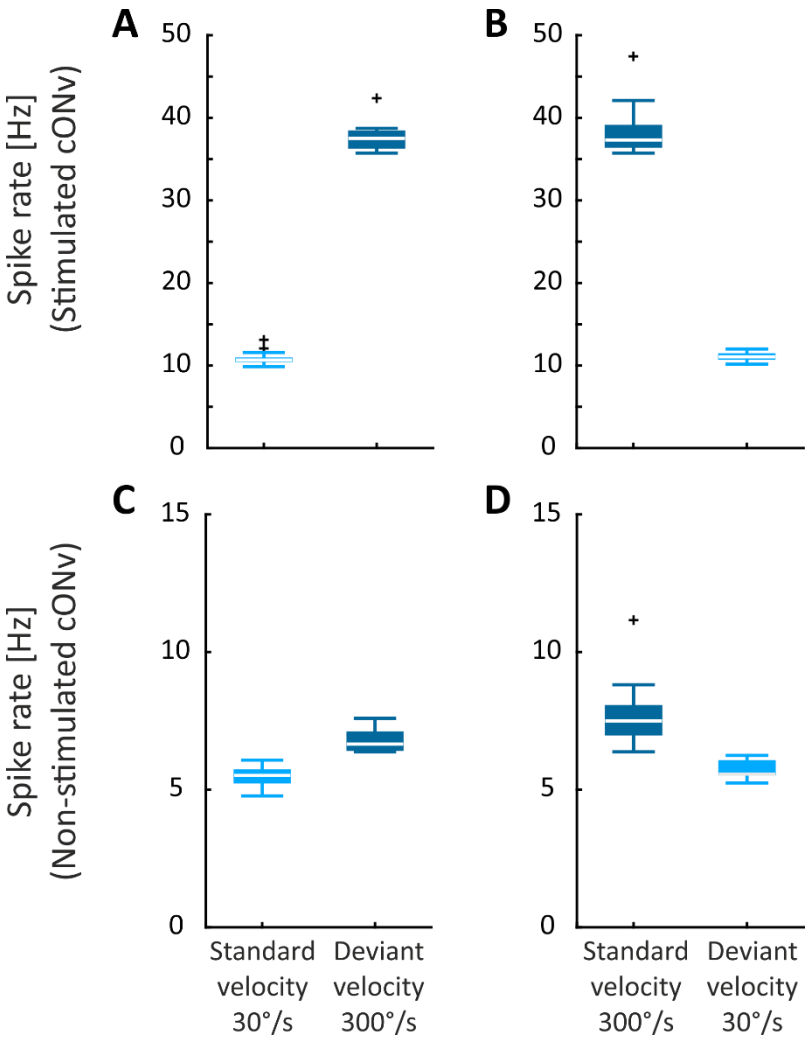


Figure S1: Activity of both stimulated and non-stimulated cONv during the standard velocity and the deviant velocity. *Top row: stimulated cONv activity. Bottom row: Response of the non-stimulated cONv. A: The stimulated neuron responds to both the standard velocity at 30 deg/s (light blue) and deviant velocity of 300 deg/s (dark blue). B: As in A except that the standard and deviant velocities have been swapped. C: The non-stimulated cONv (ipsilateral) does not react to antennal movement during either standard velocity of 30 deg/s (light blue) or deviant velocity of 300 deg/s (dark blue). D: as in C except that the velocities were swapped. White bars represent the median of the distribution. The mean activity during all the ramps composing the stimuli was used to generate the boxplots for both stimulated and non-stimulated cONv. N = 16 Animals.*

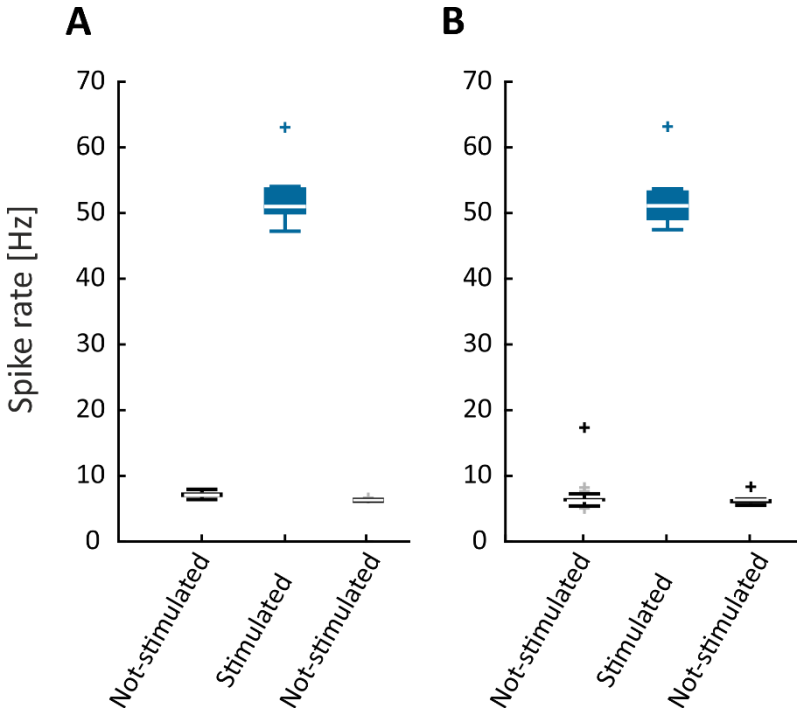


Figure S2: activity of the second cONv during deflection of the ipsilateral antenna (not stimulated) and deflection of the contralateral antenna (stimulated). *A: cONv does not react to stimulation of the ipsilateral antenna at 30 deg/s (Not stimulated) but increased its activity when its contralateral antenna was moved at 300 deg/s (dark blue; stimulated). B: As in A except that the velocity used to move the ipsilateral antenna was 300 deg/s (not stimulated). White bars represent the median of the distribution and crosses show outliers. The mean activity during all the ramps composing the stimuli was used to generate the boxplots for both stimulated and not-stimulated episodes. N = 16 Animals.*

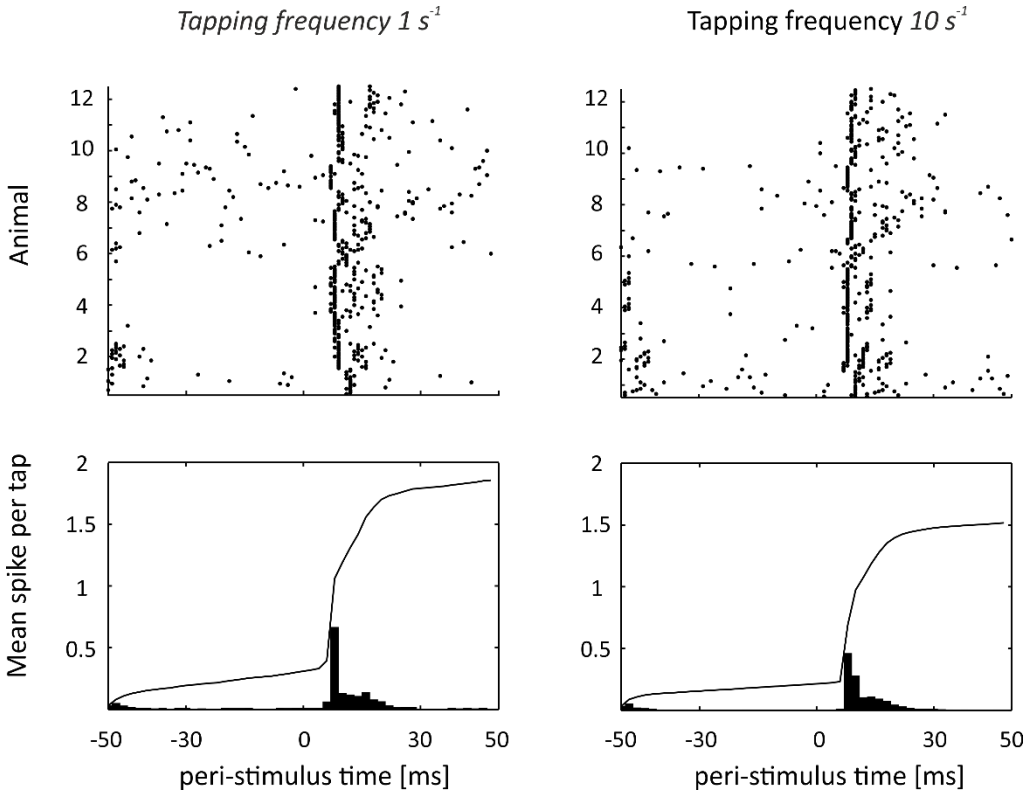


Figure S3: Raster plots (top) and peri-stimulus time histograms (bottom) of cONv activity aligned to the onset of individual taps (t = 0 s) for tapping frequencies 1 s^{-1} and 10 s^{-1}. *Raster plots show only the first 20 taps per animal, but the histograms include all data from N = 12 animals. The black line shows the mean cumulative sum of cONv spikes per tap occurring within 50 ms before and after a tap. Histograms show mean spike number per 1 ms bin. Each point in the raster plots corresponds to a cONv spike. cONv responds reliably to tapping with at least one spike per tap at bot tapping frequencies presented.*

Chapter 4

Signalling unintended movement: A means of contact detection in an active tactile sensor

Abstract

Touch is often considered as a primarily active sense for many animals. Since active touch requires a physical contact with a surface, and regardless of the origin of activation, sensory systems will be activated. Thus, it is difficult to discriminate self-generated behaviour (reafference) from externally triggered actions (exafference). A broadly used strategy in the animal kingdom relies on the corollary discharge principle. Motor actions triggered by the animal are used to inhibit the sensory signals and thus relay to downstream targets allowing the distinction between self-action and imposed actions. Here we use an individually identified descending interneuron (cONv) of the stick insect to show how animals may exploit the distinction of intended from unintended movement to reliably detect external contacts. We carried out single unit recordings in immobilized animals and recorded the activity of cONv during passive deflection of the antenna and self-generated antennal movement. Deviation from intended self-motion (e.g., a contact-induced stop) or intended rest (e.g., by contact-induced deflection) leads to reliable short-latency firing – providing an angular-velocity encoder at rest and an efficient contact detector in the behavioural state of locomotion.

Introduction

The natural activity cycles of animal behaviour include episodes of active movement as well as episodes of rest. Assuming that the information provided by sensory systems should be optimized for a particular behaviour, an important feature of sensory processing must be to discriminate stimulation caused by self-generated action (reafference) from stimulation caused by other, purely external causes (exafference). A central neural mechanism is required to account for this, as it has been proposed decades ago as the principle of corollary discharge or efference copy (Sperry, 1950; von Holst and Mittelstaedt, 1950). Since corollary discharge refers to a more general aspect of the phenomenon, we will use this term throughout this study (Crapse and Sommer, 2008). However, both phenomena rely on the same principle: A motor command is sent not only to the actuators but also – as a copy - to an element of the sensory pathway where it prevents a response to the incoming afferent activity expected to result from the execution of the motor command. Corollary discharge has been reported for various sensory systems and many animal species (for reviews see Webb, 2004; Poulet and Hedwig, 2007; Crapse and Sommer, 2008; Huston and Jayaraman, 2011; Straka et al., 2018). In insects, Kim et al., (2015) found that during a rapid turn, some visual neurons of *Drosophila* receive motor-related input that could suppress the cells responses to the turn. In crickets, a descending interneuron was found to inhibit the primary auditory afferents (Poulet and Hedwig, 2002), allowing singing male crickets to stay responsive to auditory stimuli. Compared to other sensory systems, a particularity of the sense of touch is that it requires the physical contact between a part of the body, i.e., the sensor, and an external surface. A tactile sensation requires displacement or deformation of the sensor. Therefore, the distinction of “being touched by” or “having touched” something is linked to the motion of the sensor, i.e., whether it was at rest or rather actively exploring its surrounding. Indeed, across the animal kingdom, touch is often studied considered as a primarily “active sense”. For example, rats use their whiskers (mystacial vibrissae) to detect texture and orient towards it (Diamond et al., 2008; Grant et al., 2012). They actively orient their whiskers towards objects of interest and can discriminate surface roughness by whisking (Guić-Robles et al., 1989; Mitchinson et al., 2007; Diamond and Arabzadeh, 2013). For many nocturnal animals, the sense of touch is the main sense to rely on in order to detect objects, conspecifics or predators and orient accordingly. In insects; the antennae are multimodal structures

containing all the necessary apparatus to be used both as active and passive sensor of the outside world (Staudacher et al., 2005). Crickets use their antennae to tell conspecifics from predators (Okada and Akamine, 2012) but also in agonistic behaviour, where the territorial males hit each other with their antennae (Alexander, 1961) and use tactile cues to infer the strength of their opponent (Hofmann and Schildberger, 2001). Others, like the Indian stick insect *Carausius morosus* continuously move their antennae rhythmically while walking (Dürr et al., 2001), in order to explore and sample the spatial volume ahead (Krause and Dürr, 2012; Dürr and Schilling, 2018). During such active exploration behaviour, stick insects often respond to antennal contact with targeted reaching movement of the front leg (Schütz and Dürr, 2011). As yet, like all other animals, stick insects rest for a substantial fraction of their lifetime, with remarkable camouflage behaviour in which they stay still and mimic twigs or show a death-feigning behaviour (thanatosis).

In both active and passive forms of behaviour, it may be vital for an animal to detect contact events. The present study compares active (exploratory) and passive (resting) episodes of stick insect behaviour to investigate context-dependent differences in the sensory processing of touch in general, and tactile sensor displacement in particular. Our main objective was to decide between two alternative hypotheses: According to the first hypothesis, contact-events should be processed depending on whether the tactile sensor was moved actively or passively; according to the alternative hypothesis, contacts should be signalled depending on whether the registered sensor displacement was intended or un-intended by the animal.

In stick insects, a number of descending interneurons have been characterized (Ache and Dürr, 2013) that convey information about tactile sensor movement from the brain to the thoracic ganglia where they could contribute to the execution of tactually induced reach-to-grasp movements (Schütz and Dürr, 2011). Among them, a bilateral pair of giant interneurons, the contralateral ON-type velocity-sensitive (cONv) neurons are located in the gnathal ganglia (GNG; = suboesophageal ganglion, Ito et al., 2014) and have a descending axon contralateral to the antenna they respond to (Ache et al., 2015). They encode joint angle velocity when the flagellum is deflected passively around the scape-pedicel joint in resting animals. Since locomotion in stick insects is always linked to active tactile exploration (Dürr et al., 2018), we asked whether cONv also encoded the velocity of self-generated exploratory antennal movements. Second, since obstacle contacts can lead to either displacement of a

passive sensor or stop of displacement of an active sensor, we asked whether tactile sensor displacement was signalled according to effective movement or intended movement. Considering that both resting posture and exploratory movements are voluntary actions of the animal, the neuron responded only when sensor displacement differed from the intended posture or movement, either by being faster or slower than intended. Our findings show that the same neuron could serve as disturbance detector in resting animal and as an obstacle detector during locomotion in the stick insect.

Methods

Animals and Dissection

Adult female stick insects of the species *Carausius morosus* (Sinéty, 1901), bred at Bielefeld University were used. All six legs were removed at the coxa-trochanter joint by autotomizing or using scissors when necessary. Animals were fixed on a Balsa-wood platform ventral side down using dental glue (Protemp, 3M EPSE, Neuss, Germany) and the cuticle was cut open along the dorsal midline along all three thorax segments. The gut was lifted outside the body cavity that was then filled with saline solution (NaCl: 180 mM, KCl: 4 mM, $CaCl_2$ $2H_2O$: 5 mM, $MgCl_2$ * $6H_2O$: 1 mM, HEPES: 10 mM, Sucrose: 10mM). Leg nerves that connect the mesothoracic ganglion to the middle leg were ablated and the mesothoracic ganglion was placed on a metal 'spoon' covered with wax to increase the stability of the connectives and the ganglion (for more details see Ache et al., 2015).

Recordings

Extracellular single-unit recordings from cONv were obtained using thin-walled borosilicate glass microelectrodes (Hilgenberg, wall thickness 0.125 mm). The shaft was filled with 1 M KCl. Either the left or right connective was impaled anterior to the mesothoracic ganglion. A reference electrode was placed in the body cavity in contact with saline solution. Data were recorded at 10 kHz, using a Micro1401 A/D converter and Spike 2 data acquisition software (version 8.01, both Cambridge Electronic Design, Cambridge, UK).

Identification of cONv was done as described earlier (Ache et al., 2015; Lepreux et al., 2019): cONv shows a characteristic, velocity-dependent response to passive deflection of the contralateral antenna, no response during passive deflection of the ipsilateral antenna, and a reliable response to substrate vibration as induced by taps onto the recording setup.

Antennal movements

Passive deflection. Antennae were moved passively using a paintbrush held by the experimenter. The direction of movement depended on the prior position of the antenna before the movement (i.e., upward deflection if the antenna was positioned in the ventral working range, otherwise downward deflection). Note that cONv belongs to a group of purely motion-sensitive descending interneurons that neither respond to tactile contact of the flagellum (Ache and Dürr, 2013) nor to the direction of antennal movement (Ache et al., 2015; Ache and Dürr, 2015. As a consequence, the mechanical contact caused by the paintbrush did not cause spike activity in cONv.

Active, exploratory movement. In order to observe self-generated antennal movements in stationary animals during electrophysiological recordings, animals were stimulated either by gently and repeatedly touching the thorax or abdomen with a paintbrush, blowing at the front of the animal, presenting a cold light guide for a couple of seconds, or a combination of these treatments. In the context of leg motor control, such treatments are commonly used to "activate" the animal, thus leading to physiological changes such as a reflex reversal at the femur-tibia joint (Bässler, 1988). This "active response" is commonly related to locomotor activity (for review, see Bässler and Büschges, 1998).

Interruption of exploratory movement. Once the animals showed self-generated antennal movements, an obstacle (a pen) was held still into its working-range, thus interrupting the ongoing active movement of the flagellum. Time and location of antennal contact events were analysed by visual inspection of the videos recorded (see below).

Active tactile exploration during locomotion. As walking and climbing stick insects always move their antennae (for review, see Dürr et al., 2018), we used example trials of this active tactile exploration behaviour in order to compare it with active, exploratory antennal movements of stationary animals. For this, antennal movements were recorded as stick insects walked towards the end of a horizontal walkway (4 cm wide), where they engaged in searching behaviour during which both antennae and both front legs are moved rhythmically. Typically, this searching behaviour ends with a transition into a resting posture (camouflage behaviour).

Motion capture

Irrespective of the kind of antennal motion, movements were tracked optically by means of marker-based motion capture. Typically, one retro-reflective marker was fixed on each flagellum with nail polish. In two animals, only one marker was placed on the antenna contralateral to the recording site. During electrophysiology, trajectories of the markers were recorded at 100 or 200 frames per second in two views, using a digital camera (Basler acA1920-155um, equipped with a Computar M321228C-MP lens). The camera was placed 30 cm away and to the side of the animal. In addition to the side view, the camera also recorded a top view via a mirror placed approximately 5 cm above the animal and at an angle of 45 ° relative to the camera plane. Videos were captured using the Pylon viewer software (V4.2.1.4845, x64; Basler). Both camera views were calibrated using the MATLAB Camera Calibration Toolbox.

Antennal movements of unrestrained walking stick insects were recorded by means of a commercial, marker-based motion capture system (Vicon MX10, equipped with eight T10 cameras, controlled by Vicon Nexus) at 200 frames per second, as described earlier (Krause et al., 2013).

Data analysis.

*Electrophysiology***.** All electrophysiological data were exported from Spike2 to MATLAB (version 9.2, MathWorks R2017a) and analysed with custom-written scripts.

Video analysis. For better semi-automatic tracking of the markers that were placed on the flagellum, videos were pre-processed off-line in Virtual Dub (1.10.4, by Avery Lee, www.virtualdub.org) to enhance image contrast. Marker tracking was done using a custom-written toolbox for MATLAB. Together with the camera calibration, marker coordinates yielded 3D trajectories of one point per flagellum. From those trajectories, time courses of both antennal joint angles were calculated using inverse kinematics (Krause and Dürr, 2004). To do so, the position of the flagellum tip was determined by extending the line from head to the marker to average length of a flagellum for *Carausius morosus*. Trajectories were filtered using a second-order zero-lag Butterworth filter with a cut-off frequency of 10 Hz.

Annotation of the antennal movement type (active/exploratory, passive, inter-rupted/contacts) was done manually frame by frame in Virtual Dub and logged into MATLAB for further analysis. Processing of supplementary videos 1 to 3 was done in Adobe After Effect in order to add raw signals and joint angle time courses.

Results

Active movement in restrained animals resemble the exploratory movements during walking.

During locomotion, stick insects always show rhythmic movement of both antennae (Dürr et al., 2001; Krause et al., 2013) for near-range exploration (Krause and Dürr, 2012; Dürr et al., 2018). During rest, however, they tend to keep their antennae immobile in a stereotypical resting posture. Since the central pattern generators for antennal movement are located in the brain (Krause et al., 2013), there is probably no ascending drive from the ventral nerve cord during rest. To date, rhythmic antennal movements in stationary stick insect preparations have only been described in case of pharmacological activation by pilocarpine (Krause et al., 2013). Here, we report that tactile stimulation of the abdomen of stationary stick insects could induce to bouts of active and rhythmical antennal movements (Figure 26). This is similar to tactually induced leg searching movements in stationary animals as used by Berg et al. (2015). Here, we use such rhythmical antennal movements as an indicator for locomotion-like activity of the stationary animal, related to the definition of an active locomotor state in research on leg reflexes (e.g., Hellekes et al., 2012; for review, see Bässler and Büschges, 1998). This is supported by the fact that we found a strong similarity of rhythmic antennal movements during unrestrained locomotion (Figure 26A) and those triggered in stationary, active animals as used in the present study (Figure 26B).

During bouts of active antennal movement, antennal tip trajectories of stationary animals covered a similar range and followed a similar pattern as in walking animals. In both cases, trajectories were of elliptic shape with considerable cycle-to-cycle variability. In the 12 s episodes shown in Figure 26, head-centered beating fields were of similar size and location. The slightly higher density in case of the intact animal was caused by more movement cycles per unit time. In addition to the motion-captured 3D trajectories of the left and right antennal tips, we calculated the joint angle time courses of both antennal joints (HS: head-scape joint; SP: scape-pedicel joint), using inverse kinematics (Krause and Dürr, 2004). In the two examples shown in Figure 26, the 5-to-95 percentile ranges of the joint-angle distribution were slightly larger in the stationary, active animal than in self-motivated walking animal. As yet, these differences between experimental paradigms were not larger than within-animal variation of left and right antennae or walking and searching episodes of the naturally moving animal (see Suppl. Table 1 for joint angle range data for Figure 26).

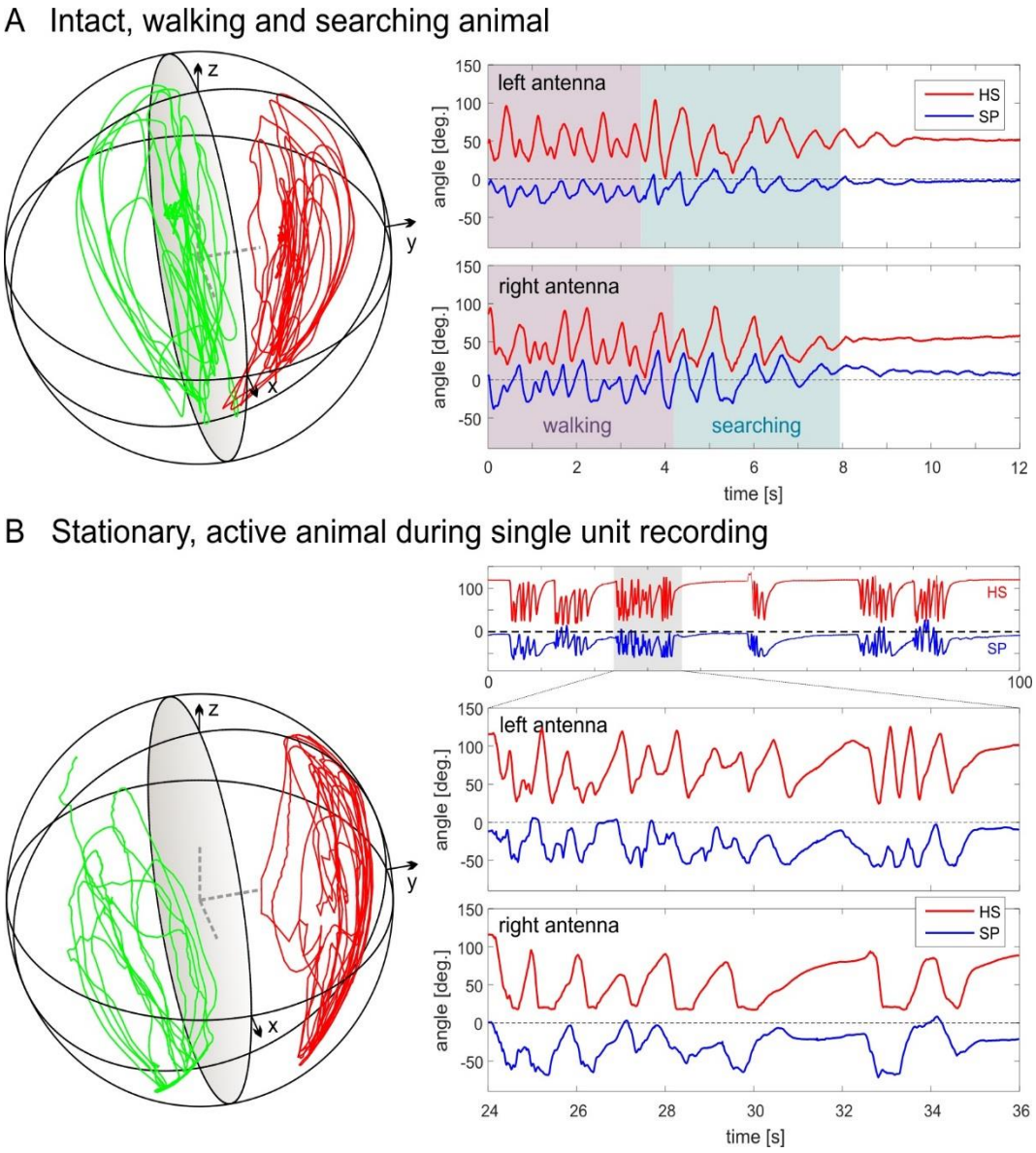


Figure 26: Antennal movement of stationary active animals is similar to that of free walking animals.

A) Intact animal. Left: 3D trajectories of the left (red) and right (green) antennae of an animal walking freely towards the edge of a horizontal walkway. Both antennae are moved rhythmically during walking and a searching episode after having reached the edge. Right: Joint angle time course of the head-scape (HS; red) and scape-pedicel (SP; blue) joints of the antennae during a 12 s episode including active exploration during walking (purple shading), searching (blue shading) and a subsequent transition into a resting posture. The onset of searching is defined as the instant at which the ipsilateral foot stepped across the edge. B) As above, but for a stationary, active animal during simultaneous recording of the cONv interneuron. The top right panel of B shows that active movement occurs in bouts of several seconds duration (here: left antenna only). The grey shaded area comprises a bout of 12 s duration. 3D trajectories (lower left) and joint angle time courses (lower right) correspond to the episode of this bout.

In summary, kinematic parameters of antennal movement in stationary animals were well within the natural inter-trial variation, indicating that the level of exploratory antennal activity of stationary, active animals was equivalent to that of self-motivated walking animals.

Self-generated antennal movements do not activate cONv.

Given these behavioural observations, we used single-unit recordings of cONv in stationary stick insects to test whether movement-induced spike activity differed between passive, experimenter-induced and active, self-induced antennal movement, i.e., whether it was an un-intended or intended movement. In general, the neuron responded to unintended passive deflection of the flagellum (Figure 27A) but did not respond to the self-generated movement at all (Figure 27B). This finding was corroborated in a sample of nine animals, with 26 trials for passive deflection and 73 trials of active exploratory movement episodes (Figure 28). As in Figure 27, the onset of either passive deflection or exploratory antennal movement corresponded to an increase of velocity (Figure 28Ai, Bi). Furthermore, cONv activity increased when passive deflection started (Figure 28A panels ii and iii). As described earlier by Ache et al. (2015) and Lepreux et al., (2019), passive deflection of the ipsilateral antenna did not activate cONv (Fig. S2).

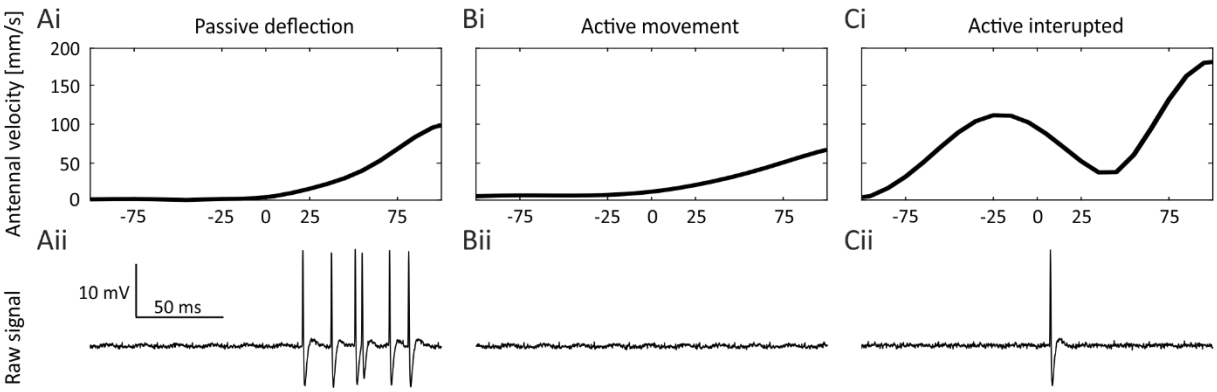


Figure 27: Representative single trial examples of cONv activity during three antennal movement types

(A) Passive deflection; (B) active, i.e., self-generated movement; (C) active antennal movement that was interrupted by a mechanical stop. Top row shows antennal tip velocity before (- 100 to 0 ms) and after (0 to 100 ms) the start of antennal movement (Ai, Bi), or the contact with the stop (Ci). Bottom row shows the raw signal of the corresponding single-unit recording. All panels are from the same preparation.

Conversely, during self-generated antennal movement, cONv activity was similar to the activity before movement onset (Figure 28B panels ii and iii, n = 73, N = 9) although the velocity was in the same range as for passive deflections (Figure 28. Ai and Bi). Note that, unlike in the example shown in Figure 27, some cONv showed spontaneous spiking activity before passive deflection and active exploratory movement in some animals (see also Lepreux et al., 2019). Nevertheless, the overall effect across trials was consistent: cONv displayed reliable stimulus licked responses during passive, unintended deflection of the contralateral antenna, but its activity did not change systematically during intended, active movement.

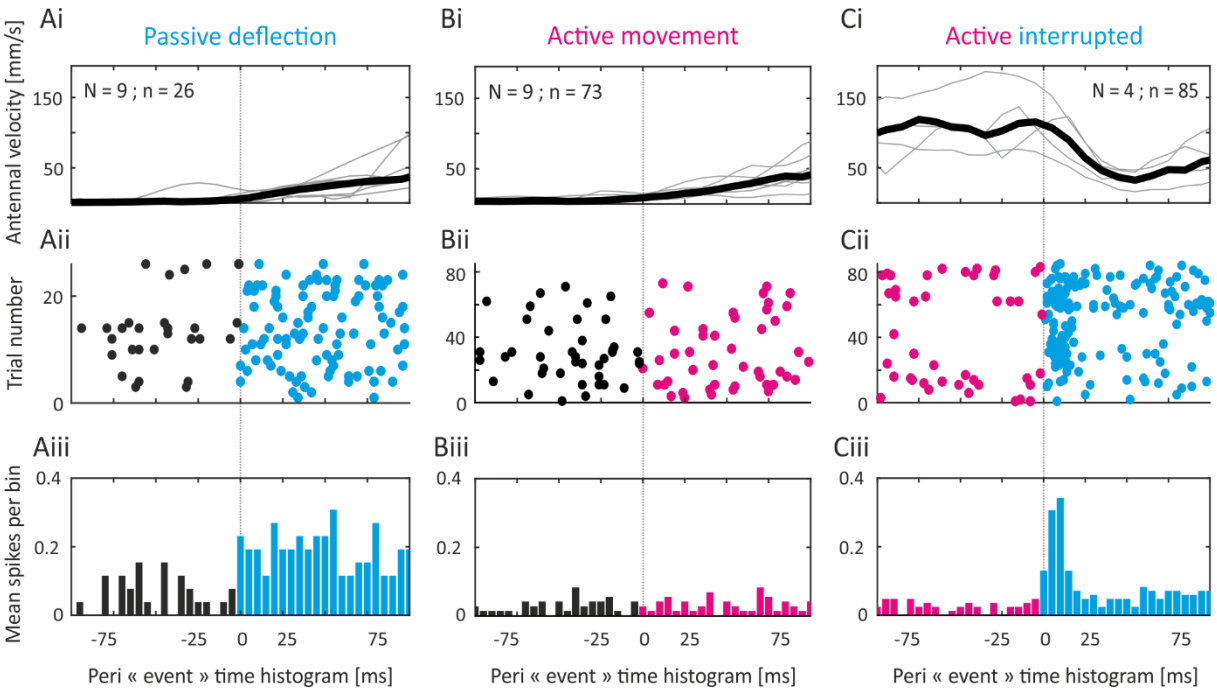


Figure 28: cONv is activated only during unintended, passive antennal movement.
Response to A) passive deflection of the flagellum, B) self-generated (active) antennal movement, or C) active antennal movement that is interrupted by an obstacle. Bold lines in the top row of panels (i) show the median antennal velocity in mm/s, aligned to the onset of each movement episode (Ai, Bi; vertical dashed line) or the first contact with the obstacle (Ci). Thin grey lines show per-animal means. Middle row of panels (ii) shows raster plots of cONv spikes per trial (one row per trial) while the bottom row (iii) shows peri-stimulus time-histograms per movement type. The coloured points and bars show episodes of intended, active (magenta) or unintended, passive (blue) movement (black: no movement). N refers to the number of animals tested per movement type, whereas n refers to the total number of trials.

cONv is activated when the antenna hits an obstacle.

As these observations focused on whether cONv was activated upon onset of movement, they did not allow us to distinguish whether it was the presence/absence of

motor action that caused the difference, or rather the deviation from an intended movement. Therefore, we tested the response of cONv to the stop of active movement.

We reasoned that an unexpected contact with an obstacle during an ongoing exploratory movement, and the corresponding interruption of intended movement, would tell us whether cONv activity was suppressed during own motor effort or whether the neuron would remain responsive to deviations from intended movement. In the first case, cONv activity should not be altered in response to the contact. In the second case, there should be a contact-induced increase of activity. To test this, we monitored cONv activity as the animal performed exploratory antennal movements. Then, we interrupted antennal movement by holding an obstacle into the action range of the flagellum. cONv did not fire before the contact (*i.e.,* during exploratory movement) but fired one spike 10 ms after the contact occurred (Figure 27C). Before contact, the flagellum was moving at an average velocity of 120 mm/s (Figure 28Ci). During this period, cONv activity did not change compared to its activity at rest or at onset of the exploratory movement (Figure 28Aii, Bii). In a total of 85 single trials from four animals, cONv started firing shortly after an involuntary interruption of exploratory

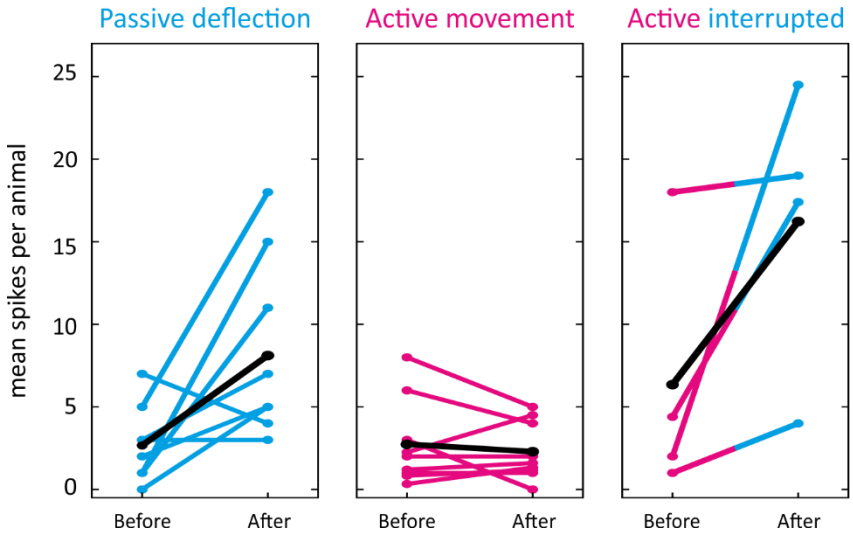


Figure 29: cONv activity before and after a change in movement.
Each point shows the spike counts of cONv per animal and movement type for time windows of 100 ms width before after the change in movement (Left: no vs. passive movement; Middle: no vs. active movement; Right: active vs. interrupted movement). Black lines show the mean number of spikes in 100 ms episodes of the population. Blue lines indicate changes to passive movement whereas magenta lines indicate onset or interruption of an active movement. Left: Number of spikes increases for passive deflections in all but one animal (N = 9). Middle: Number of spikes does not change as active exploratory movements are initiated (N = 9). Right: Interruption of active movement always leads to an increase in number of spikes (N = 4).

movement. The peak of the response occurred within 10 to 15 ms after contact. This delay is similar to the latency of 11.2 ± 2.7 ms reported for passive SP joint movement (Ache et al., 2015; mean ± sd).

Next, we wanted to know whether all neurons recorded showed the same pattern of activity for each one of the three types of antennal movement. The mean cONv activity showed a significant increase of activity during passive deflection of the antenna in seven of nine preparations when compared to no movement (Wilcoxon's test for matched pairs, $p = 0.031$) whereas no significant difference of activity was observed during episodes of active, exploratory movements ($p = 0.62$; Figure 29). In each one of the four animals tested, cONv showed an increase of activity upon antennal contact, consistent with our trial-by-trial analysis.

Spike number depends on contact parameters.

As cONv had been found previously to encode the velocity of the SP joint (Ache et al., 2015), we asked whether the velocity before contact had an influence on the activation of the neuron. Accordingly, we determined the number of spikes per contact (Figure 30A) and related them to the mean velocity in a 50 ms window before the contact (Figure 30B) as well as the duration of the contact (Figure 30C).

The number of trials and the fraction of responses with a given number of spikes recorded within 100 ms after antennal contact is shown in Figure 30A. cONv mostly fired one or two spikes per contact event (together 58 %, or 49 trials) but sometimes more. Almost one fifth of all contact events (15 trials, 19%) were not followed by a cONv spike. Overall, the number of spikes per contact was significantly correlated to the median antennal velocity before contact, but not to the median contact duration (Spearman correlation, $p= 0.017$ and 0.078, Figure 30).

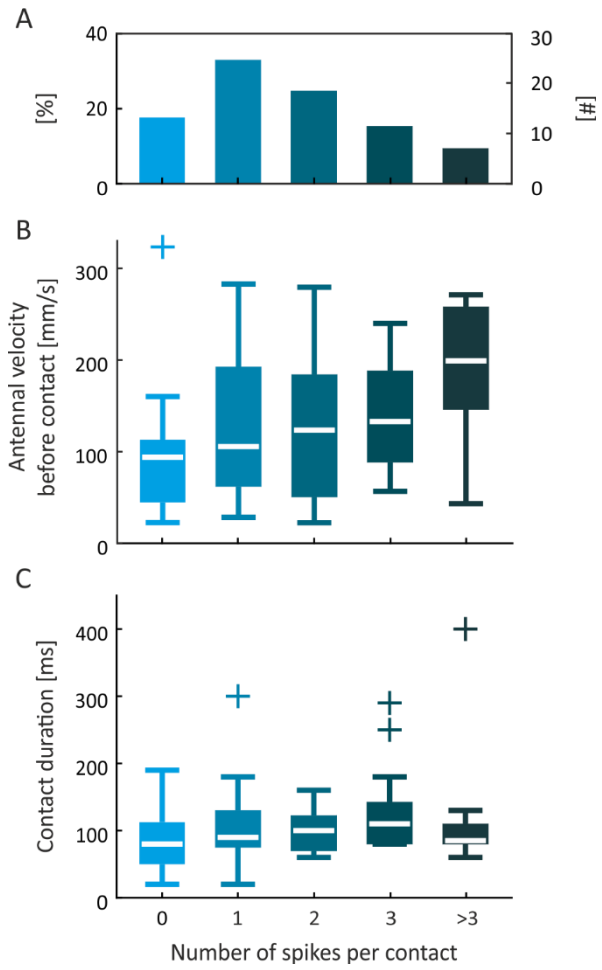


Figure 30: cONv responds to interruption of active antennal movement by tactile contact.

The number of cONv spikes elicited depends on the velocity before contact but not on contact duration. A) Upon tactile contact, cONv most often fires one or two spikes. Bars show percentages and trial numbers of responses comprising a given number of spikes. B) Faster antennal movement tends to lead to more cONv spikes upon contact . Box-whisker-plots show mean antennal velocity over 50 ms before contact in relation to the number of spikes per contact. C) Number of spikes per contact event is not correlated with the contact duration. White bars show the medians. N = 4 animals with a total of n = 85 trials.

Discussion

Exploring the world requires the capacity of an organism to detect features of the surroundings actively and passively. Flying insects can rely on optic flow to orient and detect prey and several studies have shown that visual neurons are differentially activated depending on whether the background moves as a results of the fly's own movement or as a results of artificial displacement (e.g., (Kim et al., 2015)). Nocturnal insects rely on their antennae to orient in the dark. Indian stick insects actively move their antennae during locomotion. In both examples, animals must be able to detect voluntary (active) from involuntary (passive) movements. In the stick insect, a descending interneuron called cONv was found to encode the velocity of imposed deflections of the antenna in quiescent animals (Ache et al., 2015; Lepreux et al., 2019, Figure 28). Here we found that active exploratory movements generated by the animal itself were not encoded by cONv, while interruption of an ongoing movement was accompanied by spiking activity of cONv (Figure 28 and Figure 29). Since stationary, quiescent stick insects typically do not display antennal exploratory movements unless pharmacologically activated (Krause et al., 2013), we needed to confirm

that the spontaneous antennal movements in stationary animals as observed here have the same properties as in active, walking animals. As during locomotion, both antennal joints contributed to spontaneous bouts of movement, and the joint angle ranges were similar in both stationary and freely walking animals (Figure 26 and Krause et al., 2013). This suggests that the antennal movements in our stationary animal preparations are a sign of exploratory searching activity that corresponds to active exploration behaviour during locomotion (Krause and Dürr, 2012).

As described earlier by Ache et al. (2015) and Lepreux et al. (2019), passive deflection of the flagellum strongly activated cONv (Figure 27, Figure 28 and video 1). Despite the velocity-dependence of cONv spike activity during passive deflection (Ache et al., 2015) we found that cONv activity was not modulated during exploratory antennal movements (Figure 27B, video 2). Indeed, peri-stimulus time histograms (Figure 28Biii) showed that cONv activity remained unaffected by onset of exploratory movement. Thus, we conclude that cONv is neither encoding the velocity of, nor is activated by self-generated exploratory antennal movement.

Previously, studies in crickets have shown that sensory responses of some descending brain neurons are gated depending upon the behavioural state of the animal. The neurons responded to some auditory or visual stimuli only when the animals were walking (Zorović and Hedwig, 2011), while others, such as an aversive auditory stimulus, were not gated in a similar manner (Staudacher and Schildberger, 1998). Gebhardt and Honegger (2001) showed that during active antennal movements the synaptic input of the DBNi1-2 neuron is suppressed. As the reduction of the EPSP amplitude was correlated with strong motor activity and hyperpolarization of DBNi1-2 was considered unlikely, they hypothesized that input must have been inhibited presynaptically during antennal motion.

As in the study by Gebhardt and Honegger (2001) we used a contactless stimulator designed to move the antenna around its SP-joint, in order to stimulate proprioceptive hair fields at this joint. Since ablation of antennal hair fields strongly affects antennal movement (Krause et al., 2013), hair field afferent terminals arborise in close vicinity to cONv dendritic branches (Ache et al., 2015), and a computational model of antennal hair field afferents suggesting that it is sufficient to simulate the velocity dependence of cONv (Ache and Dürr, 2015), we assume that at least part of the velocity-sensitivity of cONv is driven by afferent input of antennal hair fields. Furthermore, since mechanoreceptor transduction in hair fields

should not be dependent on whether the antennal joint is deflected actively or passively, we propose that hair field afferent input to cONv is inhibited during bouts of active antennal movement.

cONv responds to antennal contact events.

Much like what has been described for unrestrained walking stick insects (Krause and Dürr, 2012), active stationary animals responded to antennal contact with a reduction of the movement range (e.g., see Video 3) and repeated tactile sampling (Figure 30). In comparison, cockroaches repeat contact with a detected object without changing the range of movement (Okada and Toh, 2006). During initial contact and subsequent antennation, cONv responded reliably to antennal contacts with a median of one spike per contact (Figure 28C, Figure 30C). Spike number was significantly correlated with antennal velocity prior to contact, and velocity at contact was the lowest when no spike was fired (Figure 30).

In cockroaches and crickets the flagellum is important for detection and discrimination of the contact type (Comer and Baba, 2011; Okada and Akamine, 2012) and tactile surface discrimination in bees (Scheiner et al., 2005) whereas the most proximal parts of the antenna are required for an appropriate escape response of the cricket (Schöneich et al., 2011) and necessary for tactile object shape discrimination in the bee (Scheiner et al., 2005). It has also been shown in cricket that a DIN responded more to strong antennal touch than to weak antennal touch (Schöneich and Hedwig, 2015). Taken together, touch-related activity in insect interneurons often not only signal the actual occurrence of contact events, but also encode other features of the contact such as position or force. The extent to which cONv could encode other aspects of contact events than velocity prior to contact cannot be determined within the scope of this study. The fact that active exploratory movements are subject to constant modulation, in both stationary and unrestrained walking animals (Dürr et al., 2001; Krause and Dürr, 2012), makes it difficult to measure response to contact parameters in a controlled fashion.

cONv signals unintended displacement

Predicting the sensory consequences of own motor activity is one strategy that animals can adopt to distinguish their self-motivated and externally imposed movement resulting from interactions with the environment. A much simpler mechanism for adjusting sensory processing to own motor activity is state-dependent gating of sensory input. These

extremes show that the concept of corollary discharge may be implemented at different levels of complexity (for review, see Crapse and Sommer, 2008). Nevertheless, the common property for this phenomenon is the distinction of the origin of sensory activation. In order to identify whether a neuron carries a corollary discharge some rules apply (Poulet and Hedwig, 2006, 2007): The origin should come from a motor area and project to an area that is not responsible for the generation of motor activity but for sensory processing. In the visual system of *Drosophila*, Kim et al., (2015) could show direct evidence of a corollary discharge. In the auditory pathway of cricket, the corollary discharge interneuron carries information of the wing motor activity that induces chirping. It inhibits the interneuron that receives auditory afferent input, thus preventing a response of the animal to its own song.

In the present study, evidence of corollary discharge can only be indirect. As both active and passive deflection of the flagellum involve the SP-joint, we argue that proprioceptive hair field afferents must have been activated. Nevertheless, only passive deflection and interruption of active exploratory movement induced cONv spikes. Assuming a simple corollary discharge mechanism with state-dependent gating of all afferent inputs to cONv, interruption of self-generated antennal movement should not have triggered cONv spikes. A slightly more complex mechanism with specific gating of afferent input from proprioceptive hair fields only, could explain that cONv did not encode antennal velocity during active movements, but remained responsive to other input, such as campaniform sensilla on the pedicel. Since campaniform sensilla are known to respond to resisted movement of a leg (Zill et al., 2013) and to encode bending direction of the flagellum in locusts (Heinzel and Gewecke, 1979) it is possible that these mechanoreceptors responded to both passive deflection and resistance of active exploration through sudden contact with an obstacle. In both cases, the response of cONv signalled unintended antennal movement, once as “deflection of the antenna at rest”, and once as “movement stop despite activity”. No matter which mechanism underlies this signalling of unintended movement, it must involve a form of corollary discharge to prevent hair-field-related input during intended, active movement.

Detecting antennal contact as interruption of intended movement

The short latency of cONv spikes reported here (10 to 20 ms; Fig. 3) is consistent with the latency found for antennal contact in DINs of other insect species (Ye and Comer, 1996; Schöneich et al., 2011; Zorović and Hedwig, 2013) even when accounting for the size of the

species (Burdohan and Comer, 1990). It is well in the range of behavioural data showing retargeting movement of front leg 40 ms after antennal contact (Schütz and Dürr, 2011). Since we recorded cONv in the mesothorax, spikes would have reached the prothoracic ganglion even earlier (in adult *C. morosus*, the pro-and mesothoracic ganglia are more than 10 mm apart). Thus, there would be sufficient time for the prothoracic ganglion to process contact-related cONv spikes and to elicit an appropriate motor reaction of a front leg, even when considering that muscle activation dynamics and passive forces that could slow down the onset of a new movement (Guschlbauer et al., 2009; Harischandra et al., 2019). Although the role of cONv is not yet known we think that owing to its size, its fast response and its arborisation at the prothoracic ganglion, cONv is likely to play a major role in the control of locomotion. Analogous giant fibres with similarly short latency in other insects have been proposed to be involved in eliciting escape response or collision avoidance behaviour (Burdohan and Comer, 1996; Baba et al., 2010). Since or results show that cONv is not activated by self–generated, intended antennal movement but responds to unexpected interruption of the latter, we argue that cONv is suitable to detect obstacle contacts in the behavioural state of locomotion, whereas it acts more like an angular-velocity encoder of the antennae at rest.

References

Ache JM, Dürr V (2013) Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway. J Neurophysiol 110:2099–2112.

Ache JM, Haupt SS, Dürr V (2015) A direct descending pathway informing locomotor networks about tactile sensor movement. J Neurosci 35:4081–4091.

Alexander RD (1961) Aggressiveness , territoriality , and sexual behavior in field crickets (Orthoptera : Gryllidae). 17:130–223.

Baba Y, Tsukada A, Comer CM (2010) Collision avoidance by running insects: Antennal guidance in cockroaches. J Exp Biol 213:2294–2302.

Bässler U (1988) Walking Movements of Stick Insect Forelegs : J Exp Biol 147:125–147.

Bässler U, Büschges A (1998) Pattern generation for stick insect walking movements - Multisensory control of a locomotor program. Brain Res Rev 27:65–88.

Berg EM, Hooper SL, Schmidt J, Büschges A (2015) A Leg-local neural mechanism mediates the decision to search in stick insects. Curr Biol 25:2012–2017.

Burdohan JA, Comer CM (1990) An antennal-derived mechanosensory pathway in the cockroach: descending interneurons as a substrate for evasive behavior. Brain Res 535:347–352.

Burdohan JA, Comer CM (1996) Cellular organization of an antennal mechanosensory pathway in the cockroach, Periplaneta americana. J Neurosci 16:5830–5843.

Comer CM, Baba Y (2011) Active touch in orthopteroid insects: Behaviours, multisensory substrates and evolution. Philos Trans R Soc B Biol Sci 366:3006–3015.

Crapse TB, Sommer MA (2008) Corollary discharge across the animal kingdom. Nat Rev Neurosci 9:587–600.

Diamond ME, Arabzadeh E (2013) Whisker sensory system - From receptor to decision. Prog Neurobiol 103:28–40.

Diamond ME, von Heimendahl M, Knutsen PM, Kleinfeld D, Ahissar E (2008) “Where” and “what” in the whisker sensorimotor system. Nat Rev Neurosci 9:601–612.

Dürr V, König Y, Kittmann R (2001) The antennal motor system of the stick insect Carausius morosus: Anatomy and antennal movement pattern during walking. J Comp Physiol - A Sensory, Neural, Behav Physiol 187:131–144.

Dürr V, Schilling M (2018) Transfer of spatial contact information among limbs and the notion of peripersonal space in insects. Front Comput Neurosci 12:101.

Dürr V, Theunissen LM, Dallmann CJ, Hoinville T, Schmitz J (2018) Motor flexibility in insects: adaptive coordination of limbs in locomotion and near-range exploration. Behav Ecol Sociobiol 72:15.

Gebhardt M, Honegger HW (2001) Physiological characterisation of antennal mechanosensory descending interneurons in an insect (Gryllus bimaculatus, Gryllus campestris) brain. J Exp Biol 204:2265–2275.

Grant RA, Sperber AL, Prescott TJ (2012) The role of orienting in vibrissal touch sensing. Front Behav Neurosci 6:39.

Guić-Robles E, Valdivieso C, Guajardo G (1989) Rats can learn a roughness discrimination using only their vibrissal system. Behav Brain Res 31:285–289.

Guschlbauer C, Rosenbaum P, Büschges A, Blumel M, Akay T, Gruhn M, Hooper SL (2009) Neural control of unloaded leg posture and of leg swing in stick insect, cockroach, and mouse differs from that in larger animals. J Neurosci 29:4109–4119.

Harischandra N, Clare AJ, Zakotnik J, Blackburn LML, Matheson T, Dürr V (2019) Evaluation of linear and non-linear activation dynamics models for insect muscle. PLoS Comput Biol 15:1–30.

Heinzel HG, Gewecke M (1979) Directional sensitivity of the antennal campaniform sensilla in locusts. Naturwissenschaften 66:212–213.

Hellekes K, Blincow E, Hoffmann J, Büschges A (2012) Control of reflex reversal in stick insect walking: Effects of intersegmental signals, changes in direction, and optomotor-induced turning. J Neurophysiol 107:239–249.

Hofmann HA, Schildberger K (2001) Assessment of strength and willingness to fight during aggressive encounters in crickets. Anim Behav 62:337–348.

Huston SJ, Jayaraman V (2011) Studying sensorimotor integration in insects. Curr Opin Neurobiol 21:527–534.

Ito K, Shinomiya K, Ito M, Armstrong JD, Boyan G, Hartenstein V, Harzsch S, Heisenberg M, Homberg U, Jenett A, Keshishian H, Restifo LL, Rössler W, Simpson JH, Strausfeld NJ, Strauss R, Vosshall LB (2014) A systematic nomenclature for the insect brain. Neuron 81:755–765.

Kim AJ, Fitzgerald JK, Maimon G (2015) Cellular evidence for efference copy in Drosophila visuomotor processing. Nat Neurosci 18:1247–1255.

Krause A, Dürr V (2012) Active tactile sampling by an insect in a step-climbing paradigm. Front Behav Neurosci 6:30.

Krause AF, Dürr V (2004) Tactile efficiency of insect antennae with two hinge joints. Biol Cybern 91:168–181.

Krause AF, Winkler A, Dürr V (2013) Central drive and proprioceptive control of antennal movements in the walking stick insect. J Physiol Paris 107:116–129.

Lepreux G, Haupt SS, Dürr V (2019) Bimodal modulation of background activity in an identified descending interneuron. J Neurophysiol 122:2316–2330.

Mitchinson B, Martin CJ, Grant RA, Prescott TJ (2007) Feedback control in active sensing: Rat exploratory whisking is modulated by environmental contact. Proc R Soc B Biol Sci 274:1035–1041.

Okada J, Akamine S (2012) Behavioral response to antennal tactile stimulation in the field cricket Gryllus bimaculatus. J Comp Physiol A 198:557–565.

Okada J, Toh Y (2006) Active tactile sensing for localization of objects by the cockroach antenna. J Comp Physiol A 192:715–726.

Poulet JFA, Hedwig B (2002) A corollary discharge maintains auditory sensitivity during sound production. Nature 418:872–876.

Poulet JFA, Hedwig B (2006) The cellular basis of a corollary discharge. Science (80-) 311:512–522.

Poulet JFA, Hedwig B (2007) New insights into corollary discharges mediated by identified neural pathways. Trends Neurosci 30:14–21.

Scheiner R, Schnitt S, Erber J (2005) The functions of antennal mechanoreceptors and antennal joints in tactile discrimination of the honeybee (Apis mellifera L.). J Comp Physiol A Neuroethol Sens Neural Behav Physiol 191:857–864.

Schöneich S, Hedwig B (2015) Corollary discharge inhibition of wind-sensitive cercal giant interneurons in the singing field cricket. J Neurophysiol 113:390–399.

Schöneich S, Schildberger K, Stevenson PA (2011) Neuronal organization of a fast-mediating cephalothoracic pathway for antennal-tactile information in the cricket (Gryllus bimaculatus DeGeer). J Comp Neurol 519:1677–1690.

Schütz C, Dürr V (2011) Active tactile exploration for adaptive locomotion in the stick insect. Phil Trans R Soc B 366:2996–3005.

Sperry RW (1950) Neural basis of the spontaneous optokinetic response produced by visual inversion. J Comp Physiol Psychol 43:482–489.

Staudacher EM, Gebhardt M, Dürr V (2005) Antennal movements and mechanoreception: neurobiology of active tactile sensors. Adv In Insect Phys 32:49–205.

Staudacher EM, Schildberger K (1998) Gating of sensory responses of descending brain neurones during walking in crickets. J Exp Biol 201 (Pt 4):559–572.

Straka H, Simmers J, Chagnaud BP (2018) A new perspective on predictive motor signaling. Curr Biol 28:R232–R243.

von Holst E, Mittelstaedt H (1950) Das Reafferenzprinzip. Naturwissenschaften 37:464–476.

Webb B (2004) Neural mechanisms for prediction: Do insects have forward models? Trends Neurosci 27:278–282.

Ye S, Comer CM (1996) Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, Periplaneta americana. J Neurosci 16:5844–5853.

Zill SN, Chaudhry S, Büschges A, Schmitz J (2013) Directional specificity and encoding of muscle forces and loads by stick insect tibial campaniform sensilla, including receptors with round cuticular caps. Arthropod Struct Dev 42:455–467.

Zorović M, Hedwig B (2011) Processing of species-specific auditory patterns in the cricket brain by ascending, local, and descending neurons during standing and walking. J Neurophysiol 105:2181–2194.

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

Supplementary material:

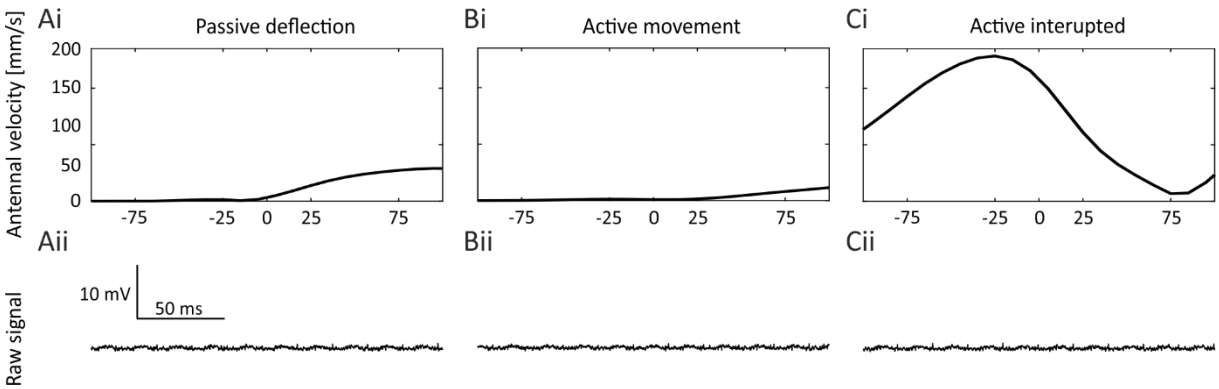


Figure S1: cONv neither responds to movement nor contact of the ipsilateral antenna. *Representative example of a single unit recording of cONv during (A) passive deflection, (B) active / self-generated movement, or (C) active movement that was interrupted by a stop. Same neuron as shown in Fig. 2 but with movement of the other, i.e. ipsilateral, antenna. Top row (i): Antennal velocity in mm/s before (-100 to 0 ms) and after the change in movement (0 to 100ms). Bottom panels (ii): Raw single unit recording of cONv in the movement episodes and time windows shown above.*

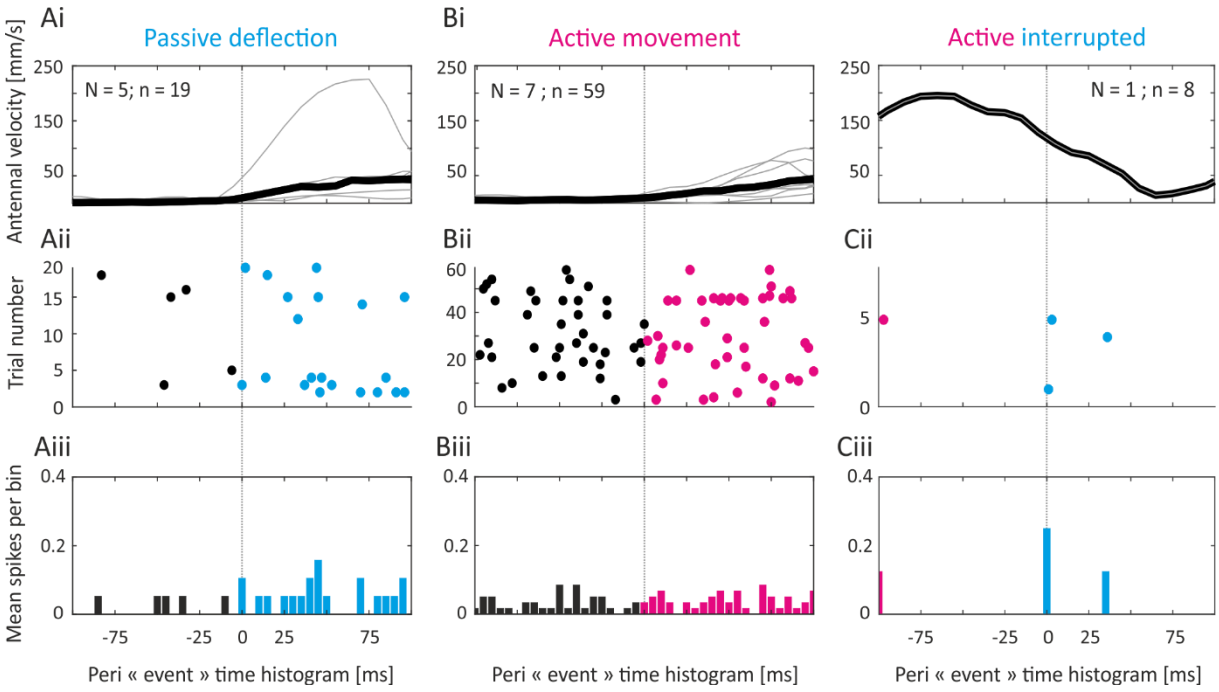


Figure S2: cONv activity is not affected by movement or contact of the ipsilateral antenna. *A) Passive deflection of the ipsilateral antenna. B) Self-generated (active) antennal movement. C) Ipsilateral antennal movements interrupted by an obstacle. Top panels (i) show the antennal velocity in mm/s before (-100 to 0 ms) and after (0 to 100 ms) the start of each movement (or interruption thereof, in C). Middle panels (ii) are raster plots of cONv activity with one row per trial, while bottom panels (iii) show the peri-stimulus time-histograms for all trials of a given type of movement. The vertical dashed line shows the onset of movement (or interruption thereof, C). Blue colour indicates passive deflection of the flagellum, magenta indicates active antennal movement and black indicates phases without antennal movement. N refers to the number of animals, whereas n refers to the total number of repetitions.*

Supplementary Table 1: Antennal joint angle ranges of the data shown in Fig. 1A. in different behavioural condition

N=3	Natural locomotion	
	Walking	**Searching**
HS joint, left	60.9° [92.1, 31.2]	61.5° [87.6, 26.1]
HS joint, right	57.1° [78.0, 20.9]	57.3° [74.3, 16.9]
SP joint, left	32.3° [0.8, -31.5]	37.6° [10.0, -27.6]
SP joint, right	40.8° [4.7, -36.1]	43.2° [12.4, -30.8]

Numbers give joint angle ranges in degrees, with 5% and 95% percentiles given in brackets

Supplementary Table 2: Antennal joint angle ranges of the data shown in Fig. 1A. in different behavioural condition for each animal

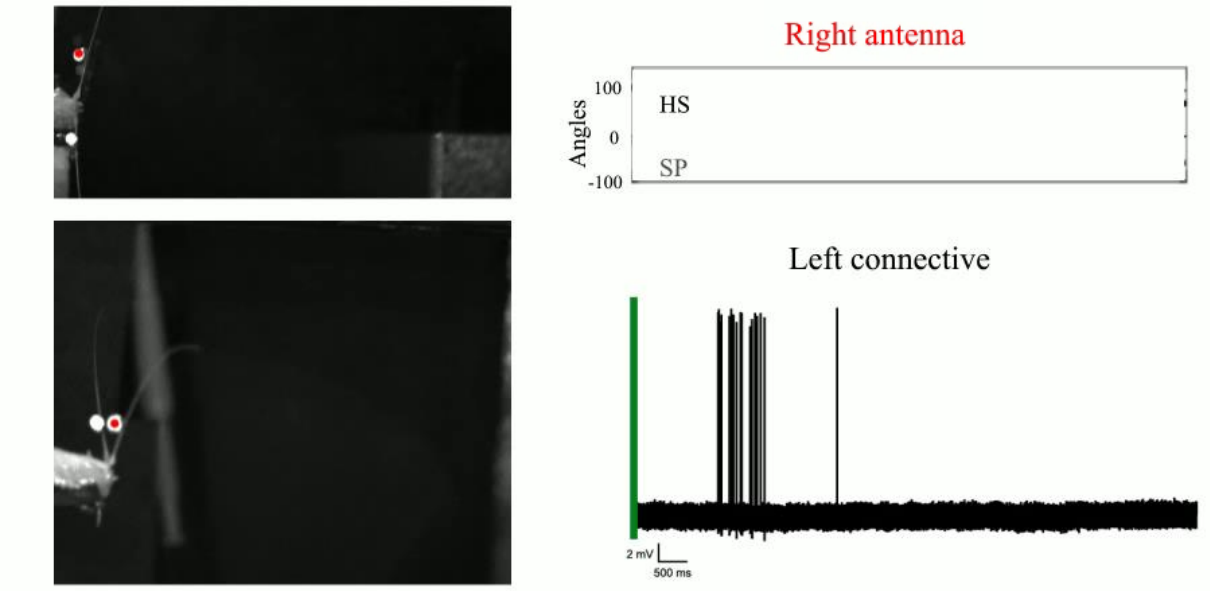
	#	N	HS joint, left	HS joint, right	SP joint, left	SP joint, right
Walk	**3**	14	61.4° [90.2, 28.8]	53.0° [72.6, 19.5]	27.8° [4.7, -23.1]	31.7° [8.4, -23.3]
	4	19	65.5° [103.7, 38.3]	73.6° [100.2, 26.6]	34.4° [-3.5, -37.9]	45.8° [-4.4, -50.2]
	11	11	55.7° [82.4, 26.6]	44.7° [61.3, 16.6]	34.7° [1.3, -33.4]	45.0° [10.2, -34.8]
Search	**3**	14	59,1° [86.3, 27.3]	54.5° [73.3, 18.8]	28.1° [1.1, -17.0]	34.6° [15.0, -19.7]
	4	19	71.8° [99.5, 27.7]	68.7° [88.5, 19.8]	43.5° [6.5, -37.0]	42.7° [1.0, -41.7]
	11	11	53.6° [76.9, 23.3]	48.8° [61.0, 12.2]	41.1° [12.3, -28.8]	52.2° [21.2, -31.0]

Numbers give joint angle ranges in degrees, with 5% and 95% percentiles given in brackets

Supplementary Table 3: Antennal joint angle ranges for each animal used in figures 27 to 30

#	HS joint, left	HS joint, right	SP joint, left	SP joint, right
1	103.3° [33.3, 135]	Not available	60.4° [-51.1, 10.5]	Not available
2	129.9° [27.2, 151.6]	Not available	74.1° [-39.5, 34.7]	Not available
3	104.4° [0, 104.5]	157.2° [0, 157.2]	90.8° [-71.7, 19.1]	76.2° [-76.2, 0]
4	88° [0, 88]	96.4° [0, 96.4]	28.3° [-28.3, 0]	54° [-54, 0]
5	85.9° [0, 85.9]	100.5° [50.5, 153.6]	59.6° [-36, 23.6]	66° [-63.5, 2.6]
6	111.3° [0 11]	101.9° [0, 101.9]	30.8° [0, 30.8]	53.9) [-54; 0]
7	98.1° [0, 98.1]	94.5° [0, 94.5]	47.7° [-47.7, 0]	61.2° [-61.2, 0]
8	110.7° [0 , 110.7]	125.9° [0 , 125.9]	47.2° [-46.4, 0.7]	55.6 °[-55.6, 0]
9	81.7° [49, 130.8]	107.2° [11.6, 118.8]	43.5° [-58.7, -15]	40.5° [-48.6, 0]

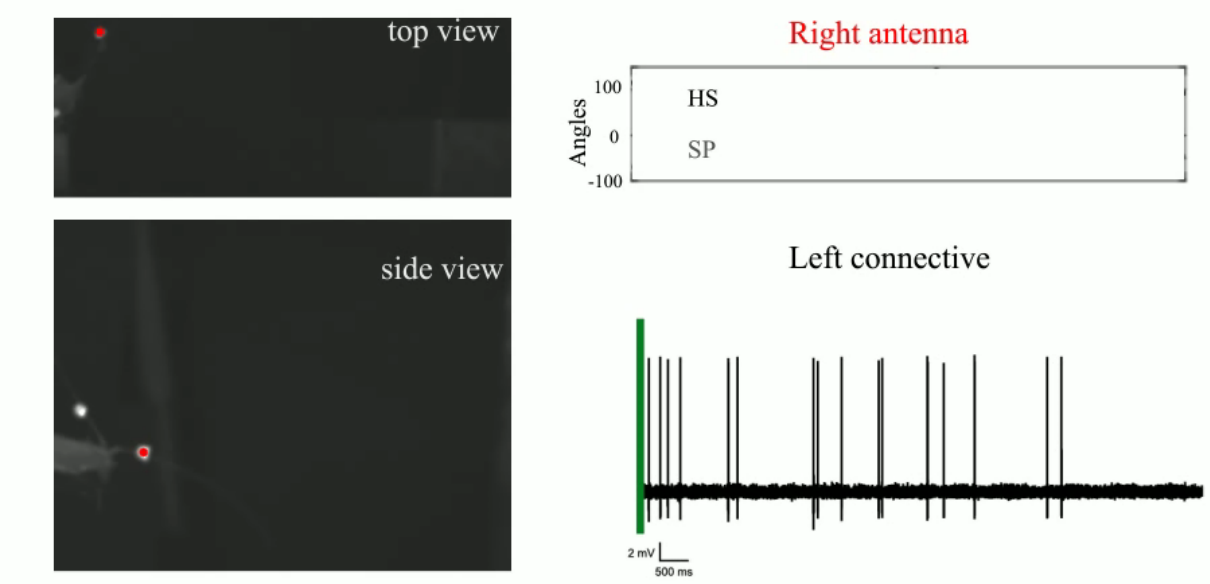
Numbers give joint angle ranges in degrees, with 5% and 95% percentiles given in brackets



Video 1[3]: Passive deflection of the contralateral antenna reliably induces spike activity in cONv. *Left: Top and side view as recorded by the setup camera. The marker of the right antenna is labelled by a red dot. Top right: Joint angle time courses of the right head-scape (HS) and scape-pedicel (SP) joints. Bottom right: Simultaneous extracellular single-unit recording of cONv in the left connective during that movement episode.*

[3] Link to download: https://usf.box.com/s/67ri8o2b78zwgp71ajalzlqynwsnzrlj

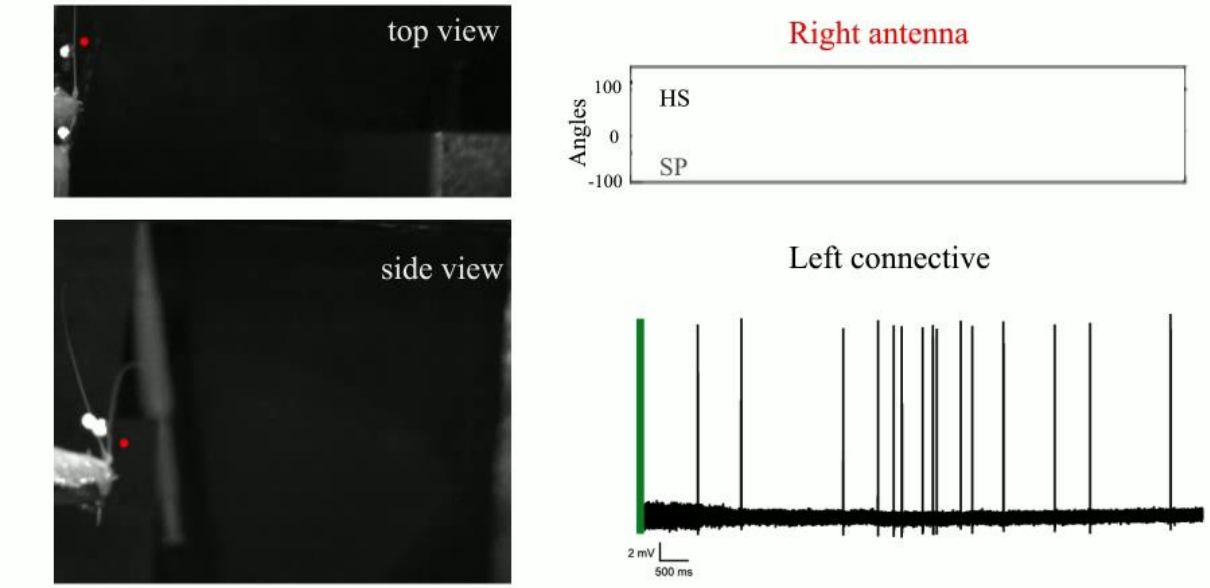
Password: Lepreux_et_al_2020



Video 2[4]: Active exploratory movement of the contralateral antenna does not induce a movement-related change of spike activity in cONv. *Left: Top and side view as recorded by the setup camera. The marker of the right antenna is labelled by a red dot. Top right: Joint angle time courses of the right head-scape (HS) and scape-pedicel (SP) joints. Bottom right: Simultaneous extracellular single-unit recording of cONv in the left connective during that movement episode.*

[4] Link to download: https://usf.box.com/s/b0p816ec6o2w8445l9nfdslp2a9ons9c

Password: Lepreux_et_al_2020



Video 3[5]: Interruption of active exploratory movement of the contralateral antenna induces spike activity in cONv. *Left: Top and side view as recorded by the setup camera. The marker of the right antenna is labelled by a red dot. Top right: Joint angle time courses of the right head-scape (HS) and scape-pedicel (SP) joints. Bottom right: Simultaneous extracellular single-unit recording of cONv in the left connective during that movement episode.*

[5] Link to download: https://usf.box.com/s/jmnt4mqj6kaebni4upwsd1l67ozxamdi

Password: Lepreux_et_al_2020

Chapter 5

General Discussion

In the present thesis I took advantage of the stick insect *Carausius morosus* and used an electrophysiological approach to study in more details a pair of identified giant descending interneurons (cONv, Ache et al., 2015) that are thought to be part of a neuronal pathway involved in the reach-to-grasp behaviour described by Schütz and Dürr (2011). Due to its size, and its response properties, cONv was a good candidate to study tactile induced behaviour in the stick insect. However, in order to understand whether cONv could be involved in this paradigm it was important to gain insight on how single neurons behaved in a restrained preparation before allowing movements with less constraints.

Thus, I studied cONv activity occurring between epochs of mechanosensory activation (chapter 2) and found that although the known antennal sensory input is separated for the pair of cONv their spontaneous activity at rest was highly correlated and could be reduced upon cross-modal activation. One hypothesis proposed was that adaptation was taking place and reduced the sensitivity of the neuron, which was then less likely to fire action potentials. As a consequence, I analysed cONv activity under constant mechanosensory stimulation that was known to cause adaptation (Chapter 3). I looked at the aspect of stimulus-specific adaptation and found that cross-modal adaptation occurred between antennal movement and substrate vibration but not the other way round. Both of these studies were carried out using quiescent animals while imposing passive antennal stimulation. In order to fill the gap between data from restrained preparations and experiments on freely walking animals, I studied the activity of cONv in animals that engaged in active antennal exploration although they were immobilized (chapter 4). In these experiments, I combined antennal motion tracking with single-unit recordings of cONv and found that cONv did not encode the velocity of self-generated antennal movements. However, I found that cONv responded during interruption of ongoing movement, making cONv a possible contact detector neuron. Although, these studies provide new insights into the encoding properties of cONv and its putative role in behaviour, some questions remain unsolved:

- Can cONv be recorded extracellularly in walking animals?
- What are the antennal inputs to cONv?
- Where does the substrate vibration input comes from?
- Finally, what is the role of cONv in motor behaviour?

In the following discussion I will try to answer these questions or bring elements of response.

cONv cannot be identified extracellularly in behaving animals

In chapter 3 and 4, I further characterized the pair of giant DINs in quiescent animals. However, there was still information missing regarding the activity of the cONv interneuron under more behaviourally relevant conditions. Since cONv was identified as having the largest amplitude action potential during extracellular neck-connective recordings (Ache et al., 2015 and chapter 2 and 3), it was believed that it could also be recorded extracellularly with hook electrodes (*e.g* as proposed by Schmitz et al., 1988) in tethered animals walking on a treadmill. The animal would be stabilized by a rod over a treadwheel that was rotated by the leg movement of the animal. The animal could walk and display exploratory antennal movements. However, as soon as the insect started to walk, the overall activity in the connective increased tremendously and was too strong to allow a reliable identification of the spikes belonging to cONv (Figure 31). Indeed, cONv was no longer the neuron displaying the biggest amplitudes action potentials in the extracellular neck connective recording. This can be explained by several factors:

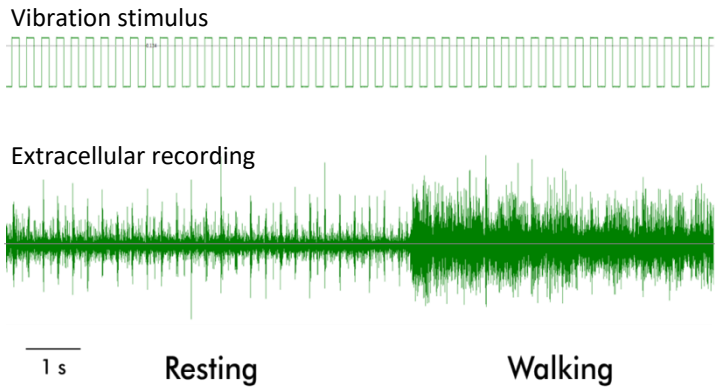


Figure 31: Whole nerve neck-connective recording with a hook electrode.
Top: trace showing the substrate vibration stimulus delivered at a tapping frequency of 5 s^{-1}. Bottom: Neck-connective recording during rest (standing) and walking. The overall activity in nerve increased during walking so much that cONv spikes could not be discerned anymore. The black line represents a threshold for the identification of the cONv spikes.

- There is a general arousal of the activity which is dependent on the state of the stick insect and the animal switched from a resting state to an active state (Bässler and Büschges, 1998).

- The neurons controlling locomotion are sending descending information from the head down to the legs, leading to an increase of the descending neural activity in the connective.
- A targeting-mechanism that guides foot-placement of the legs according to the position of the leading ipsilateral leg has been observed in freely walking stick insects (Theunissen et al., 2014). This means that there is information transfer between adjacent legs via the thoracic connectives. Equally, sensory related activity is sent via axons into the connective and the information required for the position of the targeting is related to sensory activity from the other legs.

All of these aspects may contribute to mask cONv spikes in whole-nerve neck-connective recordings in walking animals. In order to rule out at least the confounding effect of sensory afferents, I studied antennal movements in stationary animals in which the legs had been removed. The reason for that was that it would reduce the number of units recorded by the hook electrode and could allow to isolate cONv spikes from background nerve activity based in their amplitude. However, as antennal movement was induced by haptic stimulation of the abdomen, we still observed a strong increase of the overall activity in the recording (Figure 32). When the antenna was moving (as shown by the black trace in Figure 32), the number of units recorded increased. Some neurons displayed spikes that reached even higher amplitudes than cONv did at rest. As a consequence, cONv spikes could not be separated from the other units. However, hook electrodes are not the only option available for extracellular nerve recordings. Indeed, Stout (1971) used suction electrodes in order to record neuronal activity in freely moving cockroaches. These electrodes consist of a polyvinyl tube and a stainless-steel wire mounted to a syringe needle. Suction electrode recordings are suitable for neurons with large-diameter axons that are located at

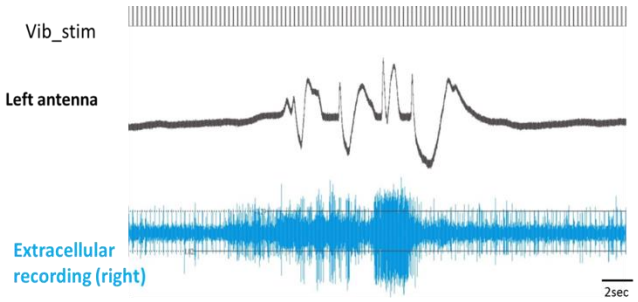


Figure 32: Neck-connective recording during active antennal movement in stationary animals.
Top: Substrate vibration induced by a tapping frequency of 10 s^{-1}. Middle: Movement of left antenna captured by the mean of a retroreflective marker placed on the flagellum which was tracked with a camera. Bottom: hook electrode recording. The two light grey lines show the amplitude of cONv when the animal was at rest.

the periphery of the nerve, much like cONv. The suction at the tip of the electrode exerts a depression on the nerve and the selectivity of the electrode permits to record only few cells at the same time. This improves discriminability of spikes from single neurons than whole-nerve recordings. Nevertheless, during pronounced movement of the animal, the tip of the electrode could move and lead to the loss of the signal. Others like Staudacher et al. (1998) or Zorović and Hedwig (2013) used glass pipette electrodes to record descending brain neurons in crickets walking on a trackball. In both cases they were able to record descending neuronal activity during spontaneous or sensory induced walking behaviour. Although these electrodes are fragile, they can be used in setups where they are well stabilized. Thus, I decided to use glass pipette electrodes for intracellular or extracellular single-unit recordings of cONv during exploratory antennal movement. However, this comes at a cost as the necessary preparation generally results in conditions that may impede the induction of active behaviours. It was critical to tune the trade-off between the possibility of observing active behaviour from the animal and the surgical procedures mandatory to allow for stable single-cell recordings.

Towards the identification of the antennal mechanosensory inputs.

In chapter 4, I combined motion capture analysis of the moving antenna with electrophysiology. Altogether, it allowed me to reconstruct in 3D the movement of both antennae while the single-unit recording cONv revealed its excitability during imposed antennal deflection and self-generated exploratory movements. I found out that contrary to the passive ramp-and-hold deflection during which cONv encoded the movement velocity (Ache et al. 2015), rhythmic and sustained antennal movements, (*i.e*., movement reminiscent of exploratory behaviour during free walking), did not lead to an increased cONv activity. The neuron showed neither a velocity dependency nor a reduction of its spontaneous activity during voluntary movement. However, when these antennal exploratory movements were interrupted by an obstacle, cONv fired each time the antenna touched the obstacle and signalled the presence of the object. As discussed in chapter 5, the interruption of the antennal movement by an obstacle is similar to the passive deflections that were used to characterize cONv (in chapter 3 and 4). It can also be considered as a deviation from intended action. According to this view, in quiescent animal the ramp-and-hold stimulus causes cONv activity because the intended position of the antenna is along the body axis, immobile and no

afferent activity is expected. Thus, in the case of a contact of the flagellum with an obstacle, there is an interruption of the ongoing movement and the actual movement is different than the intended one. One possible explanation is that corollary discharge (Sperry, 1950) , also known as efference copy (von Holst and Mittelstaedt, 1950) of the antennal motor neuron is taking place (Figure 33). Although it is a matter of debate as to whether insects have such a mechanism (Webb, 2004) there is accumulating evidence for corollary discharge mechanisms under various behavioural paradigms (Poulet and Hedwig, 2002, 2006, 2007; Huston and Jayaraman, 2011; Krapp, 2015). Thus, self-induced actions are unambiguous in comparison to external disturbance. In crickets, sound emitted by the animal itself can be distinguished from external sound through a corollary discharge mechanism (Poulet and Hedwig, 2002, 2006). Similarly, Schöneich and Hedwig (2015) showed that a giant descending interneuron responsive to wind stimulus was inhibited when the animal was singing. Corollary discharge was also found to occur in the antennal mechanosensory system (Gebhardt and Honegger, 2001). Corollary discharge occurs in systems where the input to the neuron would always be activated, regardless of whether the action was self-generated or driven by external sources. To know whether corollary discharge occurs in our system, it is necessary to know what the input to cONv is. To date it is still unclear and will be discussed in the following section.

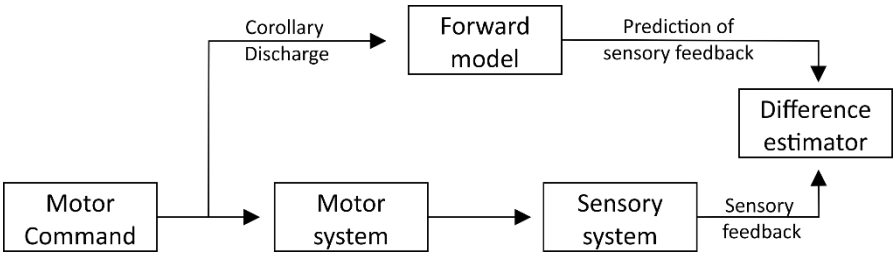


Figure 33: Schematic representation of a forward model in a motor system.
The motor command is sent to the motor system and a copy of that command is sent in parallel to a forward model. The forward model will predict what signal the sensory system should send based on that command. The difference between both feedbacks will be analyzed and the difference will be signalled

Hair field afferents: the main candidate

As mentioned above, the identification of antennal mechanosensory input to cONv is still unclear. However, one can reduce the list of input candidates based on the response properties of cONv. First of all, cONv is encoding the velocity of passive deflection of the flagellum. Among all the mechanoreceptors evoked in chapter 1, the most suitable candidate

is the antennal hair field (Figure 2). Indeed, in the cockroach Okada and Toh, (2001) showed that the response of hair field afferents depended on the angle and the velocity at which they were bent. The stick insect antennae carry seven hair fields (Krause et al., 2013). Two hair plates are located on the SP-joint together with two hair rows. Ablation of hair fields resulted in an increase of the angular range during exploratory antennal movement (Krause et al., 2013). It impaired the ability of cockroaches to orient towards stationary object following antennal contact (Okada and Toh, 2000). Thus, in different species the locomotion can be affected if the antennal hair fields are ablated. In their study, Ache et al. (2015) carried out back fill stainings of the hair field afferents together with an intracellular staining of cONv. They could show that the projections of the hair field afferents were overlapping with the dendrites of cONv in the Gnathal ganglion (their figure 1B, see also (Goldammer and Dürr, 2018). However, no direct connection between the hair field afferents and the cONv has ever been shown. Although the ramp-and-hold stimulus (used in this study and by Ache et al., 2015) is designed to mainly stimulate the hair fields of the SP-joint, it cannot be excluded that other mechanoreceptors are also activated during the ramp-and-hold stimulus. A straight-forward experiment would be to eliminate the hair plates and hair rows by shaving them and evaluate the activity of cONv with intact versus ablated hair fields. If we assume that the ablation of the hair field is successful, it should result in a loss of the velocity encoding from cONv. If this is true, then a corollary discharge as described earlier must occur in this system to inhibit the input of the hair fields during voluntary exploratory movement and not during passive antennal movement. Indeed, hair fields will be deflected irrespective of whether it is a passive or a self-generated antennal movement and should thus fire in both cases. This hypothesis is currently tested in our department by Bianca Jaske, a Master student writing her thesis on this topic.

Campaniform sensilla: the outsider

Among the mechanoreceptors that could potentially activate cONv, campaniform sensilla (CS) are another candidate (chapter 1, Figure 2). Organized as a ring around the SP-joint of the antennae (also called Hick's organ, (Hicks, 1857), they can sense the bending of the joint along their axis of orientation (Heinzel and Gewecke, 1979). As of now, there is no indication reporting a connection between the CS and cONv. However, the response properties of the CS could explain the mode of activation of cONv during the passive and active antennal movement. In the leg of the stick insect the tibial campaniform sensilla are

organized as 2 distinct group of different orientation (Zill et al., 2011). They were shown to respond only to imposed forces to the tibia and along their axis of orientation. The stimulus was presented as a ramp-and-hold stimulus using a piezo-electric probe on the tibia. The response of one CS group was recorded only during the ramps and no activity was recorded during the hold. Moreover, the intensity of the response measured for resisted movement was proportional to the force applied on the leg. Conversely, when the leg movement was not resisted and not due to muscle activation, there was no activity from the two groups of CS. If we assume that i) cONv receives information from the campaniform sensilla located at the SP-joint, ii) that the response properties of these CS resemble those of the tibial CS, it could in principle explain why cONv is activated during passive deflection of the antenna using ramp-and-hold stimulation. Accordingly, the spiking activity of cONv upon antennal contact with an obstacle could be signalled through the force that are encoded by the CS. As the animal moves its antenna the self-generated movements are not resisted and thus no CS afferent input would be expected to drive cONv. As soon as the antenna touches an obstacle, the movement becomes resisted (*i.e.* loaded) and is signalled by downstream neurons like cONv. Assuming that this is the case for cONv, it rules out the possibility of an efference copy mechanism. Indeed, contrary to hair field that are under continuous stimulation during passive and active antennal movement, the CS are only responding during resisted movement.

Johnston's organ: the unknown

The next candidate on the list is the Chordotonal organ (CO, Figure 2). Located in the pedicel of the antennae, it measures vibration of the flagellum and the descending information from the CO is used for flight stability (Mamiya and Dickinson, 2015). For terrestrial insects it may play a role in antennal-mediated control of locomotion. In the stick insect, little is known about the antennal CO. One of the most studied chordotonal organs is the femoral chordotonal organ (FeCO). Its involvement in the control of locomotion has been extensively studied (Zill, 1985; Bässler, 1988; Bässler and Büschges, 1998; Field and Matheson, 1998). Mamiya et al. (2018) showed in flies, that the FeCO afferents can measured the position, the movement and the vibration. Using *in vivo* calcium imaging, they were able to analyze the different neurons separately and found specific tuning properties. They showed that a subpopulation of neurons was encoding the joint position during ramp-and-hold stimulus and they were sensitive to vibration. Although, the kinetics of calcium imaging

is slow, they argued that it is possible that these neurons encode the velocity of the movement. In flies, the FeCO shares similar properties with the antennal chordotonal organ also called Johnston's organ (Field and Matheson, 1998). As already developed in chapter 1, the Johnston's organ detects airborne vibration, gravity and touch (Matsuo and Kamikouchi, 2013; Albert and Göpfert, 2015). Moreover, topographic study of JO afferents in the honeybee shows that they project in the GnG of the animal (Ai et al., 2007). Thus, it could be possible for Johnston's organ afferents to provide input to cONv too. Little is known about the stick insect CO and its connectivity. As of yet it is still unclear whether they carry a so-called Johnston's organ although they have a rather simpler chordotonal organ with one attachment to the cuticle (Dürr 2014). However, if cONv were to receive antennal chordotonal organ input, there should be reafference of the antennal motor activity such that during voluntary exploratory movement the chordotonal organ does not activate cONv.

Tactile hairs: the impossible option

Contrary to the mechanoreceptors discussed above the tactile hairs can already be ruled out as input to cONv. In all the experiments that involved the usage of a ramp-and-hold stimulus, the antennae were cut to their 5th proximal annulus and the stimulation was given by means of a contactless apparatus. It consisted of a minutien pin inserted in the stump of the flagellum that was moved by a magnet placed at the extremity of a motor potentiometer (Gebhardt and Honegger, 2001; Ache and Dürr, 2013; Ache et al., 2015). The activation of cONv could not arise from tactile hairs as they were removed and/or not stimulated.

The origin of substrate vibration.

In the previous section, I discussed the potential antennal input to cONv that potentially could explain the velocity encoding of passive antennal deflections. Although cONv responds mainly to external activation of the antenna, it also responds to substrate vibration (Ache et al., 2015). Contrary to antennal stimulation where only one cONv responds to the movement of its contralateral antenna, both cONv neurons respond simultaneously to substrate vibration (chapter 2, Figures 7, 8 and 9). Substrate vibration were delivered by a solenoid hitting the recording table at various tapping frequencies. Ache et al., (2015) showed that both cONv neurons respond with one action potential per tap and can follow tapping frequency from 1 tap per second up to 20 taps per second (see also chapter 2, Figure 9 and Chapter 3, Figure S3). They also showed that the encoding of this vibration was not mediated

through a descending input but rather through a direct input to the GnG. Indeed, ablation of the circumesophageal connectives (connecting the brain and the GnG) resulted in a loss of response to the antennal ramp-and-hold stimulus while the response to tapping persisted. Moreover, cONv response properties were unaffected by transection of the thoracic connectives (Ache et al., 2015) showing that cONv does not receive ascending input from leg mechanoreceptors. Therefore, the source for substrate vibration can neither be located on the antennae nor on the legs. Both antennal and femoral chordotonal organs that are sensing vibration can thus be ruled out. In an attempt to find out where the source for substrate vibration is, I tried to identify by which nerve the information reaches the GnG. The approach was to transect the nerve one by one, while having cONv under constant substrate vibration stimulation. The postulate is simple, after a nerve is cut, if the response to tapping is gone, the source of substrate vibration sends the information to cONv though that nerve. The GnG is innervated by several small nerves running from the head near the connectives, reaching the GnG dorsally or ventrally (Marquardt, 1939). Marquardt (1939) identified at least 7 nerves innervating the GnG. In a living animal the preparation required for the identification of these nerves while recording cONv activity was difficult and the results obtained were inconsistent. Therefore, I was not able to narrow down the source for substrate vibration to one nerve in particular. However, a lot of nerves observed by Marquardt (1939) were coming from the mouthparts of the animal and it is likely that the input to cONv for substrate vibration originates from chordotonal organ located on the mouthparts. As of now, the source of the substrate vibration input remains enigmatic and will need further investigation.

What role for cONv in motor behaviour?

To date, the exact role of cONv in the stick insect remains unknown. One of the reasons is the lack of information about the targets of cONv axons. One likely possibility is that cONv modulates leg movements. Büschges (1989) identified a neuron regulating leg movement which morphology in the mesothoracic resemble the arborisation pattern of cONv. Furthermore, cONv shares similarities with neurons that have been shown to regulate locomotion in other insects. For instance, in cockroach the DMI neurons identified by Burdohan and Comer, (1996) arborize in all thoracic ganglia in regions where leg motoneurons and premotor interneurons are located (Ritzmann and Pollack, 1986, 1990; Murrain and Ritzmann, 1988; Stubblefield and Comer, 1989). Similarly, the descending contralateral

movement detector of the locust DCMD (O'Shea et al., 1974; Gabbiani et al., 1999) has a big diameter contralateral axon that connects the thoracic ganglia and was shown to contribute to trigger a jump in response to a visual looming stimulus (O'Shea et al., 1974; Gabbiani et al., 1999; Gray et al., 2001). In the cockroach, the giant fibre DMI b1 responds to bilateral touch of the antennae and its activity is correlated with the direction of the turn in the escape behaviour (Burdohan and Comer, 1990; Ye and Comer, 1996). Escape behaviour has not been described and analysed in the stick insect although fast avoidance turns have been observed anecdotally. However, stick insects can react to tactile stimulation of their antennae with a fast reaching movement of their front legs (Schütz and Dürr, 2011). This movement can occur either when the front leg is already in stance or it could occur during a swing movement that is interrupted to reach the contact location. This is a retargeted front leg movement because the leg was already going towards the ground to make a step and has been redirected elsewhere. The onset of the retargeting takes 40 ms to occur after antennal contact. In comparison, cONv was shown to carry the antennal information to the prothoracic ganglion in less than 20 ms. This leaves enough time to modulate the leg movement accordingly. Another study in the stick insect, Berg and colleagues (2015) showed that a non-spiking interneuron (i4) could command the leg to start searching movement. Depolarisation of i4 neuron (increase of the membrane potential) triggered a searching movement of the leg in absence of ground contact. Injection of hyperpolarizing current in i4 (decrease of membrane potential) prevented the leg to do searching movement. There are two i4 per ganglion thus controlling locally each leg (Büschges, 1995; Sauer et al., 1996).

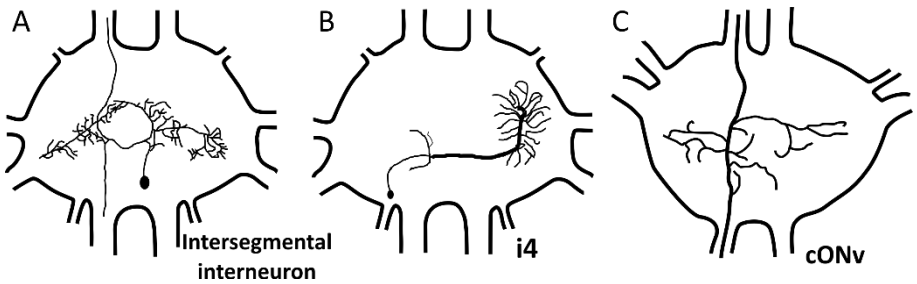


Figure 34: Morphology of three interneurons of the stick insect Carausius morosus.

A) Mesothoracic intersegmental interneuron. (Adapted from Büschges 1989). B) i4 non spiking interneuron in the mesothoracic ganglion (Adapted from Berg et al., 2015). C) Contralateral ON-type velocity sensitive interneuron in the prothoracic ganglion (adapted from Ache et al., 2015). Note that although only the prothoracic arborization of cONv is shown here it arborizes in a similar fashion in the mesothoracic ganglion.

Other thoracic interneurons found by Büschges 1989 respond to position, velocity, acceleration of the leg femur tibia joint and projects to the prothoracic and mesothoracic ganglion. cONv shares morphological similarities with some of this neurons found by Büschges (1989) that encodes properties of leg movements (Figure 34). They are descending through the thoracic nerve chord, they cross the midline of the ganglion, and they arborise bilaterally in the same regions. Thus, it is possible that cONv regulates at least some of the neurons controlling the legs. This needs to be tested in walking animals for instance by using a trackball system associated to single unit recordings of cONv and electromyogram which could provide information about the potential role of cONv during reaching movement of the front leg.

cONv has been defined as a giant descending interneuron (Ache et al., 2015) and in terms of energy, neurons displaying action potential are energetically more costly than non-spiking neurons. This applies even more to giant neurons (King, 2013). Hence, it makes sense that most giant neurons play an important role in behaviours that are decisive for the survival of the animal such as escape responses (Stubblefield and Comer, 1989; Vu et al., 1993; Wang et al., 2001; Schöneich et al., 2011). Giant axons permit fast transmission and are more reliable for the transmission of action potentials than small axons. In the stick insect *Carausius morosus* one mechanism of defence and survival is the camouflage. Animals enter the thanatosis mode which, in other words, consist to pretend to be a branch and to not move (Bässler, 1983, FIGURE3). It seems unlikely that cONv would be involved in such behaviour. Indeed, in chapter 4, I showed that cONv is activated upon antennal contacts which is not followed by interruption all types of movement. However, we can imagine that cONv interrupts the leg coordination of an animal to start a reaching movement of the front leg and allow the animal to climb. This is a crucial part of the life of *Carausius morosus* as their most favourite activity after mimicking branches is to climb bushes.

Concluding remarks

Tactually guided behaviour is a widely spread across the animal kingdom. However, the neural control of such goal-oriented actions is not well understood. *C. morosus* are obligatory walkers and show the ability to react to objects on their path by appropriate motor behaviour, *e.g.* by climbing over it. Thus, they offer an opportunity to study neuronal activity patterns during well-documented behaviour. However, as the present thesis showed it is not easy to specifically identify a unique neural substrate for such a task. Nevertheless, this thesis showed that it is still important to understand how single neurons respond under various behaviourally relevant conditions to gain insight in a more complex circuitry.

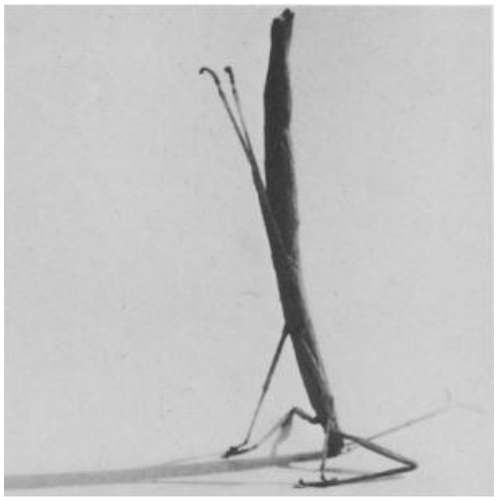


Figure 35: Catalepsy in carausius morosus
From Bässler 1983

Thus, I used an electrophysiological approach to study in more detail a pair giant descending interneurons (cONv). I studied cONv activity between epochs of mechanosensory activation (chapter 2), looked at bimodal interactions (chapter 3) and finally I tried to fill the gap between restrained preparation and experiments on freely walking animals by recording cONv in animals that performed antennal exploration although they were immobilized (chapter 4). I could show that contrary to what has been proposed, cONv is not encoding antennal velocity of exploratory antennal movement but rather acts as a sensible antennal contact detector that can participate in the reach to grasp behaviour of *Carausius morosus*..

References

Ache JM, Dürr V (2013) Encoding of near-range spatial information by descending interneurons in the stick insect antennal mechanosensory pathway. J Neurophysiol 110:2099–2112.

Ache JM, Haupt SS, Dürr V (2015) A direct descending pathway informing locomotor networks about tactile sensor movement. J Neurosci 35:4081–4091.

Ai H, Nishino H, Itoh T (2007) Topographic organization of sensory afferents of Johnston's organ in the honeybee brain. J Comp Neurol 502:1030–1046.

Albert JT, Göpfert MC (2015) Hearing in Drosophila. Curr Opin Neurobiol 34:79–85.

Bässler U (1983) Neural basis of elementary behavior in stick insects. Springer: Berlin, Heidelberg.

Bässler U (1988) Walking Movements of Stick Insect Forelegs : J Exp Biol 147:125–147.

Bässler U, Büschges A (1998) Pattern generation for stick insect walking movements - Multisensory control of a locomotor program. Brain Res Rev 27:65–88.

Berg EM, Hooper SL, Schmidt J, Büschges A (2015) A Leg-local neural mechanism mediates the decision to search in stick insects. Curr Biol 25:2012–2017.

Burdohan JA, Comer CM (1990) An antennal-derived mechanosensory pathway in the cockroach: descending interneurons as a substrate for evasive behavior. Brain Res 535:347–352.

Burdohan JA, Comer CM (1996) Cellular organization of an antennal mechanosensory pathway in the cockroach, Periplaneta americana. J Neurosci 16:5830–5843.

Büschges A (1989) Processing of sensory input from the femoral chordotonal organ by spiking interneurones of stick insects. J Exp Biol 144:81–111.

Büschges A (1995) Role of local nonspiking interneurons in the generation of rhythmic motor activity in the stick insect. J Neurobiol 27:488–512.

Dürr V (2014) Stick insect antennae. Scholarpedia 9(2):6829

Field LH, Matheson T (1998) Chordotonal organs of insects. Adv In Insect Phys 27.

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

Gebhardt M, Honegger HW (2001) Physiological characterisation of antennal mechanosensory descending interneurons in an insect (Gryllus bimaculatus, Gryllus campestris) brain. J Exp Biol 204:2265–2275.

Goldammer J, Dürr V (2018) Proprioceptive input to a descending pathway conveying antennal postural information: Terminal organisation of antennal hair field afferents. Arthropod Struct Dev 47:465–481.

Gray JR, Lee JK, Robertson RM (2001) Activity of descending contralateral movement detector neurons and collision avoidance behaviour in response to head-on visual stimuli in locusts. J Comp Physiol - A Sensory, Neural, Behav Physiol 187:115–129.

Heinzel HG, Gewecke M (1979) Directional sensitivity of the antennal campaniform sensilla in locusts. Naturwissenschaften 66:212–213.

Hicks JB (1857) On a new structure in the antennae of insects. Trans Linn Soc London 22:147–154.

Huston SJ, Jayaraman V (2011) Studying sensorimotor integration in insects. Curr Opin Neurobiol 21:527–534.

King D (2013) What can giant axons tell us about genetics and evolution. Int Soc Neuroethol Newsl.

Krapp HG (2015) How a fly escapes the reflex trap. Nat Neurosci 18:1192–1194.

Krause AF, Winkler A, Dürr V (2013) Central drive and proprioceptive control of antennal movements in the walking stick insect. J Physiol Paris 107:116–129.

Mamiya A, Dickinson MH (2015) Antennal mechanosensory neurons mediate wing motor reflexes in flying drosophila. J Neurosci 35:7977–7991.

Mamiya A, Gurung P, Tuthill JC (2018) Neural coding of leg proprioception in drosophila. Neuron 100:636-650.e6.

Marquardt F (1939) Anatomie der Muskulatur und der peripheren Nerven von Carausius (Dixippus) morosus. Abteilung für Anatomie und Ontogenie der Tiere 66: 63-128.e.

Matsuo E, Kamikouchi A (2013) Neuronal encoding of sound, gravity, and wind in the fruit fly. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 199:253–262.

Murrain M, Ritzmann RE (1988) Analysis of proprioceptive inputs to DPG interneurons in the cockroach. J Neurobiol 19:552–570.

O'Shea M, Rowell CHF, Williams JLD (1974) The anatomy of a locust visual interneurone; the descending contralateral movement detector. J Exp Biol 60:1–12.

Okada J, Toh Y (2000) The role of antennal hair plates in object-guided tactile orientation of the cockroach (Periplaneta americana). J Comp Physiol A Sensory, Neural, Behav Physiol 186:849–857.

Okada J, Toh Y (2001) Peripheral representation of antennal orientation by the scapal hair plate of the cockroach Periplaneta americana. J Exp Biol 204:4301–4309.

Poulet JFA, Hedwig B (2002) A corollary discharge maintains auditory sensitivity during sound production. Nature 418:872–876.

Poulet JFA, Hedwig B (2006) The cellular basis of a corollary discharge. Science (80-) 311:512–522.

Poulet JFA, Hedwig B (2007) New insights into corollary discharges mediated by identified neural pathways. Trends Neurosci 30:14–21.

Ritzmann RE, Pollack AJ (1986) Identification of thoracic interneurons that mediate giant interneuron-to-motor pathways in the cockroach. J Comp Physiol A 159:639–654.

Ritzmann RE, Pollack AJ (1990) Parallel motor pathways from thoracic interneurons of the ventral giant interneurons system of the cockroach,Periplaneta americana. J Neurobiol 21:1219–1235.

Sauer AE, Driesang RB, Büschges A, Bässler U (1996) Distributed processing on the basis of parallel and antagonistic pathways simulation of the femur-tibia control system in the stick insect. J Comput Neurosci 3:179–198.

Schmitz J, Büschges A, Delcomyn F (1988) An improved electrode design for en passant recording from small nerves. Comp Biochem Physiol Part A Physiol 91:769–772.

Schöneich S, Hedwig B (2015) Corollary discharge inhibition of wind-sensitive cercal giant interneurons in the singing field cricket. J Neurophysiol 113:390–399.

Schöneich S, Schildberger K, Stevenson PA (2011) Neuronal organization of a fast-mediating cephalothoracic pathway for antennal-tactile information in the cricket (Gryllus bimaculatus DeGeer). J Comp Neurol 519:1677–1690.

Schütz C, Dürr V (2011) Active tactile exploration for adaptive locomotion in the stick insect. Phil Trans R Soc B 366:2996–3005.

Sperry RW (1950) Neural basis of the spontaneous optokinetic response produced by visual inversion. J Comp Physiol Psychol 43:482–489.

Staudacher EM, Schildberger K (1998) Gating of sensory responses of descending brain neurones during walking in crickets. J Exp Biol 201 (Pt 4):559–572.

Stout JF (1971) A technique for recording the activity of single interneurons from free-moving crickets (Gryllus campestris). Z Vergl Physiol 74:26–31.

Stubblefield GT, Comer CM (1989) Organization of giant interneuron projections in thoracic ganglia of the cockroach Periplaneta americana. J Morphol 200:199–213.

Theunissen LM, Vikram S, Dürr V (2014) Spatial co-ordination of foot contacts in unrestrained climbing insects. J Exp Biol 217:3242–3253.

von Holst E, Mittelstaedt H (1950) Das Reafferenzprinzip. Naturwissenschaften 37:464–476.

Vu ET, Lee SC, Krasne FB (1993) The mechanism of tonic inhibition of crayfish escape behavior: Distal inhibition and its functional significance. J Neurosci 13:4379–4393.

Wang XJ, Gao C, Norgren RB (2001) Descending influences on escape behavior and motor pattern in the cockroach. J Neurobiol 49:9–28.

Webb B (2004) Neural mechanisms for prediction: Do insects have forward models? Trends Neurosci 27:278–282.

Ye S, Comer CM (1996) Correspondence of escape-turning behavior with activity of descending mechanosensory interneurons in the cockroach, Periplaneta americana. J Neurosci 16:5844–5853.

Zill SN (1985) Plasticity and proprioception in insects. II. Modes of reflex action of the locust metathoracic femoral chordotonal organ. J Exp Biol 116:463–480.

Zill SN, Büschges A, Schmitz J (2011) Encoding of force increases and decreases by tibial campaniform sensilla in the stick insect, Carausius morosus. J Comp Physiol A 197:851–867.

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

.

Erklärung

Erklärung Hiermit versichere ich, dass ich die vorliegende Dissertation selbstständig angefertigt, keine Textabschnitte von Dritten oder eigener Prüfungsarbeiten ohne Kennzeichnung übernommen und alle von mir benutzten Hilfsmittel und Quellen in meiner Arbeit angegeben habe. Dritte haben weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Vermittlungstätigkeiten oder für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen. Ich habe die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht. Ebenso habe ich die Arbeit zuvor weder im Inland noch im Ausland in gleicher oder ähnlicher Form bei einer anderen Hochschule als Dissertation eingereicht. Die geltende Promotionsordnung der Fakultät ist mir bekannt.

June 2020
Tampa; Florida

Gaëtan LEPREUX