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DECISION-MAKING IN INVERTEBRATES

Topic Editor
Björn Brembs

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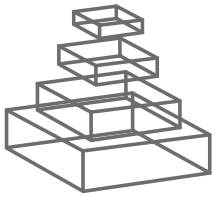

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DECISION-MAKING IN INVERTEBRATES

Topic Editor:
Björn Brembs, Universität Regensburg, Germany

Wordle depicting the frequencies of the words used in the articles of this collection.

Long before Paul The Octopus became famous during the 2010 soccer world cup, scientists realized that decision-making is a capacity which is not exclusive to the so-called 'higher' animals. Not only cephalopods, but most if not all invertebrate animals show an amazing capacity for making decisions even if the external circumstances are ambiguous, contradictory, or provide no information at all. This collection of articles celebrates the diversity of decision-making by showcasing the most well-studied cases in a range of invertebrate species.

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EDITORIAL
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Invertebrate behavior—actions or responses?

Björn Brembs*

Zoology - Neurogenetics, Universität Regensburg, Regensburg, Germany
**Correspondence: bjoern@brembs.net*

Edited by:
Scott A. Huettel, Duke University, USA

Keywords: decision making, animal, insects, mollusca, cricket, Drosophila, Aplysia, crustacean

Invertebrates have always been the reductionist neuroscientist's favorite. After all, are their nervous systems not simpler, their behavior not more stereotyped and reproducible than those of vertebrates, unfettered by cognition, and intelligence which would only serve to complicate the already tricky study of how neurons do the things they do? Until not too long ago, neurobiological study of invertebrate behavior seemed, by and large, to corroborate this view. We now believe we understand the giant fibers giving rise to fast escape behaviors from crustaceans, mollusks, or insects. We have discovered the central pattern generators controlling swimming in leeches, flying in locusts, feeding in mollusks, digesting in crustaceans, and walking in stick insects. We can now identify and characterize many of the neurons that process the visual stimuli prompting flies to turn, the courtship sounds attracting crickets and grasshoppers, or the olfactory stimuli enticing bees to extend their proboscis.

However, the apparent relative (to vertebrates) simplicity started to disappear, once scientists began to either omit parameters from the traditional experiments, add additional ones, or simply look more closely. This research topic highlights a selection of experiments which serve to demonstrate the kind of decision-making that is taking place even in invertebrates as soon as the experiment allows for sufficient degrees of freedom.

A mind-blowing list of recent examples of the kind of revelations scientists discover when they start to look in greater detail at phenomena we thought we understood is provided in Herberholz and Marquart (2012). Starting from the well-known giant fiber escape circuits in crustaceans, mollusks and insects, they show that today the role of these giant fiber systems is either questionable or only a small part of a range of different escape maneuvers with a large variety of different neural systems subserving them.

The uncrowned champions of reducing simple systems even more by eliminating as many confounding factors as possible must be the mollusks. In their riveting, neuron-by-neuron account of how even isolated ganglia of the marine snail *Aplysia* make spontaneous decisions and incorporate environmental feedback in this process of adaptive behavioral choice, Nargeot and Simmers (2012) elucidate principles of operant conditioning, habit formation, and compulsivity at a level of biological detail that will take decades to reach in most other systems.

Experiments in which parameters have been added to the traditional stimulus and its response can be grouped into two classes, those in which the internal state of the animal is taken into account and those in which stimuli are provided such as to establish a choice situation. The former class includes experiments described by Heinrich et al. (2012) on the neuronal and hormonal mechanisms influencing the decision to sing in different stages but under otherwise identical external circumstances in grasshoppers. Gaudry and Kristan (2012) explain in impressive detail the mechanisms by which different states of the medicinal leech exert their top-down influence on the processing of sensory stimuli at different stages of sensory processing, depending on the state of the animal. Far from simply being relayed to "higher" centers of the nervous system, from the sensory neurons onwards, other information is constantly being cross-correlated with and related to the sensory stream. While the coding properties of sensory neurons are the focus of Marsat and Pollack's (2012) review on ultrasound avoidance in crickets—neuronal bursts encode a "danger signal" from ultrasound often emitted by hunting bats—the work they review also shows that the final decision to initiate evasion behavior in crickets is formed in the brain of the animals, two to four synapses downstream of the sensory neurons that encode the "danger signal." Analogously, Hirayama et al.'s (2012) contribution on the predatory sea-slug *Pleurobranchea* details the neural processes by which the animal's satiation state regulates approach/avoidance behavior.

The simplest way to add a second parameter to a traditional stimulus-response experiment is to choose a stimulus that allows two different responses, even if the state of the animal is not altered. Ritzmann et al. (2012) describe such experiments in which cockroaches must decide on which side (left vs. right or above vs. below) of an obstacle to proceed. The behavior of the animals is best being described as a value-based decision in which the needs of the animal (e.g., shelter) are negotiated with the ease of mastering the barrier. This value-based negotiation of rivaling incentives for an animal was also described in Herberholz and Marquart's (2012) account of crayfish negotiating simultaneous appetitive (food) and aversive (predator) stimuli of different relative value. This well-known cost/benefit tradeoff often encountered by animals in non-laboratory circumstances was also explicitly modeled in Hirayama et al. (2012). A further step away from the simpler, traditional experiments is to not only provide choices between stimuli or behaviors, but to integrate these with variations in the state of the animal. Itskov and Ribeiro (2013) describe experiments with fruit flies deciding about whether, what and when to eat. Due to rigorous behavioral experiments combined with *Drosophila*'s genetic tool arsenal, the neuronal and molecular mechanisms underlying the processes with which various external stimuli interact with different satiation states are slowly unraveling. Probably the most complex, most difficult to control and hence most challenging class of experiments are those where the outcome of the

experiment determines the state of the animal and the stimuli are attached to other animals. Stevenson and Rillich's (2012) review of their work in cricket aggression begins to elucidate some of the neuronal components involved in mediating the simultaneous influence of experience, motivation, and sensory stimuli on the decision to fight or flee.

Perhaps to some the least surprising, but nevertheless most impressive decision-making performance can be reported from hymenopterans, arguably one of the smartest classes of invertebrates, perhaps only with a close rival in cephalopods (which are sadly not represented in this research topic). Wolf et al. (2012) remind us that the well-known navigational capabilities of desert ants are only a small aspect of their sophisticated and flexible food search and retrieval strategies. Of course, a research topic on invertebrate decision-making would not be complete without everybody's poster child for arthropod intelligence, the honey bee. In a tour de force, Zhang et al. (2012) lead us through a maze of different experiments showcasing the many levels of abstraction these animals can deploy in order to make adaptive foraging decisions. Probably among the conceptually deepest contributions is Jeanson et al.'s (2012) overview on collective decisions. Analogous to the super-organism concept of eusocial insects, it is tempting to transfer the factors guiding the emergence of a collective decision of individual invertebrates (e.g., noise amplified by positive feedback) and test if neurons in a decision-making circuit in a brain follow analogous rules when generating decisions such as the ones described above. Bringing us back to reductionism, as documented by Jeanson et al. (2012), these factors were identified mainly by reducing the contribution of the environment and relying only on the decision-making capabilities inherent in the individual animals themselves.

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Decision making and behavioral choice during predator avoidance

***Jens Herberholz*[1,2]* and *Gregory D. Marquart*[2]**

[1] Department of Psychology, University of Maryland, College Park, MD, USA
[2] Neuroscience and Cognitive Science Program, University of Maryland, College Park, MD, USA

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Cynthia M. Harley, University of Minnesota, USA
Jochen Smolka, Lund University, Sweden

***Correspondence:**
Jens Herberholz, Department of Psychology, University of Maryland, College Park, MD 20742, USA.
e-mail: jherberh@umd.edu

One of the most important decisions animals have to make is how to respond to an attack from a potential predator. The response must be prompt and appropriate to ensure survival. Invertebrates have been important models in studying the underlying neurobiology of the escape response due to their accessible nervous systems and easily quantifiable behavioral output. Moreover, invertebrates provide opportunities for investigating these processes at a level of analysis not available in most other organisms. Recently, there has been a renewed focus in understanding how value-based calculations are made on the level of the nervous system, i.e., when decisions are made under conflicting circumstances, and the most desirable choice must be selected by weighing the costs and benefits for each behavioral choice. This article reviews samples from the current literature on anti-predator decision making in invertebrates, from single neurons to complex behaviors. Recent progress in understanding the mechanisms underlying value-based behavioral decisions is also discussed.

Keywords: predation, escape, decision making, behavioral choice, neural circuits

INTRODUCTION

Successful avoidance of a predatory attack is essential for survival and future reproductive success. Failure to detect a predator before an attack initiation, failure to fight off an attack, or failure to respond to an attack with an immediate escape, can be deadly. Many aspects of nervous system function must be optimized to control anti-predator behavior, including careful sensory assessment of threat stimuli, which sometimes involves multimodal integration, rapid transmission of this information within neural structures, and finally, fast and accurate motor activation. Importantly, predator avoidance is often produced under conflicting circumstances. Many daily activities that are essential for survival, such as feeding, mate search, or habitat selection, can increase visibility and thus vulnerability to predation. Animals trying to satisfy important needs while avoiding predation face a trade-off, e.g., between eating and the risk of being eaten. Thus, the selection of the most desirable behavior requires careful calculation of costs and benefits associated with different behavioral options. For example, foraging animals must accurately measure predation risk and weigh this risk against current nutritional state. Such cost-benefit analyses are made by the nervous system through the integration of external sensory signals with current internal states, and these decisions ideally lead to behavioral choices that optimize an animal's fitness.

Invertebrates are superbly suited to measure both the behavior and neural mechanisms underlying predator avoidance. In many invertebrates, an accessible nervous system with described neural escape circuits controls discrete escape behaviors. Thus, the link between neural machinery and behavioral expression is often identifiable and quantifiable. More recently, economic decision making, i.e., costs-benefit calculations under predatory risk, has been measured and described in a number of invertebrate species. This has opened up exciting new avenues for gaining a better understanding of complex "neuroeconomic" processes at a level of analysis not feasible in vertebrates.

The first section of this review summarizes some of the foremost examples of anti-predator behavior and underlying neural circuitry found in four different arthropods. Both the specializations and shared features of these nervous systems that allow these animals to escape immediate predatory threats are discussed. The second part focuses on economic decisions made by invertebrates in situations where the risk of predation must be carefully weighed against other vitally important needs. Finally, we suggest some important future directions for the further identification of neural mechanisms underlying behavioral decisions.

MECHANISMS OF PREDATOR AVOIDANCE

While predators can provide direct cues such as visual or mechanosensory signals that alert prey to the presence of a predator, indirect cues, such as odors, also allow the assessment of a potential predatory threat. However, indirect cues are frequently more ambiguous and seldom provide information on the degree or immediacy of the danger posed. And indirect cues that signal the presence of a predator (although no predator is currently present) can divert attention from other vital activities or suppress these activities altogether. Different risk assessment behaviors, apprehension, and vigilance, are responses to indirect predator cues commonly described in vertebrate animals (Kavaliers and Choleris, 2001). Although they are likely to exist in invertebrates, these "anticipatory" predator avoidance behaviors are much less studied in invertebrates where the evolution of extremely fast and powerful escape reactions in response to immediate attack has arguably reduced the necessity for extensive predator scanning and risk

assessment. Additionally, while numerous behaviors in an animal's repertoire contribute to predator avoidance, most are subtle and difficult to subject to neurobiological analysis. For instance, an animal's decision when and where to forage is greatly shaped by the risk of predation (Lima and Dill, 1990). How an animal calculates this predatory risk and weighs it against concurrent internal and external demands is certainly an interesting question; however, the time-scale and context of such a decision make it difficult to subject to detailed electrophysiological or neuroanatomical analysis. Instead, what has overwhelmingly sufficed for the study of predator avoidance in neuroscience has been the analysis of much more discrete escape or startle behaviors. Because escape behaviors are so critical, they must interface with and frequently override the performance of any ongoing or planned behaviors. And while other behaviors may have a greater evolutionary importance over the long term, seldom are they as time-sensitive and unforgiving as escape. Thus, it is unsurprising that the circuits tasked with the sensory acquisition, computation, and action upon salient predatory cues are frequently the largest, most robust, and most highly stereotyped neural systems in an organism.

If a predator is around, it is critical to identify and react to predatory cues at an appropriate time and in an effective manner. Consequently, escape behaviors must be fast, accurate, and robust in order to be effective countermeasures against the often rapid predatory behaviors they combat. It is believed that the time-sensitive nature of these behaviors necessitates a small number of large elements in order to both maximize conduction velocity and minimize synaptic delay. Thus, escape circuits commonly have "giant fibers (GFs)," frequently the largest axons in an animal's nerve cord, which can be readily identified by their size, location, or morphology. These characteristics allow for rapid identification and often make these neurons accessible to a wide range of cell biological and electrophysiological studies.

Because of their simplicity and clear function, these circuits have been excellent models for the study of the neural basis of behavior. Recent work, however, has uncovered a surprising degree of flexibility not previously recognized in these "simple," "reflexive" systems. High-speed video recordings have exposed a previously unappreciated level of complexity to arthropod escape behaviors that has made researchers question the structure and even identity of the underlying circuits that were originally assumed to be responsible for escape (Hammond and O'Shea, 2007a,b; Card and Dickinson, 2008a,b; Fotowat et al., 2009). Additionally, wireless-recording techniques have been adapted to small invertebrate models allowing, for the first time, the correlation of neural activity from multiple identified neurons with the time-course of escape behavior in unrestrained preparations (Fotowat et al., 2011; Harrison et al., 2011). And while neural-behavioral correlations are not uncommon, escape behavior in invertebrates provides possibly one of the few opportunities to simultaneously record from all the critical elements in a neural circuit and relate it to what is now appreciated as an increasingly complex, but still tractable, behavior. This provides quite possibly one of the best current opportunities for the comprehensive analysis of the neural underpinnings of decision making surrounding a behavior.

While there is likely a broad spectrum of complexity in the circuits embedded in even the most simple nervous system, escape circuits in invertebrates are frequently divided into two broad categories: those that contain "command" or "command-like" elements and those that do not (Kupfermann and Weiss, 1978, 2001; Edwards et al., 1999; Eaton et al., 2001). In command systems, the activity of the command neuron is thought to be necessary and sufficient for the production of a behavior. Often a single spike in this neuron is sufficient for the readout of an entire escape program. While highly adaptive, these rapid behaviors are highly stereotyped, showing little variability. In contrast, the escape behaviors produced by systems ostensibly lacking a command element typically display a greater degree of complexity and flexibility and are frequently made up of a sequence of independently variable components. This flexibility affords the animal a greater degree of control over the precise timing, direction, and structure of the escape behavior. Traditionally, however, this is assumed to come at an additional computational cost that adds to the latency of the action (Bullock, 1984). Alternatively, variability may be added to behavioral decisions by sequential neural processing. For example, in the medicinal leech decision neurons can be active during competing behaviors (e.g., swimming and body shortening), and stimulation of one decision neuron can produce two different behavioral outputs, swimming and crawling. Hypothesized to be organized in a hierarchical order, the first neuron in the chain would drive general behavioral action, the next one would command selection from a pool of discrete motor patterns, and the next one would initiate the most desirable behavioral choice (Esch and Kristan, 2002).

GIANT-NEURON MEDIATED ESCAPE

Crayfish

Crayfish are equipped with powerful escape reactions mediated by rapidly responding neural circuits (reviewed in Wine and Krasne, 1982; Krasne and Wine, 1984; Edwards et al., 1999). These circuits control at least three distinct motor programs that propel the animals in different directions, but always away from real or assumed threats. Circuits and their associated tail-flips can be divided into two major categories, giant and non-giant. Two circuits, the lateral giant (LG) and medial giant (MG) system contain giant interneurons as key "command" components, are made for speed, and require strong and phasic input for their activation. In contrast, a poorly elucidated non-giant system is believed to control slower, but more variable escape tail-flips (Edwards et al., 1999). These escape circuits have been the focus of 65 years of intensive research since they were first described by Wiersma (1947, 1952) in his pioneering work.

The LG interneurons, two large fibers consisting of a series of gap junction-linked neurons that project from tail to head, are activated by tactile and strong hydrodynamic stimulation of sensory hairs and proprioceptors located on the abdomen. The LG interneurons also receive excitatory inputs from rostral sensory organs, but these inputs alone are insufficient to fire the LG. If these inputs sum with strong caudal inputs, however, a single LG action potential (in one of the two fibers) is sufficient to produce an escape motion that thrusts the animal upward and away from the point of caudal stimulation (Liu and Herberholz, 2010). The motor program is activated within milliseconds after stimulation and speed and accuracy is guaranteed through several

structural and functional specializations within the circuit (Herberholz et al., 2002). Once activated, the LG interneurons drive giant motor neurons via rectifying electrical synapses, which activate fast flexor muscles in the last two thoracic and first three abdominal segments causing a bending of the abdomen around the thoracic-abdominal joint and thus the stereotyped "jack-knife" motion that propels the animal upward (Wine and Krasne, 1972). Latency is minimal, with 5–15 ms between stimulation and start of the behavioral response, and varies according to both internal (e.g., animal size: Edwards et al., 1994) and external conditions (e.g., water temperature: Heitler and Edwards, 1998). This short latency is accomplished by the high transmission velocity due to the diameter of the GFs and by electrical coupling among most circuit components (**Figure 5A**).

The MG interneurons, a pair of large fibers projecting from head to tail, are activated by strong, phasic visual or tactile inputs directed to the front of the animal. The MG interneurons receive their excitatory inputs in the brain where both neurons are electrically coupled to each other. One action potential in one of the MGs is sufficient to drive the fast and stereotyped backward escape response. The MG interneurons connect electrically to giant motor neurons, which activate fast flexor muscles in all abdominal segments, causing the bending of the entire abdomen and propelling the animal backward away from the point of stimulation. MG tail-flips in response to tactile stimulation are as fast as LG-mediated tail-flips and happen within a few milliseconds (Wine and Krasne, 1972). Visually activated MG tail-flips are slower, but are still produced as quickly as 50 ms after detection of a visual danger stimulus (Liden and Herberholz, 2008; Liden et al., 2010).

Non-giant-mediated tail-flips are controlled by a circuit that lacks giant interneurons. These tail-flips are elicited by a variety of different stimuli, typically more gradual and less forceful in presentation than those activating giant-mediated tail-flips. They are produced with longer latencies, usually up to 10-fold slower than giant-mediated tail-flips, and considered, in a way, "voluntary" because the animal "chooses" to activate certain patterns of fast flexor muscle groups. Thus, the timing and direction of non-giant tail-flips can be modulated, resulting in a much more variable behavior compared to the giant-mediated tail-flips (Wine and Krasne, 1982; Wine, 1984). Non-giant tail-flips are also used during "swimming," where a series of tail flexions and extensions propels the animal backward through the water.

Although our understanding of the neural underpinnings of tail-flip escape, especially tail-flips produced by the LG circuit, is extensive and essentially unmatched by that of other experimental models, our knowledge of escape circuit activation in response to real predatory danger is virtually non-existent. Using dragonfly nymphs as natural predators, Herberholz et al. (2004) showed that all three escape circuits of juvenile crayfish were activated in response to attacks (**Figure 1A**). Initial escape responses to predatory strikes were primarily mediated by giant tail-flips; frontal attacks evoked MG tail-flips whereas attacks directed to the rear of the crayfish elicited LG tail-flips. While few attacks elicited non-giant tail-flips initially, overall escape performance improved substantially when non-giant tail-flips were produced following capture. Overall, crayfish were successful at evading dragonfly nymphs, avoiding the predator's strike with giant tail-flips in 50% of all cases and escaping, after being captured, using a series of non-giant tail-flips in more than 75% of the remaining cases (**Figure 1B**). Interestingly, latencies for non-giant tail-flips that were produced as initial response to the predator strike were much shorter than latencies of non-giant tail-flips elicited by tactile stimulation with a handheld probe (**Figure 1C**). This suggests that crayfish prepared the non-giant escape before the strike was delivered, possibly integrating visual and hydrodynamic cues from the approaching predator in anticipation of the attack. The study also revealed that crayfish relied entirely on their fast and powerful tail-flip escape behaviors; crayfish showed no signs of predator recognition, vigilance, or avoidance behaviors in any of the trials (Herberholz et al., 2004). Thus, the decision to escape, at least from this specific predator, is based on the activation of fixed action patterns elicited by predatory stimuli. The decision to escape is made

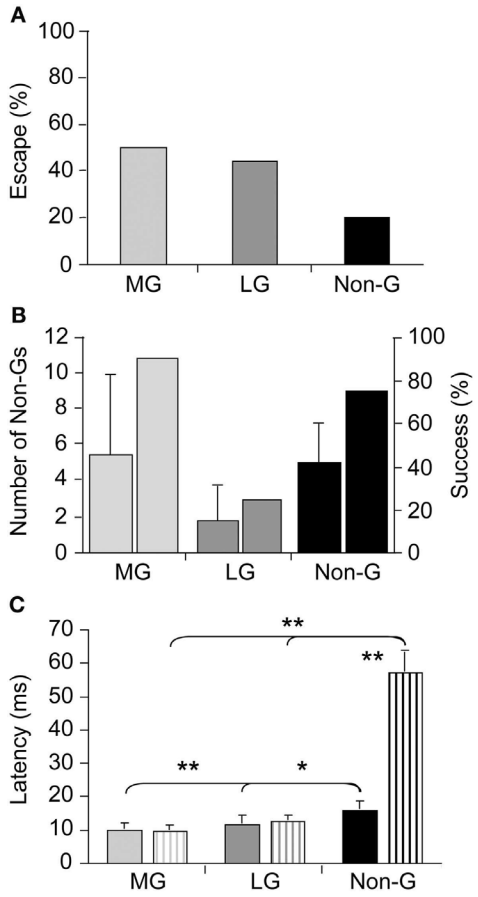

FIGURE 1 | Escape success and latencies measured in juvenile crayfish attacked by dragonfly nymphs. (A) Attacks evoking tail-flips mediated by the medial giant (MG) or lateral giant (LG) interneurons are equally effective to prevent capture whereas attacks eliciting non-giant (Non-G) tail-flips are much less effective. **(B)** Unsuccessful MG and Non-G, but not LG responses are frequently followed by a series of Non-G tail-flips (left bars), which substantially increase the overall rate of escape (right bars). **(C)** Escape latencies for crayfish attacked by predators (solid bars) or stimulated with a handheld probe (striped bars) are similar for giant mediated (MG and LG) tail-flips, but significantly shorter for predator evoked Non-G tail-flips. Modified from Herberholz et al. (2004).

at individual decision-making neurons; if the predatory signal is sufficient to activate them, escape will inevitably follow.

Drosophila

There are a number of similarities between the GF system in *Drosophila* and the MG system in crayfish. Like the MG system, the GF system contains GFs originating in the brain that project down contralaterally to primary motor neurons that control the thoracic musculature responsible for the fruit fly's escape behaviors (reviewed in Wyman et al., 1984; Allen et al., 2006). In these giant fibers, a single spike is normally sufficient for the activation of an escape jump followed by flight initiation. Despite the motor portion of both the MG and GF being well described, comparatively little is known about the visual and mechanosensory pathways that feed into the giant fiber systems of either animal (**Figures 5A,B**).

While the escape behaviors produced by these circuits are extremely fast due to high conductance velocities and the minimal synaptic delay from a preponderance of electrical synapses, this speed has generally been thought to come at the expense of flexibility (Bullock, 1984). Thus, giant-mediated escape behaviors are traditionally characterized as highly stereotyped with little variance in timing or direction; and whatever variance the result of stochastic properties of the system and not the consequence of neural computation (Bullock, 1984).

Although *Drosophila* has been a preeminent genetic model since the start of the twentieth century, its diminutive size limited its use in electrophysiology until the 1970s (Bellen et al., 2010). And while the GF system was identified in 1948 (Power, 1948), it was not electrophysiologically characterized and linked to the production of escape behavior until the early 1980s (Wyman et al., 1984). This escape behavior was initially characterized as an abbreviated form of "voluntary" flight initiation (Trimarchi and Schneiderman, 1995a). While voluntary flight initiation is preceded by a series of postural adjustments that prepare the fly for stable, directional flight, escape flight lacks these preflight postural leg, and wing movements. Instead, escape initiation consists almost exclusively in the extension of the fruit fly's mesothoracic legs that propels the insect off of the substrate, which is only then followed by the unfolding and initiation of wing movements (Card and Dickinson, 2008a).

As the GF system was the only identified *Drosophila* escape circuit, it was assumed to mediate the escape behavior elicited by all visual, chemical, and mechanosensory stimuli that elicit an escape jump (McKenna et al., 1989). However, a number of observations have accumulated that conflicted with this canonical interpretation. For instance, in the housefly GF activity was shown not to be necessary for the production of an escape jump in response to looming stimuli (Holmqvist, 1994). Additionally, Trimarchi and Schneiderman (1995b) provided evidence for an olfactory-induced flight initiation reminiscent of the fruit flies' escape behavior that was also not mediated by the GFs. More recently, the simplicity of the observed escape behavior was reassessed through high-speed video analysis (Hammond and O'Shea, 2007a,b; Card and Dickinson, 2008a,b). This work illustrated that these "simple" escape behaviors were far more complex and nuanced than originally assumed (**Figures 2A,B**). Card and Dickinson (2008a) showed that rather than a simple escape jump, the escape behavior in wild-type fruit flies involves a complex sequence of events consisting of at least four distinct subcomponents: an initial freeze followed by postural adjustments, wing-elevation, and finally an escape jump coordinated with the initial down stroke of flight initiation (**Figure 2C**). These behaviors do not appear to merely be a fixed action pattern as new information continues to be integrated into and affect subsequent components of the behaviors even after sequence initiation (Hammond and O'Shea, 2007b). These preflight behaviors were found to influence both the trajectory as well as initial flight stability of the escape behavior (Card and Dickinson, 2008b).

This newly appreciated complexity of the response suggests that this escape behavior is either not in fact mediated by the GF system or that additional unidentified pathways must be involved that are responsible for the preflight sequence that proceeds the escape jump (Card and Dickinson, 2008b). Toward this end, evidence for a previously unknown escape circuit was recorded by Fotowat et al. (2009). In the absence of GF activation, the activity of this novel circuit correlated with the production of escape behavior in response to looming stimuli. While this pathway is yet to be anatomically identified, its activity shares features similar to well-described circuits responsive to looming stimuli in both vertebrates and invertebrates (e.g., pigeon: Sun and Frost, 1998; locust: Rind and Simmons, 1992; crab: Oliva et al., 2007; bullfrog: Nakagawa and Hongjian, 2010). All of this strongly suggests that the GF system is not necessary for the production of escape behavior in the fruit fly, but that the GF system, possibly akin to the escape circuits in the crayfish, may be one of many present in *Drosophila*.

Being that sudden changes in luminance (light-off) are the only stimulus to reliably produce GF-mediated escape behavior, and then only in white-eyed fruit fly mutants, what role, if any, that the GF system plays in actual escape behavior of wild-type fruit flies is now unclear. Although stimuli that reliably recruit the GF system in wild-type flies are unknown, it seems unlikely that the GF system is simply the vestige of a lost escape circuit. While the newly identified looming sensitive pathway might be tuned to a selective set of stimulus features, the GF system could still serve as a robust, broadly tuned escape circuit capable of producing rapid escape behavior when more selective systems fail (Fotowat et al., 2009).

VISUAL INTERNEURON MEDIATED ESCAPE

Locust

While locusts produce avoidance behavior in response to a variety of noxious stimuli (Riede, 1993; Friedel, 1999), the best studied of these are escape jumps in response to looming stimuli (reviewed in Pearson and O'Shea, 1984; Burrows, 1996; **Figure 3**). Like the escape behavior of fruit flies, the locust escape jump is a complex behavior composed of a sequence of distinct components, which allow the animal to direct this jump (Santer et al., 2005b). In preparation for a jump, tilting postural movements mediated by the pro- and mesothoracic legs rotate the long axis of the locust toward the direction of the eventual jump (Hassenstein and Hustert, 1999; Santer et al., 2005b; **Figure 3A**). The actual jump is produced through the cocking of the hindlegs, storage of energy

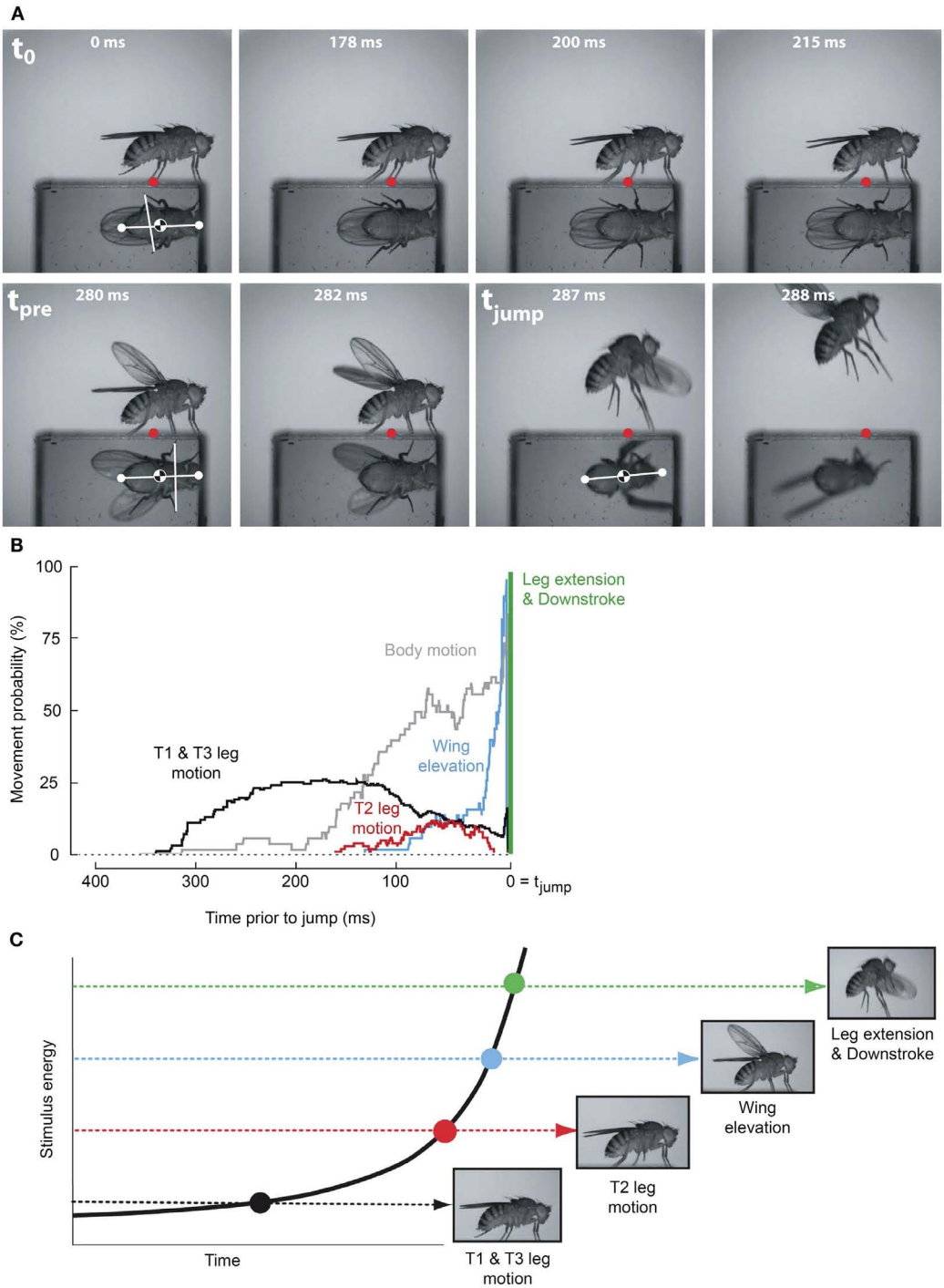

FIGURE 2 | Escape flight planning and execution in *Drosophila.* **(A)** High-speed video sequence shows a typical escape to a looming frontal stimulus with a prism allowing for simultaneous observation of ventral and side profiles. Time stamps are milliseconds elapsed since stimulus onset. Red dots mark the initial contact point of the second leg tarsi with substrate. White dots mark head and abdomen points. **(B)** Probability that body parts of the fly (black, T1 and T3 legs; red, T2 legs; blue, wings; gray, body) were moving prior to takeoff (green line). **(C)** As stimulus intensity increases, independent motor programs are activated eliciting discrete escape subbehaviors prior to takeoff. Adapted with permission from Card and Dickinson (2008b).

by the co-contraction of tibia flexor and extensor muscles, and finally the release of this energy, triggered by flexor inhibition (Burrows and Morris, 2001). Given the time required to store sufficient energy in the animal's hindlegs, co-contraction must begin as soon as possible in order to allow for a timely escape. In contrast, the adjustment of pro- and mesothoracic limbs can

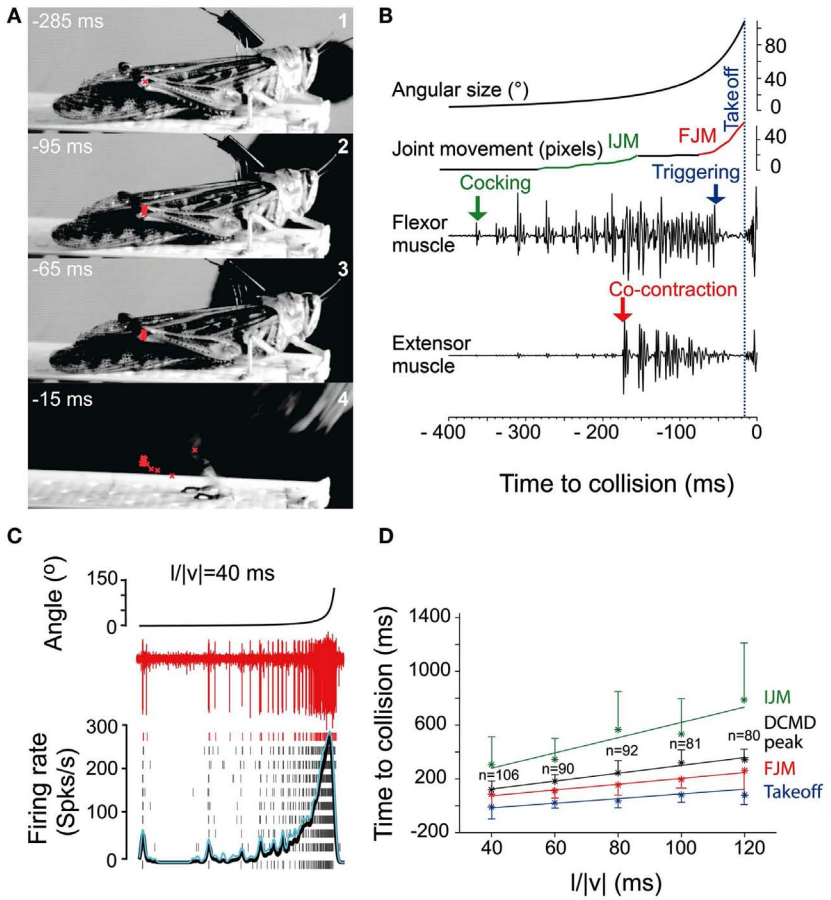

FIGURE 3 | Escape jump and DCMD activity in locusts in response to looming stimuli. (A) Four high-speed video frames from a locust producing an escape jump with time to collision listed in milliseconds. The position of the femur-tibia joint is marked in red to calculate pixel movements of the joint. **(B)** Muscle recordings from the same trial. Stimulus angular size is shown on top with joint movements and flexor and extensor recordings below. (IJM, initial joint movement; FJM, final joint movement.) **(C)** DCMD activity measured extracellularly in the nerve cord from one locust (red traces). Raster plots show DCMD spikes recorded in 10 repetitions of the stimulus. Black and blue traces show average DCMD firing rate and its standard deviation, respectively. **(D)** Timing of joint movements, DCMD peak and takeoff obtained from seven locusts. The DCMD peak occurred after the IJM and before the FJM and takeoff for all *l*/|*v*| values (*l*/|*v*| = ratio of stimulus radius (*l*) to the velocity (*v*) of the stimulus). Adapted with permission from Fotowat and Gabbiani (2007).

continue throughout co-contraction, allowing for alterations of escape trajectory up until the escape jump is triggered (Santer et al., 2005b). On the other hand, if the hindlegs were used to control direction, it is thought that the decision of where to jump would have to be made over 100 ms before the jump is produced.

Not only are locusts able to direct these jumps up to 50° to either side of their long axis, but their escape circuitry allows them to control the timing, distance, and elevation of these jumps (Santer et al., 2005b; Simmons et al., 2010). Similar to *Drosophila*, this complex sequence of events does not appear to be a fixed action pattern that once initiated must be taken to completion as the locust can relax this co-contraction and release the stored up energy without the production of an escape jump (Heitler and Burrows, 1977).

Motor areas controlling these escape jumps are innervated by a pair of large interneurons, the descending contralateral movement detectors (DCMDs) which receive excitatory inputs from lobula giant movement detector (LGMD) neurons that are responsive to looming stimuli. With a one-to-one relationship with the LGMDs, the DCMDs produce action potentials in response to looming stimuli, with their firing rate increasing as the looming object gets closer. Thus, the DCMDs were originally thought to play a major role in jump production, sometimes compared to the giant fibers in crayfish and fruit flies that control their fast escape maneuvers (Burrows, 1996). However, locusts prepare for jumps by co-contracting flexor and extensor tibiae muscles for ∼100 ms before the jump is released by relaxation of the flexor muscles. Thus, the jump is not simply triggered by suprathreshold excitation of the DCMDs, because withdrawal of excitation and inhibition are needed during the preparatory phase of the jump (**Figure 3B**). Nevertheless, the DCMDs seem to participate in all phases of the jump. Fotowat and Gabbiani (2007) compared electrophysiological recordings with high-speed video recordings and found that the rising phase of the firing rate of the DCMDs coincided with the preparatory phase of the jump, whereas the peak firing rate coincided with the co-activation period of flexor and extensor muscles, and decay of firing rate to less than 10%

coincided with takeoff. This suggests that different stages of jump production could be controlled by distinct phases in the firing pattern of the DCMDs (**Figures 3C,D**). Hindleg flexion in preparation for the jump, however, is not dependent on DCMD activity. When the connective containing the DCMD neuron was severed, hindleg flexion still occurred, and it could also be evoked with visual stimuli that did not cause high firing activity in the DCMDs. This showed that while the activity of the DCMDs may contribute to hindleg flexion, it was not necessary for it and, thus, other descending pathways would seem to be involved (Santer et al., 2008). Using a telemetry system to record DCMD and motor neuron activity in freely behaving locusts, it was found that the number of recorded DCMD spikes predicted motor neuron activity and jump occurrence, and the time of peak firing rate predicted time of takeoff (Fotowat et al., 2011). Although this underlined the role of the DCMDs as neurons exhibiting discrete firing responses to looming stimuli, which in turn affected discrete stages of escape motor output, jump production remained intact, and occurred at the same time as in control animals following DCMD ablation. Thus, another neuron for jump production must exist, and this may be the descending ipsilateral movement detector neuron (DIMD), which responds to looming targets, similarly to the DCMD (Fotowat et al., 2011). Additionally, another descending interneuron that responds to looming stimuli has recently been described. Thus visually mediated escape behavior in locusts is likely controlled by at least three different descending neurons (Gray et al., 2010). How these neurons interact to produce the escape behavior remains to be determined (**Figure 5C**).

Locusts also produce an avoidance behavior during flight. When looming stimuli are presented, flying locusts produce a gliding dive similar to the dives used by other insects to evade aerial predators. After DCMD neurons are activated by a looming stimulus, they produce short-latency excitatory postsynaptic potentials (EPSPs) in a motor neuron that raises the wing into the gliding posture. Stimuli that evoked high-frequency firing in the DCMDs also reliably elicited the gliding response, and the behavior was less frequently observed when high-frequency DCMD spikes were absent (Santer et al., 2005a). However, similar to the escape jump, DCMD activity was not always sufficient to evoke gliding. Most likely, its high-frequency activity must be precisely timed with wingbeat phase because glides can only be produced during wing elevation. In addition, other neurons that are implicated in jump production (e.g., the DIMDs) may also be involved in escape gliding in flying locusts (Santer et al., 2006).

Crabs

The role of identified neurons in visually mediated escape behavior has also recently been studied in grapsid crabs (reviewed in Hemmi and Tomsic, 2012). The firing rate of these motion-sensitive lobula giant (LG) neurons in response to looming stimuli corresponds with the intensity of the crab's escape behavior. Four distinct classes of these neurons have been anatomically and physiologically described. All four classes show wide-field tangential arborization in the lobula, somata located beneath, and axons that project toward the midbrain; however, they are uniquely identifiable due to differences in morphology and response preferences (Medan et al., 2007).

Three of these LG classes receive proprioceptive inputs from the legs, and thus could potentially integrate some contextual information during predator escape (Berón de Astrada and Tomsic, 2002). Oliva et al. (2007) tested the escape behavior of grapsid crabs on a freely rotating styrofoam ball and recorded escape movements (i.e., running) while looming stimuli were presented. They also recorded intracellularly from the LG neurons in restrained crabs and compared these recordings with the behavioral data. Escape runs were initiated soon after the LG neuron increased its firing rate, and after maximum stimulus expansion, the LG neurons stopped firing, coinciding with run deceleration in freely behaving animals. Moreover, the spike frequency of the LG neurons reflected the timing and speed of the escape response (**Figures 4A,B**). Interestingly, the activity of the LG neurons is strongly affected by season with responses weaker in winter when predation risk is typically low and the animals are less active (Sztarker and Tomsic, 2008).

The relation between LG neuron activity and escape behavior was also nicely demonstrated in experiments that tested short-term and long-term visual memory in crabs. Tomsic et al. (2003) showed that LG neurons changed their responses to a visual threat (displacement of a black screen above the animal) in correspondence with the behavioral changes observed in unrestrained animals. Modification of LG neuron activity occurred during learning and persisted, after spaced training, for 24 h. However, while the memory of freely behaving crabs reflects a strong stimulus-context association, LG neurons generalize the learned stimulus into new spatial locations. Thus, despite being able to clearly distinguish the learned stimulus from other similar stimuli (i.e., stimulus memory), the LG neurons do not appear to be involved in processing contextual visual information (i.e., where the stimulus was learned; Sztarker and Tomsic, 2011). In summary, the LG neurons are sensory neurons located in the eyestalk, and their neural activity patterns closely match escape behavior produced in unrestrained crabs (Medan et al., 2007). Their exact role in producing the behavior, however, is unknown. To answer this question, detailed investigation of the descending pathways that connect the LG neurons to the motor centers that control escape runs will be required (**Figure 5D**).

VALUE-BASED DECISION MAKING

Adaptive behavioral decisions are essential for the survival and reproductive success of most animals, including humans. Animals can typically choose from several behavioral alternatives, which need to be evaluated before the most desirable option is selected. To determine what behavior is most desirable at any given point, the nervous system must integrate external conditions (e.g., predation risk) with current internal drives (e.g., hunger state), thus trading off the costs and benefits of different alternatives before deciding which one to choose. For example, a hungry animal is more likely to choose a behavioral option that involves risks because the value placed on foraging is greater than the value placed on other alternatives such as hiding. If the benefit of finding a meal outweighs the estimated cost of being attacked by a predator, the decision is to forage. If the value placed on foraging is low because the animal is satiated, other behavioral options become more valuable and behavioral output will shift toward less risky activities. The

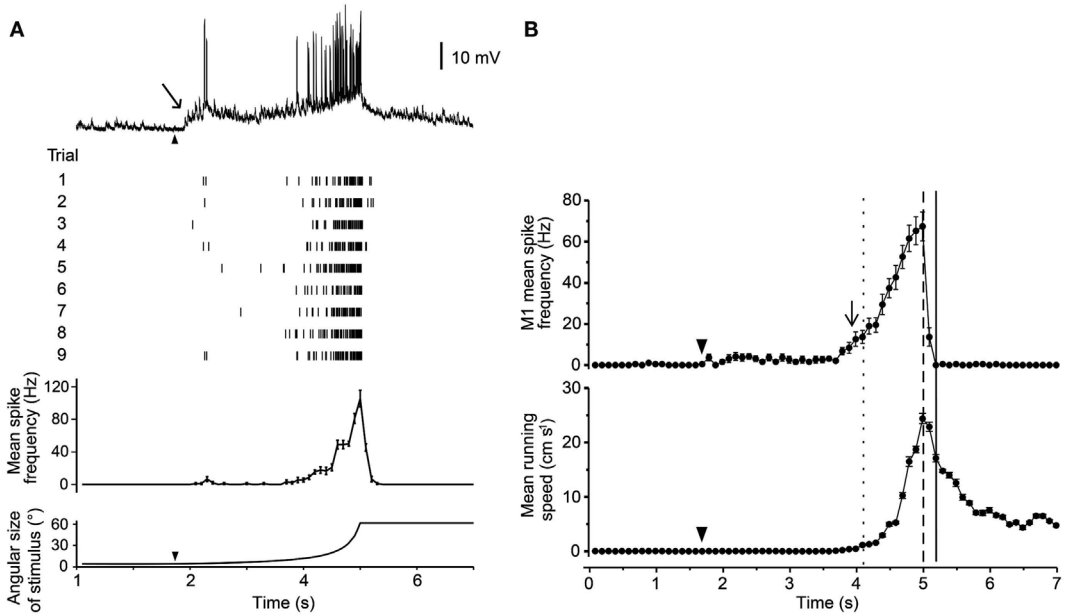

FIGURE 4 | Response of a crab's LG neuron to looming stimuli and correlation with escape run. (A) Intracellular trace from one LG neuron in response to a looming stimulus. Raster plot shows responses from one neuron to nine repetitions of the stimulus. Histogram shows mean spike rate obtained from all nine trials. Angular size of the looming object is shown in bottom trace. **(B)** Mean spike rate from a single LG neuron (top) and mean escape running speed (bottom). Arrowheads mark the start of stimulus expansion and long arrows mark increase in spike rate above resting level. Adapted with permission from Oliva et al. (2007).

literature on value-based decision making, especially with a focus on prey behavior in predator-prey interactions, is extensive and covers a wide range of organisms (e.g., Ydenberg and Dill, 1986; Lima and Dill, 1990).

The relatively new field of "neuroeconomics" is concerned with the neural underpinnings of value-based decision making in humans and other non-human primates (Schall, 2001; Rangel et al., 2008) and there is now fast growing interest in understanding the neural mechanisms that govern cost-benefit calculations. An increasing number of studies performed in humans and other primates are combining non-invasive techniques such as functional magnetic resonance imaging or cortical recordings with discrimination tasks or cognitive experiments (Glimcher and Rustichini, 2004; Huettel et al., 2005; Sugrue et al., 2005). The complexity of the mammalian brain, however, presents many challenges. It is difficult to directly correlate neuronal activity and behavioral expression and to obtain detailed information on neural circuit organization, cellular mechanisms, and the interplay between sensory and motor systems. Decision-making circuitry has been studied quite extensively in various invertebrates, but descriptions of neural mechanisms underlying value-based (economic) behavioral decisions are rare (Kristan and Gillette, 2007; Kristan, 2008). This is surprising because behavioral experiments have shown that invertebrates make decisions that are not always simple and reflexive, but are often the product of careful cost-benefit calculations (Ydenberg and Dill, 1986; Lima and Dill, 1990; Chittka et al., 2009). Thus, invertebrates are ideally suited to study the neural mechanisms underlying value-based decision making. In the following section, we will review some recent experiments on value-based decision making in response to predatory threat, and provide two examples where economic decisions can be linked to identifiable neural circuitry.

CRAYFISH

When juvenile crayfish are exposed to fast-moving shadows while foraging in an artificial stream environment, they respond by choosing one of two behavioral actions: they either freeze in place and remain motionless for several seconds before resuming foraging or they produce a tail-flip mediated by the MG neuron that propels the animal backward and away from the approaching shadow and the expected food source (Liden and Herberholz, 2008; **Figure 6A**). Thus, crayfish respond to visual threat signals that simulate the imminent attack of a predator with defensive behaviors that are discrete and incompatible. When Liden and Herberholz (2008) exposed groups of juvenile crayfish to different shadow velocities, they found that the frequencies of the two behavioral responses were dependent on shadow speed. Slower moving shadows evoked more tail-flips than freezing, but as shadow speed increased the frequency of tail-flips decreased and crayfish primarily produced freezing behavior. The study also showed that different individuals choose different anti-predator strategies when exposed to one type of shadow. Some animals decided to freeze in response to the danger signal while others decided to tail-flip. This suggests that different crayfish have different thresholds for each behavioral action, but what underlies this difference remains to be determined. Because all tested animals were of identical size and shared the same social

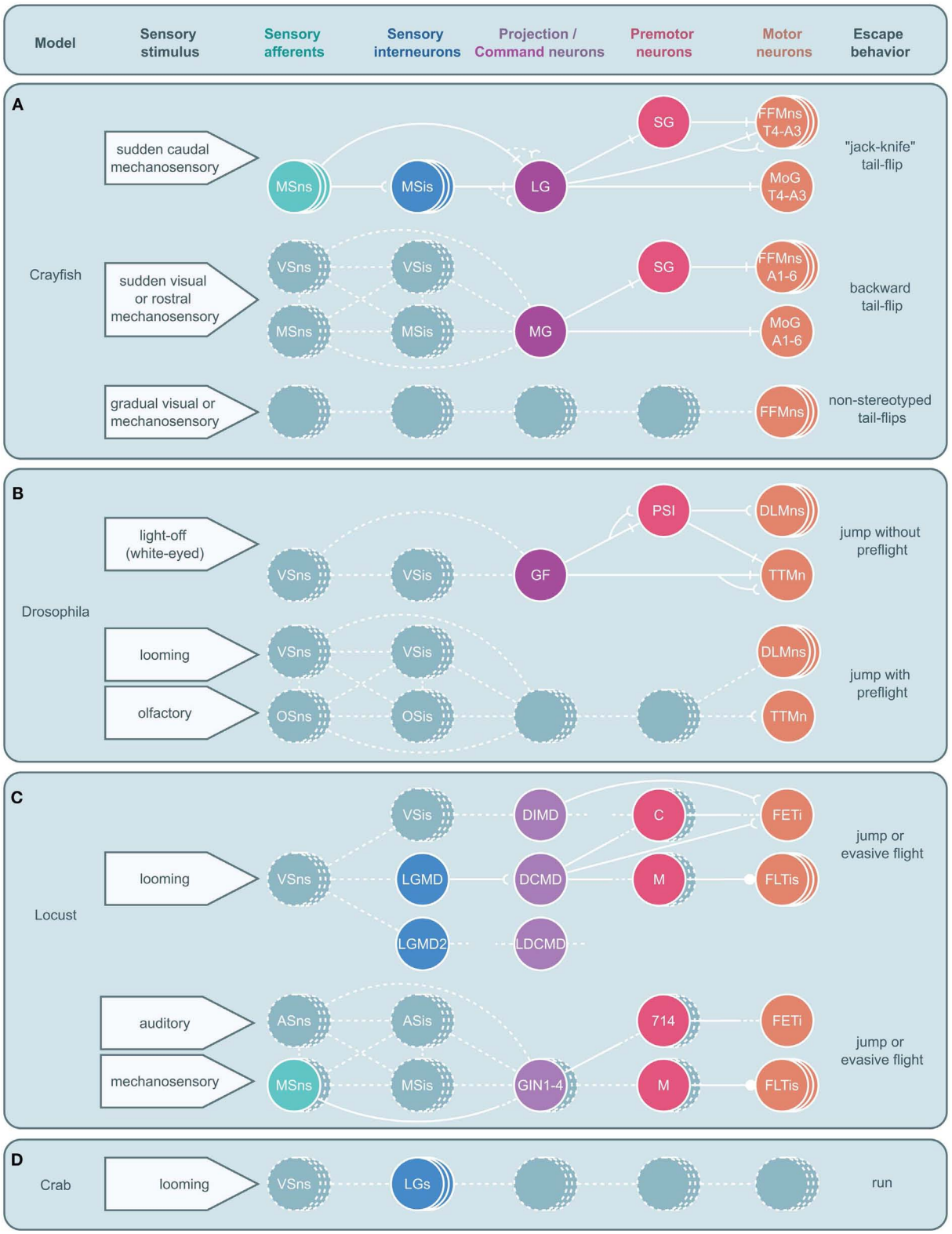

FIGURE 5 | Circuitry for arthropod escape behavior. Neural circuits underlying escape behaviors for crayfish **(A)**, *Drosophila* **(B)**, locust **(C)**, and crab **(D)** are illustrated. Circuits are divided into five levels: sensory neurons, sensory interneurons, projection (ascending or descending) or command neurons, premotor neurons, and motor neurons with associated sensory

(Continued)

FIGURE 5 | Continued
stimuli on the left and motor output on the right. Solid circles and lines represent identified neurons and connections while dashed circles and lines represent neurons and connections yet to be identified. Stacked circles represent a population of neurons. Lines end in four ways: with a perpendicular line, a concave cup, a circle, or dashes. Perpendicular lines represent electrical synapses. Concave cups represent electrical synapses. Circles represent inhibitory synapses. Dashes indicate an unknown synapse type. Generic abbreviations: MSns, mechanosensory neurons; MSis, mechanosensory interneurons; VSns, visual sensory neurons; VSis, visual sensory interneurons; OSns, olfactory sensory neurons; OSis, olfactory sensory neurons; ASns, auditory sensory neurons; ASis, auditory sensory interneurons. **(A)** Crayfish tail-flips are controlled by one of three circuits, the lateral giant (LG), medial giant (MG), and non-giant escape circuit. While the LG system is almost fully elucidated and the abdominal motor outputs of the MG are also well described, very little beyond the fast flexor motor neurons (FFMns) are known to play a part in non-giant tail-flips. SG, segmental giant neuron, MoG, motor giant neuron. **(B)** *Drosophila* escape jumps are the result of at least two circuits; a giant fiber (GF) system mediating jumps lacking preparatory leg and wing movements and a yet to be identified escape circuit that produces escape jumps with preparatory preflight limb and wing adjustments. (PSI, peripherally synapsing interneuron, DLMns, dorsal lateral motor neurons, TTMn, tergotrochanteral muscle neuron.) **(C)** Locusts possess at least two escape circuits as well, one responsive to looming stimuli and another responsive to auditory and mechanosensory stimuli. While numerous neurons that are believed to play a role in these behaviors have been identified, both circuits remain incomplete. [LGMD, lobula giant movement detector neuron; LGMD2, lobula giant movement detector neuron 2, DCMD, descending contralateral movement detector neuron; DIMD, descending ipsilateral movement detector neuron; LDCMD, late descending contralateral movement detector neuron, C, C ("cocking") neuron, M, M-neuron, FETi, fast extensor tibia motor neuron, FLTis, flexor tibia motor neurons, 714, neuron 714.] **(D)** In crabs, a class of visual interneurons, the lobula giants (LGs), have been identified that are thought to play a role in the crab's escape behavior; however, no other elements in this escape circuit have been elucidated.

experiences and feeding history, other intrinsic factors must be responsible.

Recently, Liden et al. (2010) used the same experimental design to show that crayfish base their escape decisions on the values of each behavioral option. They measured escape latencies for shadow-induced MG-mediated tail-flips by comparing photodiode signals with bath electrode recordings that non-invasively captured neural and muscular activity produced during tail-flips (**Figure 6B**). They found that very fast approaching shadows become inescapable because they collided with the animal before a tail-flip could be generated. Moreover, tail-flips are costly because they move the animal away from the expected food source. Thus, the observed suppression of tail-flipping in favor of freezing in animals facing inescapable shadows, where the value of a tail-flip would be low, reflects the output product of an "economic" decision-making process. Although tail-flipping is considered a less risky strategy when experiencing a predator attack, crayfish also defaulted to freezing behavior when the expected reward became more valuable. When food odor concentration in the artificial stream was increased 10-fold, shadows that evoked mostly tail-flips under standard conditions now generated mainly freezing behavior. Interestingly, if high food value was paired with a strong predator signal (a slow moving shadow) that reliably evoked tail-flips under regular conditions, the behavioral shift toward freezing was less pronounced. Thus, a strong predator signal was able to override the exaggerated food incentive (**Figure 6C**). This illustrates that crayfish calculate the costs and benefits of different behavioral options and they carefully weigh predation risk against expected reward, eventually selecting the most valuable behavioral choice (Liden et al., 2010). Because these observed tail-flips are always generated by activation of MG neurons and the MG circuit is accessible for neurophysiological and neurochemical experiments, the neural workings underlying value-based decision making in crayfish can now be investigated on the cellular level. This establishes the crayfish as an important new model for studying the neuroeconomics underlying predator avoidance. However, to understand the decision-making process on the network level, identification of interneurons that form the descending visual pathway for freezing behavior will be required.

SEA SLUG

The marine snail has been a fruitful model for studying the neural mechanisms underlying decision making and behavioral choice. Using a "competing behaviors" paradigm, early work suggested that different incompatible behaviors were organized in a hierarchical model, each controlled by command-like neurons that produced one behavior while inhibiting others. For example, when the sea slug was feeding, avoidance withdrawal in response to a tactile stimulus was suppressed (Kovac and Davis, 1977). This suppression is caused by identified interneurons that are part of the motor circuit that generates feeding. Thus, feeding behavior takes precedence over withdrawal, while escape swimming dominates most other behaviors, including feeding (Jing and Gillette, 1995). The A1 neurons, a bilateral pair of interneurons located in the cerebropleural ganglion of the snail, are necessary elements of the escape swimming behavior, and their activity also inhibits feeding behavior.

Recent work, however, has shown that sea slugs base their decisions on cost-benefit computations (Gillette et al., 2000; **Figure 7**). When presented with food stimuli, feeding behavior or avoidance behavior can be activated, depending on the concentration of the food stimulus and the current behavioral state of the animal. At low concentrations and in satiated animals, food stimuli typically evoked avoidance behavior. When the threshold for feeding was exceeded, avoidance behavior was suppressed, and in hungry snails, even nociceptive stimuli elicited feeding behavior (**Figure 7A**). This suggests both appetitive and noxious stimuli provide inputs to neural networks underlying feeding and avoidance behavior, but the final behavioral decision is determined by hunger state. Thus, in partially or fully satiated animals, the value placed on feeding behavior is low while it is high for avoidance behavior that protects the animal from predators. Using a simple cost-benefit analysis, the animal weighs nutritional needs against predator risk and selects the most desirable choice (Gillette et al., 2000; **Figure 7B**). Importantly, feeding and avoidance can be observed as fictive motor patterns in isolated central nervous systems of the snail and some of the neurons controlling these behaviors have been individually

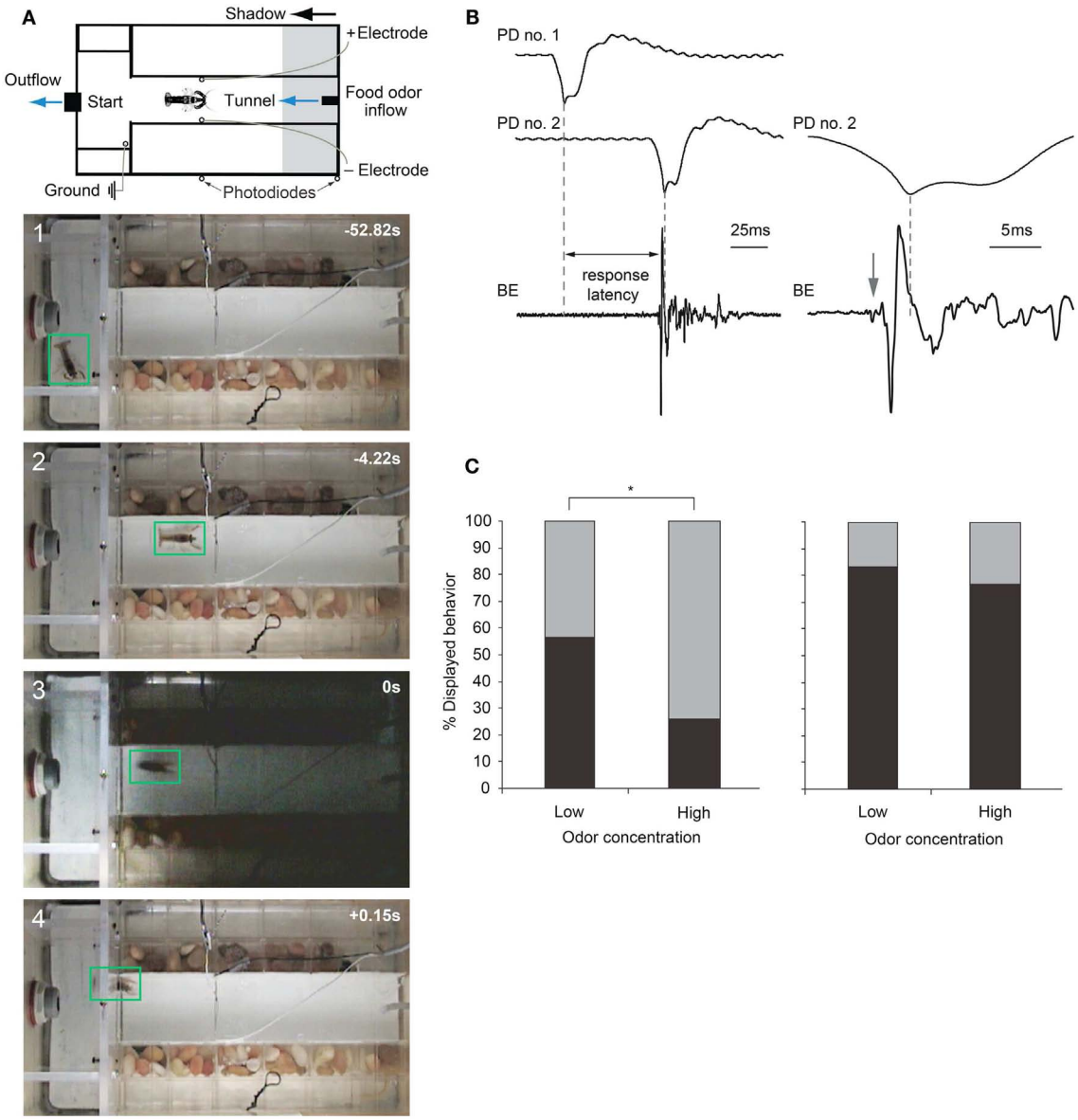

FIGURE 6 | Escape choices and neural activation in crayfish exposed to approaching shadows. (A) Experimental diagram and four video frames illustrating a crayfish foraging (first two panels) and then tail-flipping (last two panels) in response to a fast approaching shadow with time in seconds. **(B)** Left: example recordings from photodiodes positioned on the tank walls (PD no. 1 and PD no. 2) when a shadow passes by, and from bath electrodes (BE) located inside the tank that capture field potentials generated during a tail-flip. Right: Traces from PD no. 2 and BE at higher temporal resolution. In this example, animal initiated a tail-flip response (arrow) 4 ms before the shadow collided with the animal and produced the peak response in PD no. 2. The first small deflection (arrow) in the BE trace is due to MG neuron activation, while the large phasic potential and the smaller more erratic potentials that follow are due to muscular activity during tail-flips. **(C)** Left: when exposed to a medium speed shadow (2 m/s), crayfish produce fewer tail-flips (black bars) and more freezing (gray bars) when food odor concentration flowing through the tank is high. Right: when exposed to slower (1 m/s) shadows, the effect of food odor concentration on behavioral choice is less pronounced. **(A)** Modified from Liden and Herberholz (2008). **(B,C)** Modified from Liden et al. (2010).

identified (Jing and Gillette, 2003). Moreover, in isolated central nervous systems, spontaneous feeding network activity reflects feeding thresholds of the nervous system donors (for proboscis extension and biting); while orienting turns were more frequent in low-feeding threshold donors, avoidance turns dominated in high-feeding threshold donors. When a "command" neuron in the feeding network of a high-feeding threshold donor was electrically stimulated, avoidance turns were converted to orienting turns (Hirayama and Gillette, 2012). Thus, the neurophysiological and neurochemical mechanisms underlying cost-benefit calculations can now be investigated in the isolated nervous system of this animal. This is expected to substantially contribute to our cellular understanding of value-based decision-making processes.

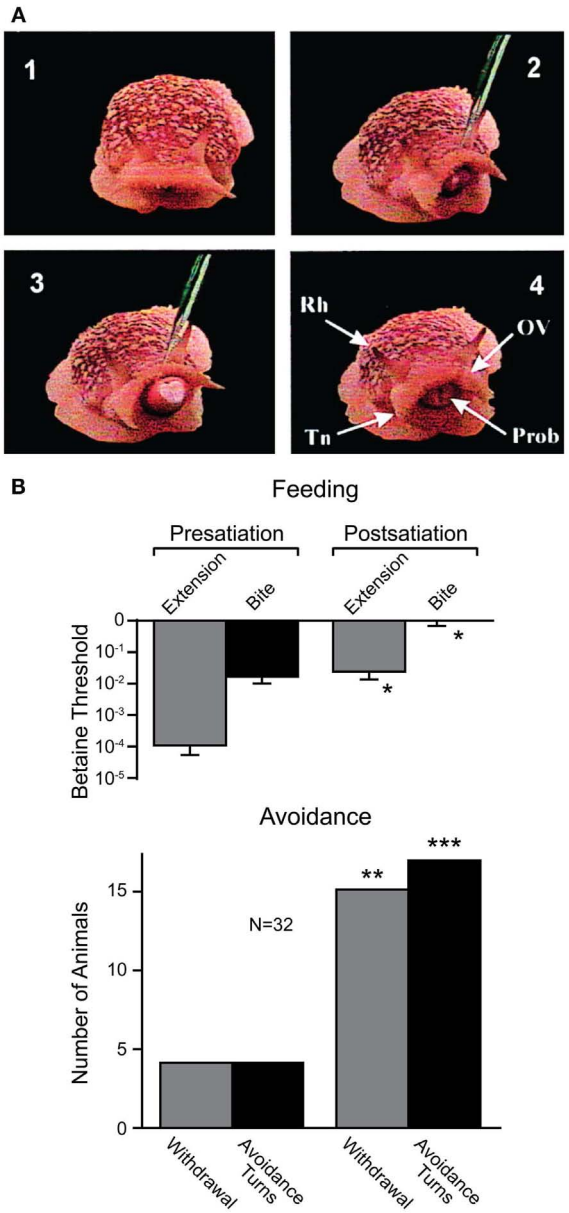

FIGURE 7 | Effects of internal state on behavioral choice in a sea slug. **(A)** Four video frames showing feeding behavior in *Pleurobranchaea californica*. Betaine application induces an orienting turn (panel 2) followed by proboscis extension and biting (panel 3). Chemosensory structures (panel 4): rhinophore (Rh), oral veil (OV), tentacle (Tn), and proboscis (Prob). **(B)** Partial satiation raised the threshold for proboscis extension and biting (i.e., feeding), and increased the frequency of withdrawal and turns (i.e., avoidance) in response to betaine. Modified from Gillette et al. (2000).

CONCLUSION AND FUTURE DIRECTIONS

Recent work in the arthropods discussed suggests that the escape behavior of all may be more complex and varied than has generally been assumed. Quantitative ethograms that divide complex escape maneuvers into a sequence of simpler events can help identify variability within each system. Moreover, combining ethograms with measures of neural structure or neural activity can elucidate the link between discrete motor actions within a series of behavioral events and the corresponding underlying neural mechanisms (Harley et al., 2009; Harley and Ritzmann, 2010).

Based on the high-speed video analysis of the behavior of fruit flies and locusts, a reexamination of the "simple" escape behavior of other arthropods is warranted. Perhaps an analysis at a temporal resolution comparable to that of the speed of production of these behaviors will uncover a degree of flexibility and control not previously appreciated in these animals as well. For example, while the escape tail-flip and freezing behavior of the crayfish in response to visual stimuli have been assumed to be two distinct behaviors, which has been supported by video analysis at 250 fps (Liden et al., 2010), possibly higher speed analysis will show that these distinct decisions are in fact part of a single escape sequence. Such an observation could provide direction in the search for the neural circuit(s) responsible for freezing, the identification of which would provide a unique opportunity to explore decision making between two circuits underlying known behavioral alternatives.

While this new appreciation for the complexity of arthropod escape behavior has reinvigorated work on giant fibers and escape behavior, it raises two significant issues. First, if the giant fiber systems previously assumed to underlie observed escape behaviors are not in fact necessary or sufficient for the production of these behaviors, what circuits are? While Fotowat et al. (2009) have made initial progress toward characterizing the activity of part of an additional putative escape circuit, the neurons will have to be anatomically identified and the circuit fleshed out in future work. Second, if the giant fibers are not involved in escape behaviors produced under existing experimental contexts, what contexts elicit their recruitment? It would be exceedingly wasteful for the largest axons in the fruit fly's nerve cord to go unused. There must be some combination of internal states and external stimulus conditions that lead to GF-mediated escape response and work should be directed toward identifying these constraints.

It is likely other arthropod models will have a similar redundancy in escape circuitry as has been described in the crayfish. Thus, a comprehensive understanding of decision making during predator avoidance will have to wait until all pathways and not just parts of some are fully characterized (**Figure 5**). While the identification of all escape circuits in any one arthropod is non-trivial, that parts of both command and non-command systems have been successfully identified in various arthropods is evidence of the feasibility of such a research program. For example, the LG neurons in grapsid crab are fully characterized and individually identifiable cells that can be accessed for intracellular recordings in live animals. The activity of these neurons is highly correlated with behavioral output, which suggests that they play a major role in mediating escape decisions. However, relevant analysis of the complete escape circuit is still missing and descending pathways that orchestrate motor actions need to be identified.

As such, future work should focus on completing the picture of currently known circuits, where often substantial sensory or motor elements remain poorly characterized, as well as identifying unknown but hinted at command or non-command circuits. This hunt for currently uncharacterized circuits might be aided by the possible similarity to and knowledge of already characterized systems found in related species (**Figure 5**). For instance, the poorly studied non-giant tail-flip circuit in crayfish might share

characteristics with that of the DCMD circuit in locusts and knowledge of the structure and function of the DCMD circuit could aid in the identification and characterization of this escape system.

Due to the assumption that giant fiber systems were a singular system responsible for the production of all escape behaviors, there is currently much confusion as to what discrete escape behavior is subserved by what specific circuit. Since it now appears that there are likely many circuits that produce a range of escape behaviors, the spectrum of these behaviors and the stimulus conditions that lead to their display will need to be carefully cataloged and behavioral assays developed that can differentiate them. However, without the ability to simultaneously record both escape behaviors and neural activity, it will be difficult to ascribe a discrete escape sequence or subcomponent of escape behavior to a particular circuit or set of neurons. For this, the use of telemetry that allows for *in vivo* recordings in freely behaving animals (Fotowat et al., 2011; Harrison et al., 2011) will have to be expanded to other invertebrates. While it will be some time before these techniques can be adapted to all models, some should be able to benefit immediately. Arguably, these techniques might have the most to offer in models like the crayfish where large parts of a number of well-described escape circuits have long been worked out (**Figure 5A**). In such a model, not only can the function of identified neurons be correlated to the performance of distinct components of a complex behavioral sequence, but also how an animal chooses between a range of escape behaviors might be elucidated. Recordings with implanted electrodes or bath electrodes, which non-invasively record neural and muscular field potentials in freely behaving animals, have begun to reveal some of the basic neural patterns underlying escape decisions in crayfish (Herberholz et al., 2001, 2004; Liden and Herberholz, 2008; Liden et al., 2010).

There is a notable lack of neuroethological studies focused on escape mechanisms produced under natural conditions. While staged encounters with natural predators in the laboratory provide some insight into the interplay between neural function and ecologically relevant escape behavior, these studies are sparse. Field studies on the other hand are often focused on ecology and behavior and not designed to investigate neural processes. Occasionally, data sets obtained separately in the field and laboratory allow for a comparative view and for correlating firing patterns of individual neurons and natural escape behavior (e.g., Hemmi and Tomsic, 2012); however, the development of new technologies that permit direct measures of nervous system function in natural settings is highly desirable.

Finally, the neuromodulation of escape behavior by monoamines such as octopamine, serotonin and dopamine is worth further exploration. Although a number of the escape circuits discussed have been shown to be responsive to the application or removal of monoamines (Glanzman and Krasne, 1983; Bustamante and Krasne, 1991; Stern et al., 1995; Pflüger et al., 2004; Harvey et al., 2008; Rind et al., 2008), little is known about the context in which these monoamines affect the performance of behavioral decisions. Since most invertebrate aminergic effects are mediated by metabotropic receptors that can have a gradual but pronounced impact on behavior, monoamines are an attractive candidate for how a nervous system may be biased toward the production of one behavior over another (Crisp and Mesce, 2006; Mesce and Pierce-Shimomura, 2010). Through these monoamines, escape behaviors might modulate or be modulated by competing behaviors. Monoamines (e.g., dopamine and serotonin) have been targeted for roles in decision making and the encoding of punishment and reward (Daw et al., 2002). Thus, the study of monoamines in the context of the evolutionarily critical task of predator avoidance provides an excellent opportunity to explore the postulated neurochemical currency of neuroeconomic decision making. Unfortunately, little work on value-based decision making has been undertaken with invertebrates despite the description of numerous value-based decisions that are likely to involve identified circuits including those mediating escape or avoidance behavior. Research in this field is currently limited to a few invertebrate species, namely the previously discussed sea slug and crayfish, where basic neural mechanisms underlying cost-benefit computations have been partially uncovered. It is surprising that researchers interested in neuroeconomics have not taken greater advantage of these highly tractable models, as they are likely to contribute much to this new field, as they have contributed to neuroscience in general (Clarac and Pearlstein, 2007). Possibly we have just begun to realize that invertebrate models are ideally suited to answer some of the most challenging questions faced today by neuroscience research.

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Functional organization and adaptability of a decision-making network in *Aplysia*

Romuald Nargeot [1,2] **** and John Simmers*** [1,2]

[1] *Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Université Bordeaux, UMR 5287, Bordeaux, France*
[2] *CNRS, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, UMR 5287, Bordeaux, France*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Benjamin Hayden, University of Rochester, USA
Avraham Susswein, Bar-Ilan University, Israel
Riccardo Mozzachiodi, Texas A&M University – Corpus Christi, USA

****Correspondence:***
Romuald Nargeot, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, Université Bordeaux Ségalen, CNRS UMR 5287, 146 rue Léo Saignat, 33076 Bordeaux, France.
e-mail: romuald.nargeot@u-bordeaux2.fr

Whereas major insights into the neuronal basis of adaptive behavior have been gained from the study of automatic behaviors, including reflexive and rhythmic motor acts, the neural substrates for goal-directed behaviors in which decision-making about action selection and initiation are crucial, remain poorly understood. However, the mollusk *Aplysia* is proving to be increasingly relevant to redressing this issue. The functional properties of the central circuits that govern this animal's goal-directed feeding behavior and particularly the neural processes underlying the selection and initiation of specific feeding actions are becoming understood. In addition to relying on the intrinsic operation of central networks, goal-directed behaviors depend on external sensory inputs that through associative learning are able to shape decision-making strategies. Here, we will review recent findings on the functional design of the central network that generates *Aplysia's* feeding-related movements and the sensory-derived plasticity that through learning can modify the selection and initiation of appropriate action. The animal's feeding behavior and the implications of decision-making will be briefly described. The functional design of the underlying buccal network will then be used to illustrate how cellular diversity and the coordination of neuronal burst activity provide substrates for decision-making. The contribution of specific synaptic and neuronal membrane properties within the buccal circuit will also be discussed in terms of their role in motor pattern selection and initiation. The ability of learning to "rigidify" these synaptic and cellular properties so as to regularize network operation and lead to the expression of stereotyped rhythmic behavior will then be described. Finally, these aspects will be drawn into a conceptual framework of how *Aplysia's* goal-directed circuitry compares to the central pattern generating networks for invertebrate rhythmic behaviors.

Keywords: *Aplysia*, feeding behavior, occasion setting, motor pattern selection, learning, central pattern generator, oscillatory properties, plasticity

INTRODUCTION

In a relatively constant environment, animals can express variable motor actions as a consequence of internal drives arising from the dynamic properties of central networks. These adaptive behaviors can be rhythmic and relatively stereotyped, or may be highly variable and directed toward a specific goal (Dickinson and Balleine, 1994; Marder, 2000; Pearson, 2000; Brembs, 2011a). Feeding, sexual, and aggressive behaviors in both invertebrates and vertebrates typify such goal-directed actions in which internally derived decisions to act are crucial for the spontaneous expression of behaviorally relevant action patterns (Kupfermann, 1974; Edwards et al., 2003; Dickson, 2008; von Philipsborn et al., 2011). The decision to act implies that the underlying central network possesses the structural and functional mechanisms that autonomously enable the selection of a particular action pattern from several variants and the "setting of the occasion" for its expression (Schall, 2005). Nevertheless, the central network operations that define decision-making are subject to regulation by sensory inputs and the positive (rewarding) or negative (punishing) consequences of an executed action. Through sensory feedback and associative learning, past experience modifies the internal drives which select and set the occasion for action pattern production (Baxter and Byrne, 2006; Brembs, 2011b; Kennerley and Walton, 2011; Nargeot and Simmers, 2011). Although several studies have begun to analyze the neuronal circuits implicated in such behavioral decision-making (Kristan, 2008; Gaudry and Kristan, 2009; Kemenes, 2009; Balleine and O'Doherty, 2010), it remains unclear how these circuits are able spontaneously to generate and organize the neuronal activity underlying coherent occasion setting and action pattern selection, and how these decision-making processes are regulated by learning.

Most of our current knowledge on the ability of the central nervous system (CNS) to spontaneously generate patterned motor activity has derived from the analysis of rhythmic and essentially stereotyped behaviors, such as locomotion and respiration. From these studies, a number of rhythmogenic networks, so-called "central pattern generators" (CPGs), have been identified and the synaptic and intrinsic membrane properties of their constituent neurons defined (for reviews, see Calabrese, 1995; Marder and Bucher, 2001, 2007; Nusbaum and Beenhakker, 2002; Marder et al., 2005; Harris-Warrick, 2010). However, although the ongoing operation of such automatic CPGs can be

dynamically regulated by sensory and modulatory inputs, this functional variability is not determined by mechanisms associated with any form of decision-making (Pearson, 2000; Harris-Warrick, 2011).

Insights into the functional design and properties of motor networks that are able autonomously to elaborate action pattern selection and occasion setting in their expression are beginning to emerge for the circuits governing invertebrate feeding behavior. Specifically, an increasing amount of data has allowed characterizing the synaptic organization, cellular properties, and dynamic operation of such networks in mollusks (Elliott and Susswein, 2002; Kemenes and Benjamin, 2009; Nargeot and Simmers, 2011). Here, we will review recent findings on the neuronal constructs of the buccal network which contribute to the autonomous genesis and selection of distinct feeding-related action patterns in *Aplysia*. Evidence that this goal-directed behavior, and the variable motor strategies used in the effective search for food, depends partly on internal and autonomous mechanisms will be presented. Then, the structural and functional properties of the networks mediating this motor variability will be described in order to pinpoint common and distinguishing features with previously characterized CPG networks for automatic behaviors. Particular emphasis will be placed on the contribution of neuronal diversity, and the erratic membrane properties and intercellular connections of identified neurons that select and set the occasions for motor pattern genesis. Finally, the regulation of these fundamental parameters of decision-making buccal circuitry by associative learning will be described.

SPONTANEOUS VARIABILITY IN *APLYSIA'S* FEEDING BEHAVIOR

In searching for food, the herbivorous *Aplysia* performs a variety of motor acts including locomotion, postural movement, head-waving, and buccal movements, which although variable in terms of occurrence, duration, and intensity, are all directed toward the goal of obtaining appropriate nutriment (Kupfermann, 1974). Past studies have focused on buccal movements, and particularly those of the tongue-like radula, as they are easily observable and quantifiable during ongoing feeding behavior (**Figure 1**). *Radula* movements are organized in repeating cycles that each consists of three successive actions: a protraction phase, retraction phase, and closure of the appendage's two halves (Morton and Chiel, 1993a; Neustadter et al., 2002; Horn et al., 2004). Depending on the relative durations of these phases and the timing of closure activity, a radula movement cycle can engage in at least two distinct behaviors – ingestion (biting, swallowing) and egestion. In an ingestive cycle, following a short protraction, radula closure that serves to grasp food, occurs mainly during a prolonged retraction phase, thereby drawing particles into the buccal cavity. In an egestive cycle, radula closure now occurs mainly during the extended protraction phase that precedes a shorter retraction, thus withdrawing particles back out of the buccal cavity. These different radula actions are expressed spontaneously in the absence of any food stimulus when the animal is randomly sampling its environment (**Figure 1A**), or they occur at an elevated mean frequency in the continuous presence of a non-ingestible food stimulus (**Figure 1B**).

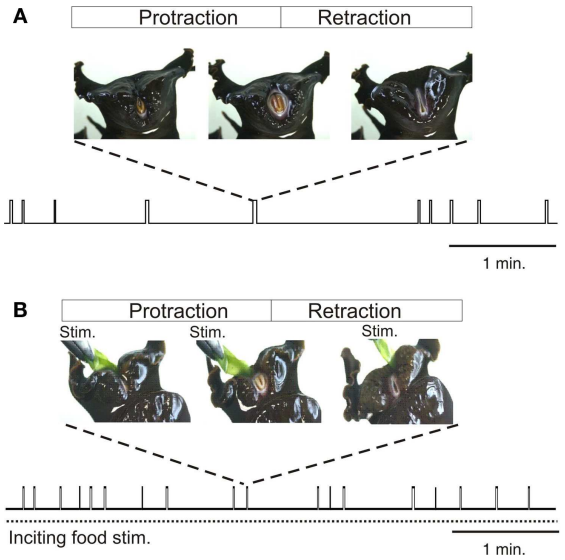

FIGURE 1 | *Radula* movements in freely behaving *Aplysia*. In the absence of food **(A)** or under continuous inciting stimulation with non-ingested food applied to the lips [Stim., **(B)**], *Aplysia* spontaneously generates repeated cycles of protraction and retraction of its tongue-like radula (vertical bars). The distribution in time and the duration of radula movement cycles are highly variable and are not determined by any explicit sensory cue, although the mean frequency of cycle occurrences increases during inciting stimulation. Between successive radula cycles the animal engages in various other actions including lip movements, head-waving or locomotion which are all directed toward effective food seeking.

In freely behaving animals, both the expression and temporal features of radula movement cycles are highly variable within a given feeding sequence. This behavioral variability is evident in the changing delay with which a transient food stimulus triggers a given radula cycle (Susswein et al., 1976), and in the unpredictable occurrence and structure of cycle emissions in the absence or presence of a constant food stimulus (Horn et al., 2004; Lum et al., 2005; Brezina et al., 2006; Nargeot et al., 2007). Thus, successive radula movements occur in an unpredictable mixture of opposing ingestive and egestive action patterns, and the intervals between the onsets of successive cycles may vary considerably, without being related to any explicit sensory cue (**Figure 1**). In addition to this spontaneous variability in the initiation and selection of radula actions, the movements within individual cycles (protraction, retraction, closure) change considerably on a cycle-to-cycle basis in terms of their duration and strength. This flexibility does not correspond to random noise in feeding behavior, but rather, is associated with the changing efficiency with which the radula is cyclically protracted and retracted in a trial-error strategy directed toward the successful seeking and consumption of food (Lum et al., 2005).

Without excluding the possible contribution of extrinsic sensory information in this behavioral flexibility via influences on both the initiation and selection of radula action, several lines of evidence suggest that the motor variability arises primarily from an autonomous central process for accomplishing effective feeding. First, the variability in feeding behavior occurs *in vivo*

in the absence of any food stimulus (Nargeot et al., 2007). Second, the parameters that characterize the behavioral irregularity continue to be expressed by radula output patterns in isolated neuromuscular and *in vitro* CNS preparations (Morton and Chiel, 1993b; Nargeot et al., 1997; Horn et al., 2004; Zhurov et al., 2005). Third, in the isolated buccal nervous system, essential aspects of the behavioral variability were found to be correlated to spontaneous, cycle-to-cycle changes in the bioelectrical activity of identified elements of the underlying central network (Hurwitz et al., 1997;Nargeot et al., 1999a,b, 2009; Jing and Weiss, 2005). Fourth, learning processes which substantially diminish or even suppress the behavioral irregularity induce corresponding changes in the endogenous properties of the network's neurons (see below). Thus, a major determinant of the spontaneous irregularity in *Aplysia's* feeding behavior appear to be encoded within the functional properties of the elements comprising the central circuitry that autonomously organizes, selects, and sets the occasion for the production of goal-directed movement cycles.

ORGANIZATIONAL PROPERTIES OF A MULTIFUNCTIONAL NETWORK

The interneurons, motor, and sensory neurons responsible for producing and adjusting radula movements are distributed in two interconnected and essentially identical neuronal circuits located within the bilateral buccal ganglia situated on the buccal mass (Kupfermann, 1974; Elliott and Susswein, 2002). In isolated *in vitro* buccal ganglia preparations, whether spontaneously active or subjected to tonic electrical stimulation of peripheral sensory nerves, this bilateral network continues to generate the motor patterns that underlie the essential features of radula movements and their variability observed in the freely behaving animal (**Figures 2A,B**; Morton and Chiel, 1993b; Nargeot et al., 1997; Jing et al., 2011). These "fictive" *in vitro* patterns are therefore composed of successive protraction and retraction phases of changeable durations and in variable overlap with closure motor nerve discharge. Thus, according to the phase position of the latter and the relative durations of the protraction and retraction phases, the distinct motor patterns that normally produce ingestive or egestive movements can be readily distinguished (**Figures 2B,C**).

In correspondence with actual behavior, the selection, initiation, and structure of radula motor patterns recorded from *in vitro* preparations also vary spontaneously from cycle-to-cycle, and in a highly irregular manner (**Figure 2B**). Not only are successive patterns comprised of burst activities of variable durations and frequencies, but the pattern phenotype (i.e., fictive ingestion or egestion) and the interval between the onsets of successive patterns

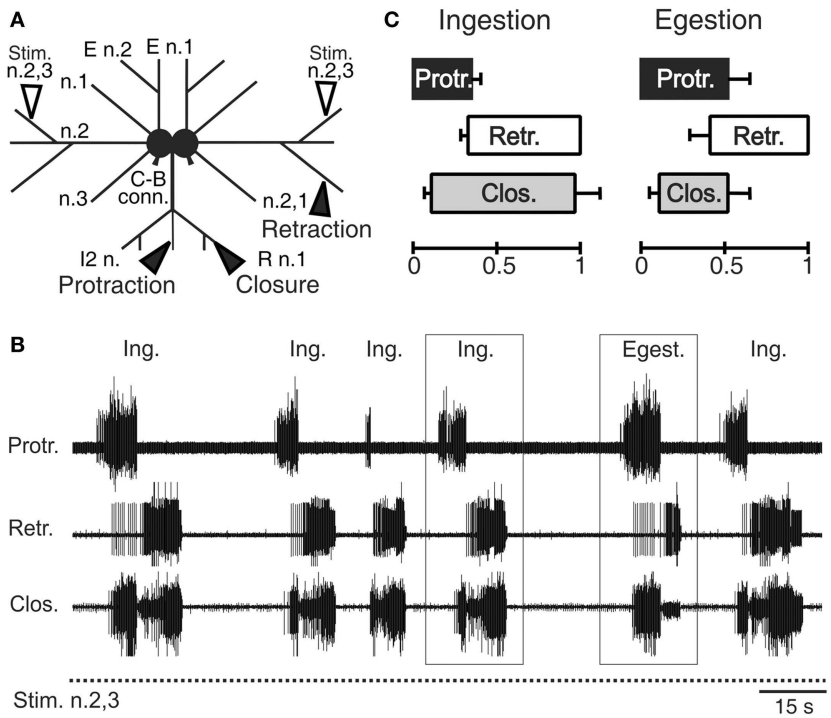

FIGURE 2 | *Radula* motor pattern generation in isolated buccal ganglia. **(A)** Schematic of the *in vitro* ganglia. Stimulating electrodes (unfilled arrowheads) are positioned on the buccal nerves 2,3 (n.2,3) containing sensory fibers that are normally activated by food stimuli within the anterior part of the buccal cavity (Nargeot et al., 1997). Recording electrodes (filled arrow heads) are positioned on the intrinsic nerve 2 (I2 n.), the buccal nerve 2,1 (n.2,1) and the radula nerve (R n.), which carry axons of protractor, retractor, and closure motor neurons, respectively. **(B)** Simultaneous extracellular recordings of spontaneous cycles (two are indicated by rectangles) of radula protractor (Protr.), retractor (Retr.), and closure (Clos.) motor activity during tonic (2 Hz) inciting stimulation of the input nerve 2,3 (Stim. n.2,3). The distribution in time of these radula motor pattern cycles and the durations of the different activity phases are highly variable [see also **(C)**] and are not determined by any sensory cue. **(C)** Based on the relative durations of the protraction/retraction phases and the phase position of closure activity, two distinct motor patterns are distinguishable (also see rectangles in **(B)**] that correspond to ingestion (Ing.) and egestion (Egest.) radula movements during actual feeding in the intact animal.

varies considerably and unpredictably. Thus, in isolation, the multifunctional buccal network is able to autonomously organize, select, and set the occasions for emitting the distinct biologically relevant patterns of radula motor activity that occur *in vivo*.

BASIC CONSTRUCTS OF THE BUCCAL NETWORK

The key components of the buccal feeding network have been identified and characterized in a variety of cellular studies using intracellular recordings (Gardner, 1977; Susswein and Byrne, 1988; Plummer and Kirk, 1990; Hurwitz and Susswein, 1996; Hurwitz et al., 1997; Kabotyanski et al., 1998; Jing and Weiss, 2002; Dembrow et al., 2004; Jing et al., 2004; Sasaki et al., 2009; see also Elliott and Susswein, 2002). Together these neurons constitute a pattern generating ensemble that shares several features with previously described CPGs for automatic rhythmic behaviors in invertebrates (**Figure 3A**; Getting, 1989; Nusbaum and Beenhakker, 2002; Marder and Bucher, 2007; Selverston, 2010). First, buccal circuit neurons generate spontaneous bursts of action potentials that are associated with at least one of the phases of overall network output. Second, experimental manipulation of their electrical activity can modify the cycle frequency of motor pattern expression via influences on two fundamental network properties: the intrinsic bioelectrical properties of constituent neurons and their synaptic interconnections (**Figures 3B,C**). As in other CPGs, specific membrane properties of buccal CPG neurons include post-inhibitory rebound, regenerative plateauing mechanisms, and endogenous oscillatory properties (Plummer and Kirk, 1990; Evans et al., 1999; Susswein et al., 2002; Nargeot et al., 2009) that underlie burst generation and autonomous network functioning. In addition, the coordination of cellular bursting into behaviorally appropriate motor output is conferred by the synaptic connections among the circuit neurons. Network synapses are generally reciprocal, although individual neurons may also exert complex synaptic influences on variable proportions of their circuit partners via a diversity of electrical and/or chemical, excitatory and/or inhibitory, conventional fast and/or modulatory actions.

Sixteen bilateral pairs of identified cells, including sensory, interneurons, and motor neurons, have been classified as integral members of the buccal CPG (**Figures 3A,B**). They are grouped into three different functional subsets, each dedicated to the genesis of a specific phase of buccal motor activity. The protractor generator subset contains those neurons that are active during, and trigger aspects of protractor motor output (Susswein and Byrne, 1988; Hurwitz et al., 1997; Kabotyanski et al., 1998; Jing and Weiss, 2002; Dembrow et al., 2004; Jing et al., 2004), while the retractor generator contains the corresponding neurons for retractor output (Gardner, 1977; Plummer and Kirk, 1990; Hurwitz and Susswein, 1996; Sasaki et al., 2009). These two functional groups are connected by reciprocal synapses that ensure the strict succession of protraction and retraction phases of activity. A third neuronal subset, composed of inhibitory cells, transiently inhibits both the protractor and retractor generators and thereby terminates each pattern cycle (Plummer and Kirk, 1990; Evans et al., 1999; Nargeot et al., 2002). Most of these CPG neurons are monosynaptically connected to corresponding populations of motor neurons controlling radula protraction and retraction (Church and Lloyd, 1994; see Elliott and Susswein, 2002), while several CPG elements excite motor neurons that drive closure muscle contractions in phase with protraction and/or retraction movement.

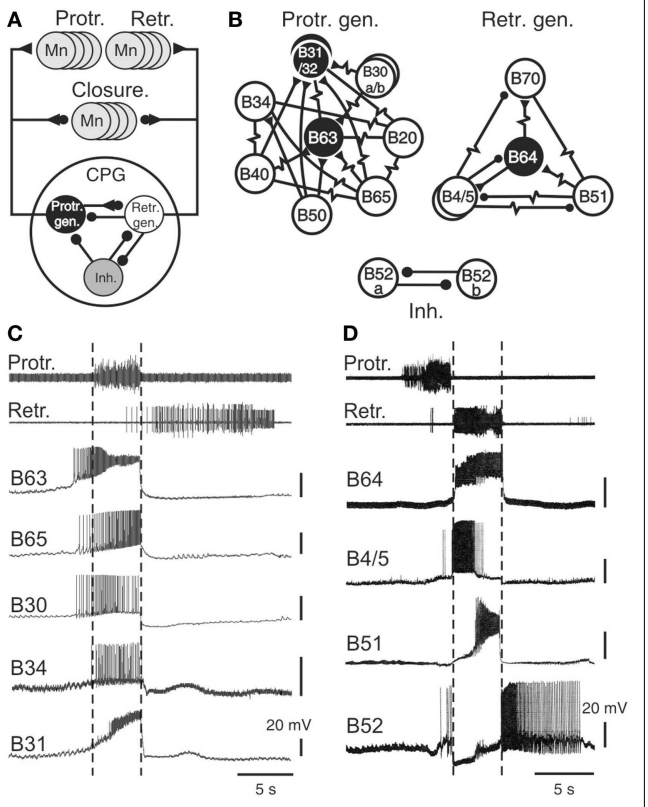

FIGURE 3 | he radula motor pattern generating network. (A) Simplified schematic of the buccal central pattern generator (CPG) and its synaptic connections with protractor, retractor, and closure motor neurons (Mn); the small filled circles represent inhibitory connections while triangles denote excitatory connections. The CPG is composed of three distinct and interconnected groups of neurons: a protractor generator (Prot. gen.), retractor generator (Retr. gen.), and a group of inhibitory neurons (Inh.). **(B)** Detailed schematics showing the neuronal diversity and synaptic connectivity within the protractor and retractor generators, and the inhibitory group. Neurons that are necessary and sufficient for radula motor pattern genesis are in black (see text). Simple resistor symbols represent non-rectifying electrical coupling; resistor symbols associated with a circle or triangle represent non-rectifying electrical coupling associated with an excitatory or inhibitory chemical synapse, respectively. For simplification, not all known synaptic connections including those between the different neuronal groups of neurons [see **(A)**] are shown. **(C)** Simultaneous extracellular recordings of radula protraction and retraction motor output and intracellular recordings from neurons of the protractor generator. The B34 and B31 cells were recorded in a different preparation from the other protractor neurons, but during a motor pattern of similar protractor phase duration. Note that the burst onsets of B63, B65, and B30 anticipate protractor motor nerve activity (indicated by vertical dotted lines). **(D)** Simultaneous extracellular recordings of protraction/retraction motor output and intracellular recordings of retractor generator neurons (B64, B4, B51) and an inhibitory neuron (B52).

FUNCTIONAL DIVERSITY OF BUCCAL NETWORK NEURONS

A striking feature of buccal network design is the diversity of neuron types within each functional subset (**Figures 3B–D**). For

example, of the 10 bilateral pairs of protractor generator neurons, 8 of these are distinctly different in terms of their intrinsic membrane properties, patterns of synaptic connectivity, and specific roles in the generation of protractor and closure motor activity. Similarly, four of the five bilateral cell pairs in the retractor generator differ significantly in their membrane properties, synaptic connections and contributions to retractor, and closure output. Although the neurons comprising each functional group are most often coupled via electrical or excitatory chemical synapses, due to a general weakness of these connections and/or individual differences in membrane properties, cells within a given group are able to express impulse bursting with distinct durations, frequency, and timing (**Figures 3C,D**).

In addition to a functional division according to their participation in the different phases of radula motor pattern production, buccal CPG neurons also play primary or secondary roles in the actual pattern generating process, with the former being necessary and sufficient for producing a specific pattern phase while the latter are not. Essential (primary) roles are restricted to the B63 and B31/32 neurons of the protractor generator and the B64 cell of the retractor generator (**Figure 3B**), their spontaneous impulse bursts being responsible for triggering the protraction and retraction phases, respectively. Accordingly, an experimental hyperpolarization by intracellular current injection of any one of these cells to prevent its bursting activity suppresses production of the corresponding phase of buccal output. Conversely, an experimental depolarization of a previously silent essential cell triggers its bursting and instigates the corresponding phase of radula activity.

The secondary cell subtype contributes to motor pattern genesis but is not necessary for its expression. This group includes the B20, B30, B34, B40, B50, B65 neurons of the protractor generator, and the B4/5, B51, B70 cells of the retractor generator. Similar to CPG neurons generally, these non-essential elements can produce spontaneous bursting in time with buccal motor output and their experimental depolarization in an otherwise silent preparation can trigger protraction or retraction phases of activity. However, in contrast to the essential B63, B31/32, and B64 neurons, a hyperpolarization to prevent their spontaneous bursting does not impair overall motor pattern genesis. Moreover, about half of these follower neurons (specifically B30, B34, B40, B50, B51) are not systemically active during successive cycles of radula motor output, further indicating that these cells serve as occasional contributors to setting the intensity and/or type of pattern expressed (see for example, B51 in **Figure 5A**).

Although *Aplysia*'s feeding network shares common organizational and functional features with CPG networks responsible for more stereotyped rhythmic behaviors, the latters' functional subcircuits are usually considered to be composed of individual neurons or cell assemblies that are similarly necessary and sufficient for motor pattern genesis (Getting, 1989; Syed et al., 1990; Marder and Calabrese, 1996). Furthermore, the cellular components of a given functional group are most often strongly connected through electrical coupling or excitatory chemical synapses, thereby ensuring tightly coordinated, and often synchronized, bioelectrical activity throughout the subset. In the buccal CPG, by contrast, necessity, and sufficiency for generating the protraction and retraction phases of radula motor output is vested in small neuronal subpopulations of the wider feeding network. This essential kernel is synaptically connected to the remaining cohort of second-order neurons that also have specific synaptic connections, membrane properties and patterns of firing, and which thereby exert varying influences on the activity of the essential neurons and motor pattern genesis. Thus, an important distinguishing feature between the multifunctional network responsible for *Aplysia*'s goal-directed feeding behavior and CPGs engaged in stereotyped automatic behaviors is the neuronal diversity that, by imparting cycle-to-cycle variability to the motor command, provides the substrate for a potential decision-making capability for when (occasion setting) and how to act (motor selection).

OCCASION SETTING VIA THE ASSOCIATION OF ASYNCHRONOUS BURSTING AND IRREGULAR MEMBRANE PROPERTIES

The buccal CPG elements that set the occasions for radula motor pattern expression would be reasonably expected to be those cells that are active before or during protraction activity, the initial phase of each pattern cycle. Within the protractor generator circuit, three neuron pairs have been found to generate spontaneous impulse bursts with onsets that consistently precede the protraction phase of each buccal motor pattern by up to several seconds (Nargeot et al., 2009). This anticipatory cell group consists of the essential B63 neurons and the optional neurons B30 and B65 (**Figure 3C**). The B63 cells are electrically coupled to the latter (Kabotyanski et al., 1998; Jing et al., 2004; Nargeot et al., 2009) and all three cell types make excitatory synapses with protractor motor neurons B31/32. However, due to their particular membrane properties and monosynaptic connectivity with other CPG elements and motor neurons, bursting in each of these anticipatory neurons contributes differently to buccal network operation (Nargeot et al., 2009). For example, spike bursts induced by depolarizing current injection into a previously silent B63 neuron are able to elicit complete motor patterns, without necessarily triggering activity in B30 and B65. In contrast, burst discharge evoked in either B30 or B65 systematically triggers bursting in B63 and consequently motor pattern emission. Alternatively, when B63 is held silent with hyperpolarizing current, an experimental activation of either B30 or B65 is no longer able to elicit a complete pattern, and at most, only closure or protractor motor output, respectively, occurs. Thus, the anticipatory protractor neurons constitute a heterogeneous subset of electrically coupled neurons of which the B63 cell pair, activated intrinsically or driven extrinsically by the B30 or B65 neurons, are the sole necessary instigators for motor pattern production.

Despite the electrical coupling between these anticipatory neurons, the onsets of their spontaneous bursts are generally highly uncoordinated and vary randomly on a cycle-to-cycle basis (Nargeot et al., 2009). Consequently, the first active cell as well as the order in which bursting commences in the other subset partners can change considerably and in an unpredictable manner from one cycle to another (**Figure 4A**). Several lines of evidence indicate that this lack of coordination is the major determinant of the variability in the central drive that sets the occasions for radula motor pattern emissions. First, a regularization of spontaneous

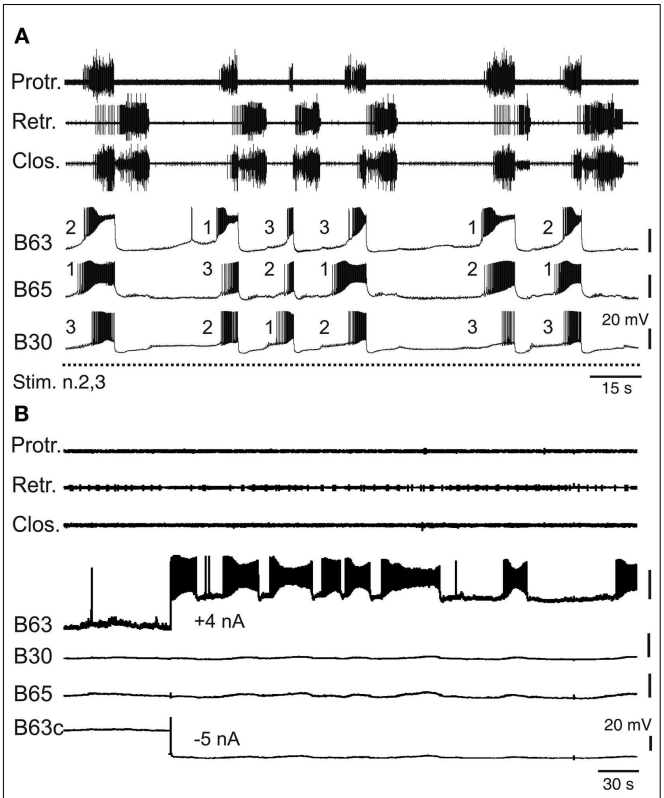

FIGURE 4 | ariability of endogenous anticipatory neuron bursting. **(A)** Simultaneous extracellular recordings of radula motor patterns (top three traces, same layout as in **Figure 2B**) and intracellular recordings of protraction initiating neurons (B63, B65, B30) during tonic (2 Hz) stimulation of n.2,3 (Stim. n.2,3). The irregular occurrences of motor patterns were associated with uncoordinated bursting and cycle-to-cycle variability in the order of burst onsets (indicated by numbering) in B63, B65, B30 prior to each motor pattern emission. **(B)** Endogenous bursting capability of an anticipatory B63 neuron under conditions of functional isolation *in situ*. In the absence of n.2,3 stimulation and with the contralateral B63 (B63c) held hyperpolarized (by −5 nA current injection) to prevent buccal CPG operation and therefore motor pattern emissions (see top three traces), a tonic depolarization of the other B63 (with +4 nA injected current) elicited repetitive, albeit erratic, bursting in the anticipatory cell.

radula movement cycles *in vivo* and of the underlying motor patterns *in vitro* can be induced by operant learning processes (see below). This stabilization of buccal CPG output was found to be correlated with an increased coordination of burst onsets in the B63, B30, B65 neurons, whereby bursts in the essential neurons B63 now invariably precede, with a brief delay, the burst activity of the other anticipatory cells in each motor pattern cycle (Nargeot et al., 2009). Moreover, this learning-induced cellular plasticity is associated with an increase in the electrical coupling amongst the anticipatory neuron subset. Second, and in a related manner, the highly erratic genesis of buccal motor patterns can be switched to regular rhythmic emissions by experimentally enhancing the electrical coupling between the anticipatory neurons: in isolated buccal ganglia preparations, an artificial increase in the electrical coupling of B63-B30 and B63-B65 was found to coordinate their bursting activity and regularize the subsequent expression of radula pattern cycles (Nargeot et al., 2010; Sieling et al., 2010; also see below).

Bursting in the anticipatory neurons, which occurs in absence of sensory input or under tonic sensory nerve stimulation, is driven principally by an endogenous oscillatory mechanism that was previously revealed under conditions of *in situ* isolation of individual cells (**Figure 4B**) by continuously hyperpolarizing a single B63 neuron to block activity in the protractor generator and the remaining CPG network (Nargeot et al., 2009). In such functional isolation, each anticipatory neuron was found capable of generating repetitive bursts of action potentials when subjected to tonic depolarizing current injection. Moreover, in close accordance with the normal erratic emissions of radula motor patterns, bursting in isolated neurons occurred with highly irregular durations and inter-burst intervals (**Figure 4B**). Thus, this erratically expressed intrinsic property of the anticipatory neurons, in combination with their weak electrical coupling, offer plausible substrates for the irregular central drive that sets the occasions for the expression of radula movements *in vivo*. Interestingly, the decision to act has also been partly attributed to the B31/32 neurons that are synaptically coupled to the B63, B30, B65 subset and which govern the transition between the onset of anticipatory activity and protractor phase production. The relative timing of this transition is highly variable and again is dependent on the active contribution of membrane conductances that in this case are specific to the B31/32 neurons (Hurwitz et al., 2008).

Together these findings therefore indicate that the irregular bioelectric behavior of a heterogeneous and asynchronously active core circuit can provide the internal drive that autonomously sets the unpredictable occasions to act in a goal-directed behavior.

MOTOR PATTERN SELECTION BY CENTRAL NETWORK RECONFIGURATION

In a given feeding sequence, *Aplysia* expresses different and even opposing action patterns that underlie cyclic ingestive and egestive radula movements. The animal' ability to switch between these two behaviors is presumably related to its trial-and-error feeding strategy, serving as a maneuver to more efficiently shear off food particles or to ensure the radula's correct alignment in the buccal cavity (Horn et al., 2004; Lum et al., 2005). Again, while the "choice" of radula action must be adaptable to the sensory environment, several arguments indicate that the selection process is mainly conferred by the inherent functional properties of the buccal CPG network itself. In the absence of sensory stimulation or under a constant electrical activation of peripheral input nerves, the isolated buccal ganglia continue autonomously and interchangeably to emit the motor patterns that underlie ingestive and egestive movements *in vivo* (Nargeot et al., 1997; Horn et al., 2004). Thus, buccal circuitry not only inherently sets the occasion for motor pattern emission, but also decides on the specific pattern phenotype expressed.

The clearly distinguishable features of ingestive and egestive outputs have enabled the neuronal basis of this pattern selection process to be investigated *in vitro* (**Figure 5A**). Intracellular recordings have identified several neurons within the buccal network that are responsible for specifying ingestion versus egestion motor patterns (Hurwitz et al., 1997; Kabotyanski et al., 1998;Nargeot et al.,

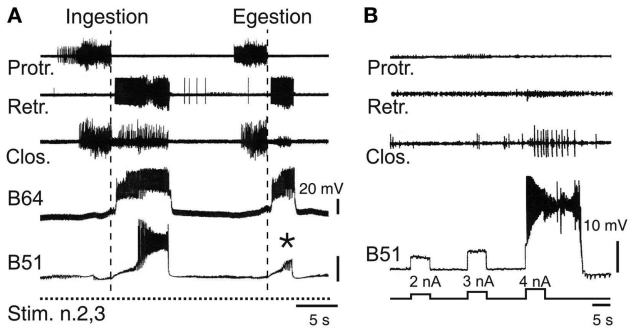

FIGURE 5 | Neuronal correlates of spontaneous motor pattern switching. (A) Extracellular recordings of radula motor patterns (top three traces) and intracellular recordings of the essential B64 neuron and the optional B51 cell of the retractor generator. Switching between ingestion and egestion motor patterns during tonic (2 Hz) stimulation of n.2,3 (Stim. n.2,3) was associated with the spontaneous, all-or-none activation or absence (*) of bursting in B51. **(B)** Endogenous plateau property of B51 revealed by brief intracellular current injections in the absence of n.2,3 stimulation. Note that a burst-generating plateau in B51 also elicited closure motor activity that normally occurs during the retraction phase of ingestion patterns [see **(A)**].

1999a,b, 2002; Cropper et al., 2004; Jing et al., 2004). In contrast to the anticipatory cell kernel, which participates in all buccal motor patterns and thereby contributes to their common features such as protraction and retraction phase alternation, the CPG neurons involved in pattern selection are able to regulate the durations of the protraction and retraction phases and their temporal relationship with radula closure activity. These latter cells, which are not essential for motor pattern genesis, are only active during the expression of a specific pattern. A well-established example is the bilateral pair of B51 neurons (Nargeot et al., 1999a), which remain inactive during egestion pattern genesis, but fire intense bursts during ingestion patterns (**Figure 5A**). This pattern-specific bursting of B51 in turn triggers closure motor activity in phase with the prolonged retraction phase of the ingestion pattern via monosynaptic excitation of the B8 closure motor neurons and the essential B64 retractor generator neuron (Nargeot et al., 1999b). Significantly, a brief sub-threshold depolarization of B51 by current injection during each spontaneous pattern emission was found to bias buccal circuit output toward expression of the ingestion pattern. Conversely, suppression of B51 activity with transient hyperpolarizing current biases the selection process toward non-ingestive pattern emissions. Similarly, other neurons of the buccal network (B34, B52) only generate bursts during the protraction phase of egestion patterns and through their synaptic connections with closure motor neurons and protractor or retractor generator neurons, are able to instruct the buccal CPG to produce egestive behavior (Hurwitz et al., 1997; Nargeot et al., 2002).

Thus, motor pattern selection in the multifunctional buccal network is determined, at least in part, by the dynamic recruitment of specific components whose activity defines the different phenotypes of circuit output. On this basis, the buccal CPG does not constitute a prescribed and constant population of reliably active neurons responsible for generating a single program of motor output. Rather, the functional composition of the network varies substantially on a cycle-to-cycle basis and in an unpredictable manner, thereby specifying individual motor programs from the variants that the overall circuit is capable of producing. It is well known that CPGs in general are not fixed entities, but that depending on sensory or modulatory influences, individual neurons can be dynamically recruited into, or excluded from, a given network in order to generate different forms of the same behavior, or even distinct behaviors (Hooper and Moulins, 1989; Dickinson et al., 1990; Meyrand et al., 1991; Nargeot, 2001; see also Morton and Chiel, 1994; Cropper et al., 2004; Harris-Warrick, 2011). However, in contrast to such extrinsically instructed circuit reconfigurations, the buccal CPG is capable of a remodeling that can occur independently of external sensory or modulatory cues, but instead it arises from the particular synaptic and active membrane properties of the circuit neurons themselves. In the case of the B51 neuron, for example, its pattern-selecting burst occurrences derive from the cell's electrical coupling with the retractor generator B64 neuron, which in turn triggers all-or-none firing in B51 as a result of the latter's intrinsic plateauing property (**Figure 5B**; Nargeot et al., 1999b).

LEARNING-INDUCED RIGIDIFICATION OF BUCCAL NETWORK FUNCTIONING

Although the motor patterns for motivated behaviors can originate autonomously from the underlying central networks, the internally driven incentive to act is regulated by sensory inputs and learning. Associative learning, including both classical and operant conditioning, plays a critical role in altering the neuronal processes that set and select behavioral action (Taylor and Lukowiak, 2000; Brembs, 2003, 2011b; Baxter and Byrne, 2006; Balleine and O'Doherty, 2010; Nargeot and Simmers, 2011).

In appetitive classical conditioning of *Aplysia's* feeding behavior, pairing an unconditional food stimulus with a tactile conditional stimulus (CS) to the lips increases the probability of a subsequent CS to elicit an ingestive radula cycle (Lechner et al., 2000a). The basis for this learning is a synaptic facilitation which enhanced ability of the CS pathway to trigger the motor pattern-initiating neurons B31/32 and pattern-selecting bursts in the B51 neuron (Lechner et al., 2000b; Mozzachiodi et al., 2003; Lorenzetti et al., 2006). Thus, through a pairing-specific occasion setting for a feeding response via B31/32, and pattern selection by B51, the buccal CPG is "instructed" to more reliably produce output of an ingestive nature.

Learning not only modifies sensory-elicited responses, but may also regulate the internally driven impulse for motor pattern production. In operant conditioning, an animal learns to make the contingent association between the spontaneous emissions of an action and its outcome (either rewarding or punishing). As a consequence, the probability of the designated behavior's expression is persistently modified and in some cases, particularly with highly appetitive rewards, may lead to a rhythmic, compulsive-like expression of the rewarded action.

Several operant conditioning paradigms have also been developed for *Aplysia's* feeding behavior and the resulting plasticity analyzed at the cellular and network levels (Susswein et al., 1986; Brembs et al., 2002; Nargeot et al., 2007). In an appetitive form

of this learning, spontaneous ingestive radula cycles were associated with the delivery of a food reward during training. After several minutes of such action/reward associations, the rate of the spontaneous occurrences of ingestive cycles was found to increase dramatically and the highly irregular expression of ingestive motor patterns switched for several hours to regular rhythmic occurrences (Nargeot et al., 2007). This behavioral plasticity was not observed when the food reward was replaced by a neutral food stimulus or when the reward was delivered independently of the timing of radula movement cycles. Such behavioral findings therefore indicated that appropriate sensory stimuli can modify through learning processes the central network's ability to "decide" when and how to act and convert the decision-making buccal circuitry into seemingly rigid and stereotyped rhythmogenic operation.

TRANSFORMATION OF SPORADIC ANTICIPATORY BURST ACTIVITY INTO SYNCHRONIZED RHYTHMICITY

The operant learning-induced acquisition of rhythmic radula pattern generation was found to be associated with a synchronization of bursting activity in the anticipatory B63, B30, B65 neurons. In isolated buccal ganglia from operantly conditioned animals, not only were the delays between burst onsets in these neurons considerably decreased compared to their activity in ganglia from untrained animals, but also the order in which they became active in each pattern cycle became regularized (**Figure 6A**) such that bursts in the essential B63 neurons systematically commenced slightly before the burst onsets of the B65 and B30 neurons (Nargeot et al., 2009; Nargeot and Simmers, 2011).

The regularization of buccal network output was correlated to specific changes in the intrinsic membrane properties and the electrical coupling of the anticipatory protraction cells (Nargeot et al., 2009). In ganglia isolated from previously trained *Aplysia*, each of these neurons under conditions of functional isolation (as described above) spontaneously generated stereotyped, rhythmic bursts of action potentials, in contrast to the same cells in ganglia from untrained animals, which produced irregular and sporadic bursting. Concomitantly, the reduced variability in the process of motor pattern initiation was associated with a strong increase in the electrical coupling between the B63-B30 and B63-B65 cell pairs. The functional significance of this synaptic plasticity has been further investigated using the dynamic clamp technique (see Sharp et al., 1993) to artificially modify the strength of electrical coupling between these neurons (Nargeot et al., 2010; Sieling et al., 2010). In buccal ganglia from naïve preparations, which generate desynchronized anticipatory neuron activity and motor patterns with an irregular temporal distribution, an experimental increase in the electrical coupling between the B63-B30 and B63-B65 cell pairs regularized and synchronized their bursting and consequently, induced rhythmic motor pattern genesis. Conversely, in ganglia from trained animals, an artificial decrease in electrical coupling among the anticipatory neurons desynchronized their spontaneous burst onsets and switched their bursting and hence motor pattern genesis to erratic and irregular occurrences.

These recent findings therefore provide compelling evidence that cell-wide plasticity in the bioelectrical and synaptic properties of the anticipatory neuron subset serves as the causal link by which operant learning regulates the autonomous setting of occasions

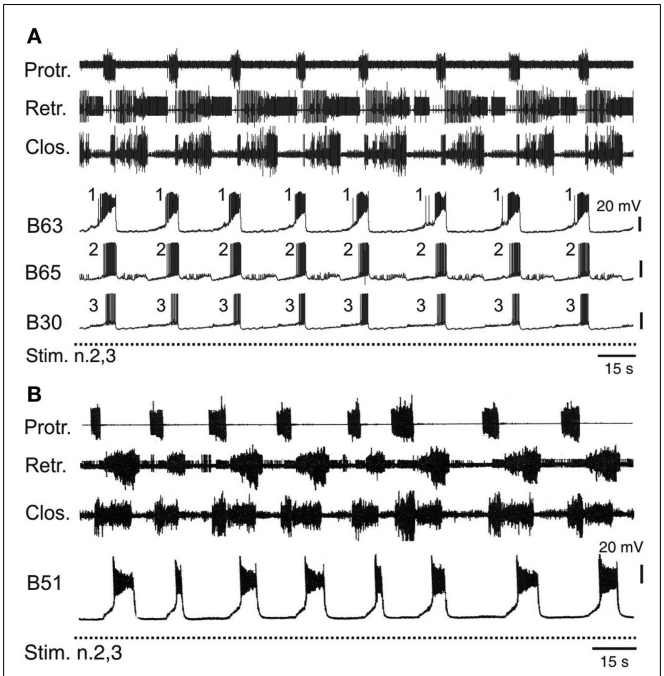

FIGURE 6 | Accelerated and stereotyped ingestive pattern genesis induced by appetitive operant learning. (A) Simultaneous recordings of radula motor patterns and underlying bursting in the anticipatory B63, B65, B30 neurons in a buccal ganglia preparation isolated from a food reward trained animal. The rhythmic expression of motor patterns was associated with regular bursting in the anticipatory neurons, with each cycle being systematically led at a brief interval by burst onset in B63 (c.f. **Figure 4B**). **(B)** The regularization of ingestion pattern expression in an *in vitro* analog of appetitive learning was also associated with a systematic participation of B51 bursting in buccal CPG functioning. Compare with **Figure 5A**.

for the expression of radula feeding behavior. In a comparative context, it is also interesting that the synchronized neuronal and regularized network activity occurring in the buccal motor system after learning, now shares very similar features with other CPGs responsible for more conventional rhythmic behaviors. In the pyloric network of the crustacean stomatogastric nervous system, for example, rhythmogenesis arises from the endogenous oscillatory behavior of a tightly electrically coupled cell subset consisting of the AB and 2 PD pacemaker neurons (Selverston and Miller, 1980). The strongest oscillator AB entrains synchronized bursting in the 2PD cells and together they drive the rest of the pyloric circuit in a stereotyped triphasic pattern that produces rhythmic ingestive movements of the animal's foregut (Selverston and Miller, 1980; Miller and Selverston, 1982; Bal et al., 1988).

STABILIZATION OF NETWORK CONFIGURATION

In addition to modifying decisions about when to act, learning modifies the decision of how to act. After *Aplysia* makes the association of food reward with ingestive radula movements, the occurrence of this motor act increases at the expense of other actions in the animal's behavioral repertoire (Nargeot et al., 2007). Correspondingly, in isolated buccal ganglia from such operantly conditioned animals, or in an *in vitro* analog of this associative learning, the buccal network generates the rewarded (ingestive)

motor pattern to the detriment of the unrewarded egestion pattern (**Figure 6**; Nargeot et al., 1997, 2007; Brembs et al., 2002). In other words, learning modifies the process of motor pattern selection by "rigidifying" the buccal network into the specific functional configuration that ensures the continuous expression of the rewarded action.

Experimental evidence has indicated that this functional rigidity arises from a corresponding stabilization of the buccal circuit's neuronal content. In ganglia from untrained animals, the B51 cells responsible for specifying ingestive pattern expression are only occasionally incorporated into network operation. In contrast, in previously trained preparations, B51 burst occurrences are strongly enhanced so as to systematically contribute, in a cycle-by-cycle manner, to motor pattern generation, and therefore to the predominance of ingestive pattern emissions (**Figure 6B**). Again, this reliable participation of B51 cells in buccal circuit functioning was attributable to learning-induced changes in their intrinsic membrane properties. In operantly trained preparations, the input resistance of these neurons and their probability of generating burst-producing plateau potentials were increased compared to B51 cells in untrained preparations (Nargeot et al., 1999a,b; Brembs et al., 2002; Mozzachiodi et al., 2008). Consequently, the depolarization of these cells via their electrical coupling with the retraction generator B64 neuron more reliably triggered bursting in time with each retraction phase, thereby resulting in the preponderance of ingestive pattern production.

Although a similar cellular analysis has not yet been extended to other neurons, such as B34 and B52 that are known to occasionally participate in buccal network operation and pattern selection processes, the above findings suggest that the reliable expression of a specific motor pattern phenotype is linked to a learning-derived specification and stabilization of the appropriate underlying circuitry. Here again, this rigidification in response to learning bestows the buccal CPG with functional features that are reminiscent of the relatively stable neuronal specification of the auto-active central networks responsible for typical rhythmic behaviors.

CONCLUSION

The data summarized in this review indicate that the simpler and more accessible invertebrate CNS is endowed with neuronal correlates of elementary decision-making processes, including the selection and initiation of specific behavioral acts, which are amenable to cellular analysis. While sampling its environment in the search of food or during food consumption, *Aplysia* produces highly variable and sporadic movements of its buccal mass and rasp-like radula. This irregular goal-directed behavior is partly governed by the autonomous functioning of a central network that spontaneously selects and sets the occasions for the expression of distinct, sometimes opposing, radula actions.

Observations on still functioning buccal ganglia *in vitro* indicate that the auto-active network driving radula feeding movements shares fundamental properties with previously described CPG circuits responsible for automatic rhythmic behaviors. Major common features include: (1) a synaptic compartmentalization of the central network into distinct functional subcircuits that are each dedicated to a specific component of the CPG's global output; and (2), a striking similarity in the dynamic membrane properties of the constituent neurons that underlie spontaneous network operation and the resulting patterned motor drive (see Marder and Calabrese, 1996; Selverston, 2010). However, several distinguishing features are also evident, particularly in relation to the capacity of a goal-directed CPG, such as *Aplysia's* feeding network, to make internal decisions about the timing and nature of its behavioral expression. Thus, a salient feature of buccal network design is its functional complexity, involving a diversity of neuronal types within each functional subcircuit that are distinctive in terms of their individual patterns of synaptic connectivity, specific membrane properties, and therefore the spontaneity, timing and structure of their activity. A second distinguishing feature resulting from the first is the unpredictable variability of activity within and between the buccal CPG subsets. In the case of the protractor generator, irregular and weakly coordinated bursting amongst this unit's anticipatory elements is able to govern the spontaneous setting of occasions for overall motor output expression. In an equivalent manner, the variability in burst expression and coordination between neurons that are essential to motor pattern genesis and non-essential circuit elements determines the selection between the different radula motor programs (**Figure 7**).

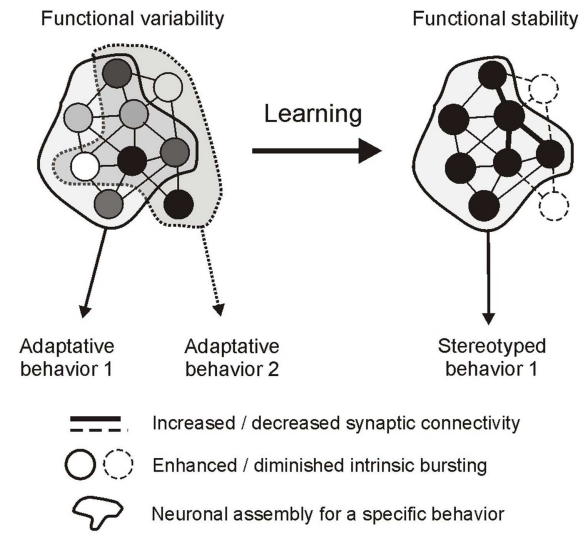

FIGURE 7 | ypothetical representation of a decision making network and its regulation by operant learning in *Aplysia*. Left, the global circuit is composed of a pool of neurons (circles) that generate erratic and weakly coordinated impulse bursts (indicated by different shading intensities) as a result of specific intrinsic properties and pattern of connectivity. The network can generate different adaptive behaviors depending on the participation of individual neurons. Some cells contribute to common features of the different behaviors (neurons in the overlapping area), others contribute selectively to a single behavior (neurons in the non-overlapping areas). Behavioral occasion setting is at least in part governed by the variability in burst coordination in the former subset of neurons, while behavioral selection depends on burst recruitment/exclusion in the latter subset. Right, learning rigidifies network functioning by modifying synaptic connectivity and intrinsic bursting properties. As a result, coordinated bursting now reduces the variability in occasion setting and pattern selection, allowing the expression of a single stereotyped rhythmic behavior.

On this basis, therefore, an elementary process in decision-making that enables the selection of goal-directed output and setting the occasion for its occurrence resides with the coordination of erratic spontaneous bursting within the functional subsets of the central network. It is also significant in this context that learning paradigms that regulate decision-making also regulate neuronal coordinating processes. As a result of sensory-mediated changes in synaptic connectivity and intrinsic membrane properties, variable cellular, and subcircuit activity is transformed into tightly coordinated and regular discharge (**Figure 7**), thereby converting otherwise unpredictable and erratic network output into a stereotyped rhythmic drive that now resembles the output commands of CPGs responsible for automatic behaviors.

Thus, in contrast to vertebrates where occasion setting and the selection of relevant goal-directed actions are thought to rely on distinct and functionally dedicated neural structures (see Balleine and O'Doherty, 2010), the buccal ganglia of *Aplysia* provide an intriguing example of how a single network, through autonomous multifunctioning mediated by specific synaptic and intrinsic neuronal properties, is able to achieve both tasks. Moreover, recent studies on this model system have offered a different conceptual framework for how reward learning can alter decision-making processes that may eventually lead to a habitual or compulsive expression of a particular goal-directed behavior. While data from vertebrates have suggested that learning switches the activation of decision-making circuitry to the recruitment of distinct automatic networks for habitual behavior (see Balleine and O'Doherty, 2010), recent findings in *Aplysia* indicate that the conversion of a goal-directed act to an automatic and rhythmic behavior can arise from a learning-induced rigidification in the functional properties of the decision network itself. A greater understanding of the cellular and sub-cellular mechanisms underlying decision-making in goal-directed behaviors of invertebrates may therefore provide general insights into the neuronal basis of decision-making processes and their regulation by learning or their deregulation in behavioral disorders.

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Reproduction-related sound production of grasshoppers regulated by internal state and actual sensory environment

***Ralf Heinrich*[1]*, *Michael Kunst*[2] *and Andrea Wirmer*[3]**

[1] *Department of Cellular Neurobiology, Institute for Zoology and Anthropology, University of Göttingen, Göttingen, Germany*
[2] *Department of Cellular and Molecular Physiology, Yale School of Medicine, New Haven, CT, USA*
[3] *Institute for Neurobiology, University of Ulm, Ulm, Germany*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Varvara Vedenina, Russian Academy of Sciences, Russia
María-Dolores García, Universidad de Murcia, Spain

***Correspondence:**
Ralf Heinrich, Department of Cellular Neurobiology, Institute for Zoology and Anthropology, University of Göttingen, Berliner Strasse 28, Göttingen, Germany.
e-mail: rheinri1@gwdg.de

The interplay of neural and hormonal mechanisms activated by entero- and extero-receptors biases the selection of actions by decision making neuronal circuits. The reproductive behavior of acoustically communicating grasshoppers, which is regulated by short-term neural and longer-term hormonal mechanisms, has frequently been used to study the cellular and physiological processes that select particular actions from the species-specific repertoire of behaviors. Various grasshoppers communicate with species- and situation-specific songs in order to attract and court mating partners, to signal reproductive readiness, or to fend off competitors. Selection and coordination of type, intensity, and timing of sound signals is mediated by the central complex, a highly structured brain neuropil known to integrate multimodal pre-processed sensory information by a large number of chemical messengers. In addition, reproductive activity including sound production critically depends on maturation, previous mating experience, and oviposition cycles. In this regard, juvenile hormone released from the corpora allata has been identified as a decisive hormonal signal necessary to establish reproductive motivation in grasshopper females. Both regulatory systems, the central complex mediating short-term regulation and the corpora allata mediating longer-term regulation of reproduction-related sound production mutually influence each other's activity in order to generate a coherent state of excitation that promotes or suppresses reproductive behavior in respective appropriate or inappropriate situations. This review summarizes our current knowledge about extrinsic and intrinsic factors that influence grasshopper reproductive motivation, their representation in the nervous system and their integrative processing that mediates the initiation or suppression of reproductive behaviors.

Keywords: reproductive behavior, reproductive states and readiness, sound production, grasshopper, neurotransmitters and hormones, central complex, corpora allata

INTRODUCTION TO THE MATING BEHAVIOR OF ACOUSTICALLY COMMUNICATING GRASSHOPPERS

The mating behavior of acoustically communicating grasshoppers (Orthoptera, Acrididae, Gomphocerinae) has been subject to various scientific investigations for some decades. Especially the subgroup Gomphocerinae contains numerous species that generate communication signals by hind leg stridulation and/or wing clapping (Elsner, 1974; Elsner and Wasser, 1995; Lorier et al., 2010). The most complete set of data concerned with the generation, perception, and nervous processing of the species- and context-specific songs has been established in the species *Chorthippus biguttulus*, in which both males and females are capable of sound production by hind leg stridulation.

Mature male *C. biguttulus* generate calling songs to attract females, courtship songs to establish female readiness for copulation and rival songs in situations of competition with other males (von Helversen and von Helversen, 1975). In contrast, females only sing in the state of active reproductive readiness that is regulated by maturation, previous mating experience, and oviposition cycles (Wirmer et al., 2010). All songs contain individual song sequences (2–6 s duration) consisting of repetitions of a species-specific basic subunit ("chirp"; 50–70 ms duration) that is typically generated by three up- and down-movements of a hind leg (von Helversen, 1972; Elsner, 1974).

Acoustic signals are generated by scraping a row of pegs on the insides of the hind legs against a prominent cuticular vein on the front wings (=stridulation). Rhythmicity of stridulatory hind leg movements that determines the species-specific temporal acoustic patterns is generated by rhythm generating circuits in the metathoracic ganglion complex (Ronacher, 1989; Hedwig, 1992). Each of the hind legs is driven by a hemiganglionic network and right-left coordination is maintained by a set of connecting neurons (Ronacher, 1989; Heinrich and Elsner, 1997). Stridulation is initiated and maintained by command neurons that connect the brain with the thoracic rhythm generators (Hedwig, 1994). Each type of command neuron invariantly activates only one song pattern from a species' repertoire (Hedwig and Heinrich, 1997). Thus, the command neurons transmit the activating signal from the brain to the thoracic pattern generators but brain neuropils located presynaptically to the command neurons mediate the decision about when

and which pattern to sing. Studies on grasshoppers including the species *C. biguttulus* and on other insects indicated that the decision about time, type, and intensity of stridulation is mediated by the central complex (Heinrich et al., 1997, 2001; Popov et al., 2005). This set of midline-spanning neuropils has been demonstrated to process multimodal sensory information (visual, acoustic, and probably others) in order to select one and inhibit other conflicting sensorimotor pathways that compete for behavioral expression (Wessnitzer and Webb, 2006).

The occurrence and intensity of grasshopper songs in response to male calling songs (to stimulate females) or female songs (to stimulate males) has been used as a measure of an individual's reproductive readiness (von Helversen, 1972; von Helversen and von Helversen, 1997; Wirmer et al., 2010). Several environmental conditions (e.g., temperature, illumination, air pressure, ambient noise) and more specific signals (e.g., acoustic signals of conspecific grasshoppers or predators) have been shown to stimulate or inhibit grasshopper sound production on a rather short time scale in the range of seconds to minutes (**Figure 1**). In addition to this short-term regulation of reproduction-related sound production, other factors influence this behavior on a longer time scale ranging from hours to weeks. These factors include sexual maturity especially in females (Loher and Huber, 1964; Kriegbaum, 1988; Wirmer et al., 2010), female oviposition cycles (von Helversen, 1972), and previous mating activity of both males and females (Loher and Huber, 1964; Wirmer et al., 2010; **Figure 1**). Adjustment of reproductive behaviors to these behavioral states has been attributed to hormone signaling and particularly juvenile hormone (JH) produced in the corpora allata has been implicated in the control of female grasshopper and other insects' reproductive motivation (Loher, 1962; Stout et al., 1991; Hartmann et al., 1994).

Grasshopper reproductive behavior including reproduction-related sound production is regulated by both the actual situation and the internal physiological state. In the following, we present the current knowledge about the neural and endocrinal mechanisms that select reproductive behaviors and activate sound production in appropriate situations and outline possible mechanisms that adjust long-term and short-term regulatory mechanisms to provide coherent behavioral responses.

"SHORT-TERM" REGULATION OF GRASSHOPPER SOUND PRODUCTION BY THE CENTRAL COMPLEX

Pharmacological studies on restrained intact grasshoppers clearly indicated that the central complex selects and coordinates sound pattern generation in acoustically communicating species. These studies identified a number of transmitters that contribute to the processing of sensory information relevant for reproductive behaviors and hence participate in the decision about whether or not to sing in a particular situation and which pattern to produce. Sound production can be stimulated by focal injection into the central complex of acetylcholine and both nicotinic and muscarinic agonists (Heinrich et al., 1997), proctolin, and dopamine and it can be inhibited by γ-aminobutyric acid (GABA), glycine, and nitric oxide (Heinrich et al., 1998; Wenzel et al., 2005; **Figure 2A**). Except for glycine, the presence of these transmitters and some of their receptors in the central complex has been confirmed by immunocytochemical studies, suggesting that they function as endogenous signals in the processing of relevant sensory information and some of them could be associated with particular sensory input that promotes or inhibits sound production (see below).

Grasshopper sound production can also be elicited by pharmacological inactivation of inhibitory synaptic signaling in the central

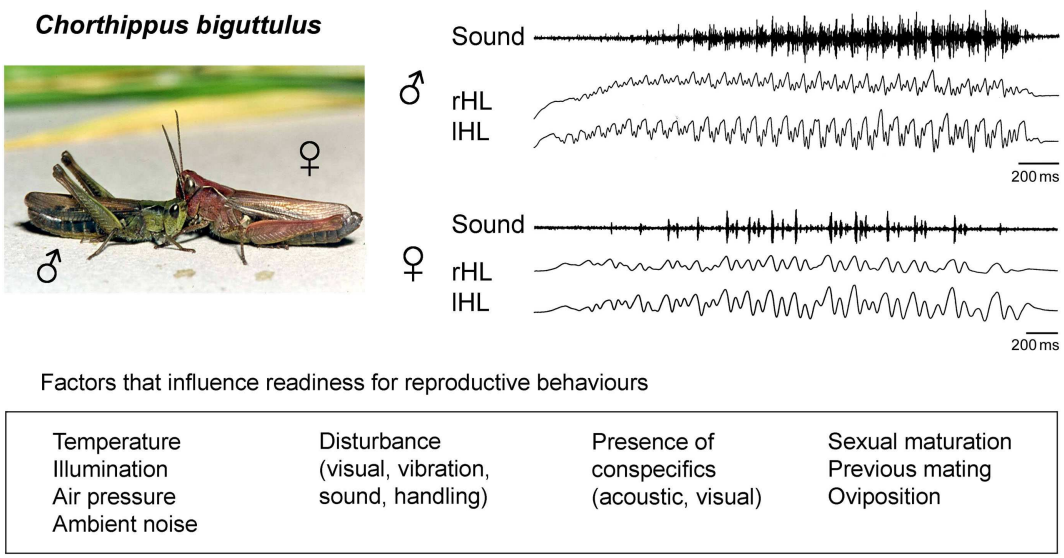

FIGURE 1 | Acoustic communication of the grasshopper *Chorthippus biguttulus*. Males and females produce sound sequences by hind leg stridulation in order to signal sexual receptivity and attract mating partners. Readiness to produce reproduction-related acoustic signals is influenced by environmental conditions, specific signals that indicate the presence predators or potential mating partners and internal physiological states resulting from maturational mechanisms and previous mating activity. Sound: oscillograms of a male calling song sequence (upper) and a female song sequence (lower); rHL, lHL: sound producing stridulatory movements of right and left hind leg.

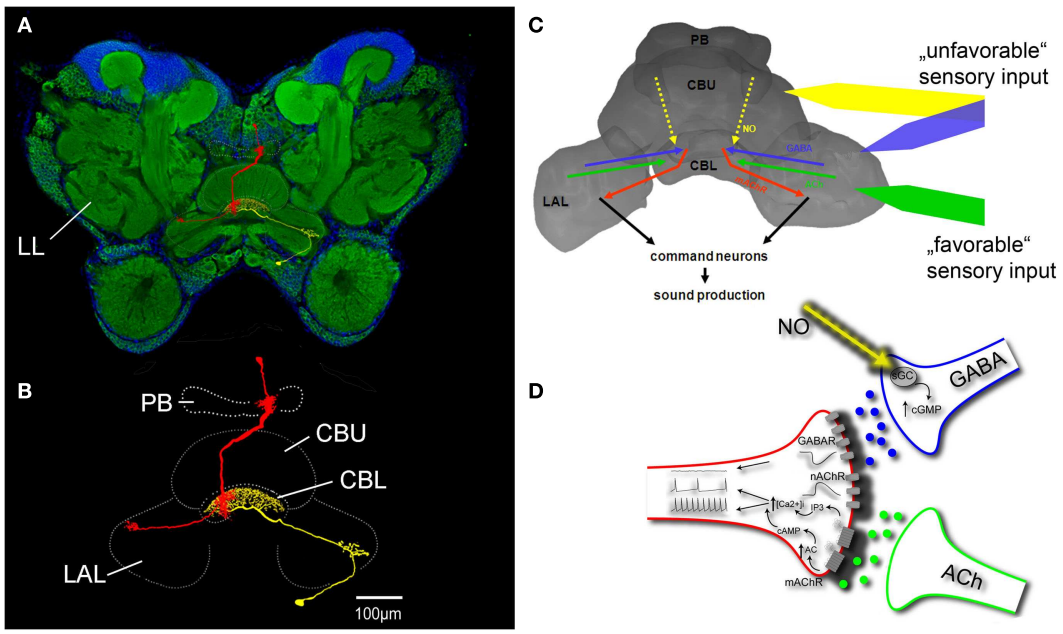

FIGURE 2 | Control of grasshopper sound production by the central complex. (A) Frontal section through a grasshopper brain labeled with the pan-neuronal marker anti-horse radish peroxidase (green fluorescence) and DAPI nuclear staining (blue fluorescence). **(B)** Innervation of central complex neuropils by one columnar (red) and one tangential neuron (yellow) with overlapping in- and output structures in the CBL. **(C)** Flow of information through the central complex related to the control of sound production. **(D)** Details of information processing in the CBL. See text for descriptions of **(C,D)**. LL lateral protocerebral neuropil containing the neural filters for sound pattern recognition; PB protocerebral bridge; CBU central body upper division; CBL central body lower division; LAL lateral accessory lobe; NO nitric oxide; ACh acetylcholine; nAChR nicotinic ACh receptor; mAChR muscarinic ACh receptor; AC adenylyl cyclase; sGC soluble guanylyl cyclase.

complex. Inhibition of chloride channel-associated receptors (e.g., $GABA_A$- and glycine-receptors) by picrotoxin (Heinrich et al., 1998), inhibition of GABA production by 3-mercapto propionic acid and inhibition of nitric oxide formation by aminoguanidine (Weinrich et al., 2008) were sufficient to release sound production by disinhibition, though the typical activity-pause structure and the clear separation of situation-specific patterns was compromised in some species. Grasshopper sound production is therefore regulated by a balance of excitatory and inhibitory input to the central complex which reflects sensory stimuli that represent favorable and unfavorable situations for reproductive behaviors (Kunst et al., 2011). Integration of promoting and suppressing information might be a universal characteristic in central nervous regions that select motor patterns (reviewed by Benjamin et al., 2010), since similar regulatory mechanisms have also been described in vertebrates, e.g., control of locomotion in mesencephalic and diencephalic locomotory regions (Takakusaki, 2008) and the selection of voluntary motor pograms by the basal ganglia (Nambu, 2009) which serve similar functions in vertebrates as the central complex in insects (Wessnitzer and Webb, 2006; Stephenson-Jones et al., 2011).

The central complex of grasshoppers (**Figure 2**) includes four interconnected subunits: the protocerebral bridge, the upper and lower divisions of the central body, and the paired noduli (Williams, 1975; Homberg, 1987). Both divisions of the central body, which are of major importance for the control of sound production, consist of individual layers intersected by 16 columns (Williams, 1975; Homberg, 1991). Layers derive from projections of groups of tangential neurons that provide input to all columns which mainly derives from the lateral accessory lobes. Columnar neurons connect the columns of the protocerebral bridge in regular ipsi- and contralateral projection patterns with those of the two central body neuropils and send information to the lateral accessory lobes, the major input/output neuropils of the central complex (Müller et al., 1997; Homberg et al., 2004). All columns include columnar neurons that express muscarinic acetylcholine receptors (mAChRs; Hoffmann et al., 2007; Kunst et al., 2011). Since no other neuron in the central complex expresses mAChRs, a subpopulation of these neurons must be directly activated when sound production is stimulated through muscarine injection into the central complex. With dendritic input regions in the lower division of the central body and synaptic terminals in the lateral accessory lobes, activity of the mAChR expressing columnar neurons seems to represent an output signal of the central complex that is sufficient (or even necessary?) to initiate sound production. It has been demonstrated that the mAChR expressing columnar neurons receive excitatory cholinergic input when grasshoppers are acoustically stimulated with songs from their own species. Male calling songs and female response songs signal high reproductive readiness and mediate strong stimulatory impact on a potential mating partner. Auditory information is first processed in the metathoracic ganglion and then relayed by ascending auditory interneurons to lateral protocerebral neuropils (LL in **Figure 2**) that include the neural filters for sound

pattern recognition (Boyan, 1983; Hedwig, 1986). A neuron that could close the loop between acoustic stimulation and production of a response song was described by Hedwig (2001). It connected the lateral neuropils with the central protocerebrum and its activation by intracellular current application initiated sound production. Pharmacological studies demonstrated that mAChR mediated excitation depends on repetitive stimulation with either acoustic stimuli (Hoffmann et al., 2007) or injections of acetylcholine (Wenzel et al., 2002). At identical stimulation sites within the central complex muscarine elicits long lasting stridulation after long latencies while ACh elicits only short lasting stridulation after short latencies, representing pure nicotinic excitatory effects (Heinrich et al., 1997). Only when the presence of ACh was prolonged through repeated injections or inhibition of acetylcholine esterase, muscarinic excitation accumulated, and eventually stimulated response songs to acoustic stimulation with conspecific song even in restrained animals (Hoffmann et al., 2007). Muscarinic excitation of mAChR expressing columnar output neurons of the central complex, elicited by prolonged exposure to sensory stimuli which favor sound production, therefore represents a basal activity upon which specific actual stimuli may add to provide sufficient excitation to initiate song production. Grasshopper song production can also be stimulated by injection of proctolin or dopamine but specific sensory input that activates these excitatory inputs has not yet been identified.

In addition to direct cholinergic excitation of columnar neurons, activation of inhibitory pathways in the central complex has a strong regulatory impact on sound production. Especially two transmitters, nitric oxide (NO) and GABA, seem to be tonically released in situations where stridulation appears inappropriate. Such an unfavorable situation for reproduction-related behavior is certainly being restrained in an experimental setup for pharmacological brain stimulation. Consequently, neither male nor female grasshoppers spontaneously respond to acoustic stimulation with songs of a potential mating partner. However, song production can be elicited through disinhibition, either by preventing NO formation with the NO-synthase inhibitor aminoguanidine (Weinrich et al., 2008) or by the chloride channel-associated receptor antagonist picrotoxin (Heinrich et al., 1998). Furthermore, systemic application of aminoguanidine increased the responsiveness of unrestrained grasshoppers to conspecific song and suppressed NO-synthase activity in the central body, suggesting that endogenous NO signaling in the central complex regulates sound production by mediating inhibition that needs to be compensated by varying amounts of specific excitation to initiate stridulation (Weinrich et al., 2008). NO mediates its inhibition via activation of soluble guanylyl cyclase and the production of cyclic GMP in its target cells (Wenzel et al., 2005). In *C. biguttulus* and other acoustically communicating grasshopper species, NO-synthase expressing neurites, that generate and release NO into central complex neuropils were exclusively located in layers II and III of the central body upper division (Wenzel et al., 2005; Kunst et al., 2011). Most of these neurites belonged to pontine neurons with their cell bodies in the pars intercerebralis and some of them were tangential neurons with somata located in the inferior median protocerebrum. In contrast, NO-responsive neurites were restricted to layer II of the lower division of the central body, overlapping with the dendrites of mAChR expressing columnar neurons and projections of GABA containing tangential cells from the inferior protocerebrum that pick up synaptic input in the lateral accessory lobes. While the columnar output neurons of the central complex were excluded as direct targets of NO signaling, essentially all neurites that accumulated cyclic guanosine monophosphate (cGMP) upon NO stimulation in the lower division were also GABA immunoreactive (Kunst et al., 2011). These immunocytochemical data were supported by pharmacological experiments in which the $GABA_A$-receptor antagonist picrotoxin prevented NO-mediated inhibition of stridulation. NO released from neurites in the central body upper division thus mediates its suppressive effects on stridulation through the activation of GABAergic terminals in the lower division that in turn inhibit mAChR expressing columnar neurons (**Figure 2**). Both, cholinergic excitation that promotes sound production and nitrergic and GABAergic inhibition that suppress sound production converge on a group of columnar output neurons of the central complex whose cumulative activity initiates stridulation and probably other reproduction-related behaviors.

In addition to its global regulation of the behavioral threshold to initiate grasshopper stridulation, GABA plays an additional role in the selective execution of song patterns associated with particular situations. Inactivation of $GABA_A$-receptor mediated inhibition in the species *Omocestus viridulus* that produces different song patterns during the process of courtship, induced irregular mixtures of normally temporarily separated song patterns (Heinrich et al., 1998). Thus GABA-mediated inhibition assures the selective production of only one pattern at a time, by lateral inhibition of descending premotor pathways that regulate the activation of different thoracic pattern generators.

"LONG-TERM" REGULATION OF GRASSHOPPER SOUND PRODUCTION BY JUVENILE HORMONE PRODUCTION IN THE CORPORA ALLATA

Reproductive readiness is characterized by specific behaviors intended to find, compare, and eventually mate with an appropriate partner of the same species and its establishment may depend on various factors. In insects, these factors include sexual maturation, exposure to stimulating signals from a potential mating partner and previous mating experience. Various studies on the regulation of reproductive readiness have been conducted on acoustically communicating grasshoppers and female responses to male calling song or male responses to female songs have been used to quantify an individual's reproductive motivation (Loher, 1966; von Helversen and von Helversen, 1983; Weinrich et al., 2008). *C. biguttulus* males maturate within 1–2 days after their imaginal molt and produce calling songs to attract females throughout their lifetime, whenever illumination and weather conditions are appropriate. After copulating with a female, male calling song activity and male responses to female songs are reduced for ∼2 days, indicating their reduced reproductive readiness for 2 days after mating (Wirmer et al., 2010). Females require post-molting maturation of ∼6–7 days ("primary rejection") before they first respond to male calling song (**Figure 3A**). If mating is prevented, this state of "active readiness" is retained throughout life, only interrupted by short periods of rejection around times of oviposition. Mating initiates

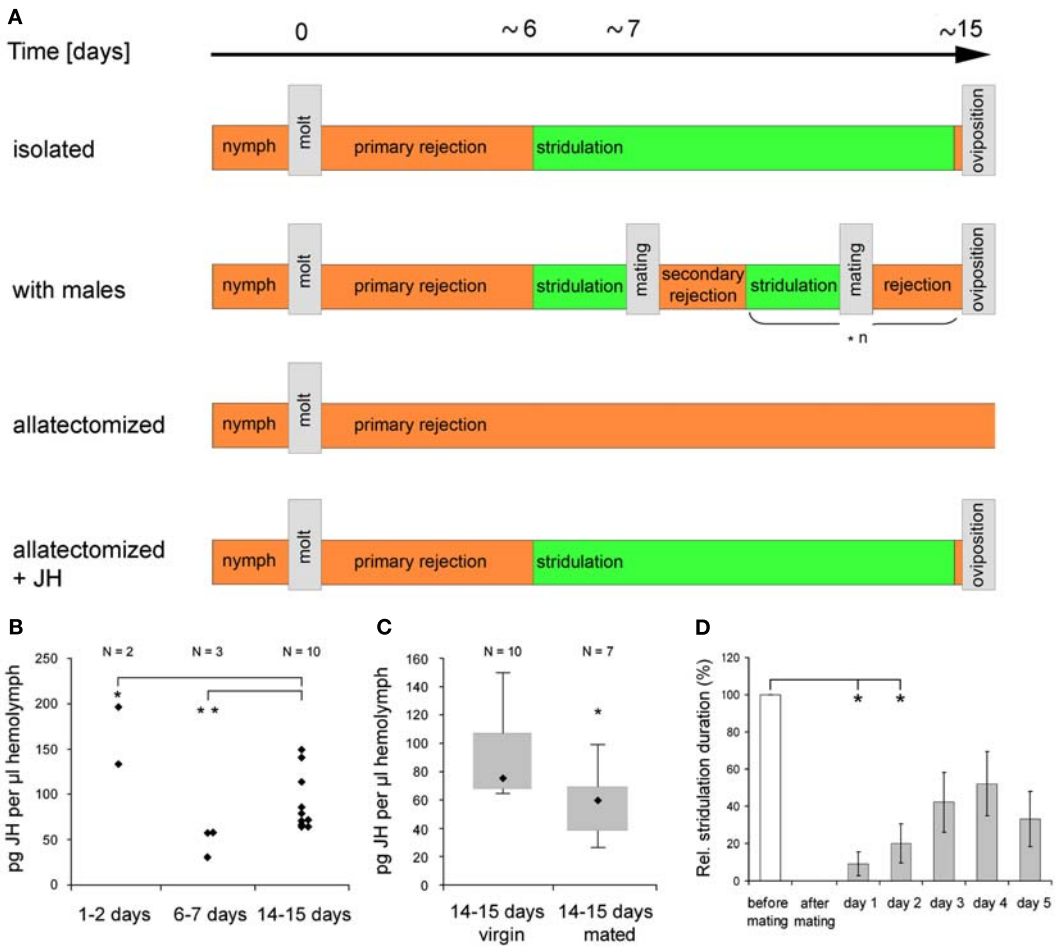

FIGURE 3 | Control of female *C. biguttulus* reproduction related sound production by juvenile hormone. (A) Reproductive states between imaginal molt and first oviposition. Allatectomy was performed within 24 h after imaginal molt. JH was diluted in acetone (23 µg JH III in 5 µl acetone) and applied to the ventral abdomen on day two after imaginal molt. Acetone alone had no effect on female reproductive behavior. **(B)** JH III titer in the hemolymph of virgin females in different reproductive states. JH concentrations were determined by radioimmunoassay following protocols of Hunnicutt et al. (1989) and Chen et al. (2007). Statistical comparison with Fisher–Pitman test. **(C)** JH III titer in the hemolymph of virgin and mated (1 day before analysis) females. Statistical comparison with non-parametric Mann–Whitney-*U*-test. **(D)** Relative duration of male song-stimulated sound production in eight females before mating, immediately after mating, and on five subsequent days. Statistical analysis with Friedman test and Wilcoxon–Wilcox test). Parts of the figure were taken from Wirmer et al. (2010).

a period of "secondary rejection" followed by re-establishment of active readiness. *C. biguttulus* show reduced responsiveness to male calling songs for up to 2 days and may pass several cycles of active readiness, copulation, and secondary rejection before their first oviposition (**Figures 3A,D**; Wirmer et al., 2010).

Establishment of female grasshopper active copulatory readiness critically depends on JH signaling. JH, a key regulator of insect development and reproduction (reviewed by Riddiford, 2008), is produced and released by parenchymal cells of the corpora allata and transported via the hemolymph to its target tissues, that include the ovaries and the nervous system (Emmerich and Hartmann, 1973; Pratt and Tobe, 1974). Direct effects of JH on reproductive biology of insect females have been documented in grasshoppers (Hartmann et al., 1994), cockroaches (Schal et al., 1997), and honeybees (Huang et al., 1991). Female grasshoppers that lack JH after surgical or chemical ablation of the corpora allata (allatectomy) never respond to male calling songs and lifelong refuse to mate with a male (Loher, 1962; own studies), indicating that JH signaling is necessary to establish reproductive readiness. A single application of exogenous JH to allatectomized *C. biguttulus* females 1 day after imaginal molt partially rescued the lack of reproductive behaviors. Females started to answer male calling songs at the typical age of 6–7 days but did not mate until first oviposition (**Figure 3A**; unpublished results). Radioimmunoassay analysis of JH hemolymph contents in different reproductive states of *C. biguttulus* females revealed high concentrations around the time of imaginal molting, low concentrations around the time of transition from primary rejection to active readiness (6–7 days) and intermediate titers in females approaching the age of their first oviposition (**Figure 3B**). These data are in agreement with studies of Hartmann et al. (1994) who determined the rates of JH synthesis in corpora allata from *Gomphocerus rufus* females

in different reproductive states and similar time courses of JH titers were also detected in the cricket *Gryllus bimaculatus* (Westerlund, 2004) and the corn borer *Diatraea grandiosella* (Shu et al., 1997) but differences have been reported in *Locusta migratoria* (Dale and Tobe, 1986) and the cockroach *Diploptera punctata* (Tobe et al., 1985). One day after mating JH titers were significantly reduced (by ∼20%) in 14–15 days old *C. biguttulus* females (**Figure 3C**) going along with reduction of responsiveness to male calling songs described above (**Figure 3D**). Effects of mating on JH synthesis were also reported from moths, flies, and cockroaches but in contrast to grasshopper females, JH production increased in these insects (Gadot et al., 1991; Moshitzky et al., 1996; Schal et al., 1997; Cusson et al., 1999).

Taking all available results together, *C. biguttulus* females seem to require a high concentration of JH at the beginning of adulthood to initiate maturation of ovaries and probably other organs involved in reproduction but require lower JH titers to activate reproductive behaviors that characterize active readiness, including sound production and copulation. Both reduction and elevation of this permissive JH concentration, probably modulated by mating and oviposition go along with lower female reproductive readiness, though a causal regulatory connection has not yet been demonstrated. Male-derived accessory gland proteins transferred to the female during copulation may also contribute to reduce sexual receptivity of females after mating (Hartmann and Loher, 1999; Ram and Wolfner, 2007).

REGULATION OF REPRODUCTION-RELATED SOUND PRODUCTION BY BRAIN AND CORPORA ALLATA

Sound production and other behaviors that promote reproduction should only be initiated when both the reproductive physiological state and the actual situation are appropriate. As described above, the same sensory signals that initiate sound production and mating in male and especially female grasshoppers during active readiness are insufficient during rejective states. Since female reproductive states are at least partly mediated by JH signaling, humoral signals may directly or indirectly modulate neural processing in brain regions that control sound production and mating.

JH production by the corpora allata is regulated by the brain. Protocerebral neurons that innervate the corpora allata via the nervi corporis allati I have been identified in the pars intercerebralis and pars lateralis regions of various insects (Moore and Loher, 1988; Virant-Doberlet et al., 1994; Vullings et al., 1999). Whether these neurons activate or inhibit JH production seems to be different among species as are the chemical signals (summarized as allatotropins and allatostatins) that mediate this regulation (Tobe and Stay, 1980; Kataoka et al., 1989; Horseman et al., 1994; Bräunig et al., 1996; Gilbert et al., 2000; Weaver and Audsley, 2009). Each corpus allatum of *C. biguttulus* is innervated by 80–90 pars intercerebralis and pars lateralis neurons and approximately half of these release RFamide from varicose terminals (**Figure 4**; Wirmer and Heinrich, 2011; unpublished results). RFamides have been demonstrated to mediate excitatory and inhibitory effects via a number of different ionotropic and metabotropic receptors (Kobayashi and Muneoka, 1989; Cazzamali and Grimmelikhuijzen, 2002). RFamide is suggested to stimulate parenchymal cells in the corpora allata since intact brain to corpora allata connections

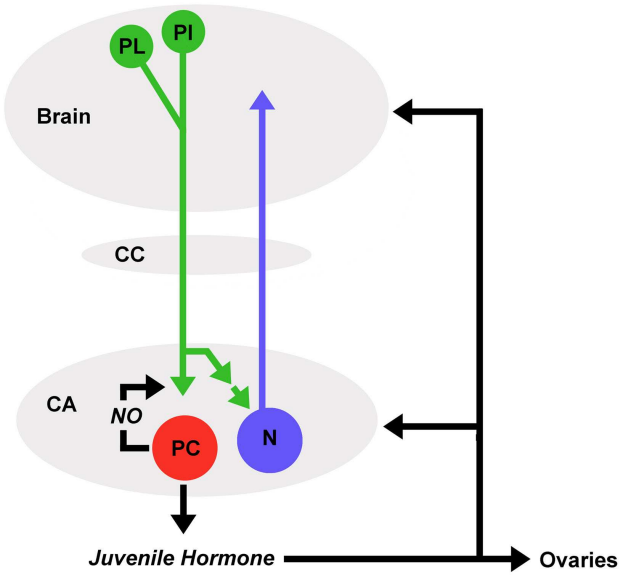

FIGURE 4 | Mutual regulatory information between brain and corpora allata. Protocerebral neurons (green) of the pars lateralis (PL) and pars intercerebralis (PI) release RFamide in the corpora allata (CA). RFamide stimulates juvenile hormone and nitric oxide (NO) production in parenchymal cells (red). As a retrograde messenger, NO induces accumulation of cGMP in RFamide releasing terminals. RFamide (directly or indirectly) stimulates action potentials in CA neurons (blue) with projections into the anterior protocerebrum. Membrane permeable juvenile hormone is released into the hemolymph and exerts pleiotropic effects on various target tissues including ovaries and central nervous system.

are required to maintain JH production in locusts (Tobe et al., 1977), oscillations of RFamide immunoreactivity correlated with changes in JH titers in locusts (Sevala et al., 1993) and RFamide stimulated JH production in some reproductive states in a cockroach (Stay et al., 2003). Prolonged courtship may actually alter the hormonal state of grasshopper females, mediating increased reproductive readiness. In species like *G. rufus*, *C. curtipennis*, females assume a state of "passive readiness" during which they do not stridulate but allow copulation after prolonged male courtship, which may last more than 1 h. A study by Riede (1983) demonstrated that exposure of *G. rufus* females to male courtship song advanced the age of first copulations compared to the start of "active readiness," suggesting that altered hormonal state may change a females behavioral response to male courtship. Whether effects like this could be mediated through activation of neurosecretory pars intercerebralis or pars lateralis neurons that modulate hormonal release from the retrocerebral complex remains to be explored.

In *C. biguttulus*, RFamide seems to stimulate both JH production and generation of NO by parenchymal cells (unpublished results). NO stimulates the formation of cyclic GMP in the terminals of RFamide releasing neurons, suggesting that it acts as a retrograde signal that modulates subsequent release of RFamide (**Figure 4**; Wirmer and Heinrich, 2011). In addition, RFamide also stimulates neurons located in the corpora allata that have recently been identified by immunocytochemistry against neuron-specific

markers in *C. biguttulus* (Wirmer and Heinrich, 2011; unpublished results) and were suggested to exist by earlier studies on other insects (McQuiston and Tobe, 1991). Anatomical studies on locusts revealed that each corpus allatum contains less than 50 neurons and at least 24 of them, that where backfilled in the same preparation from an incision into the median bundle in the protocerebrum, project into anterior portions of the brain (**Figure 4**; Wirmer et al., unpublished). While the postsynaptic targets of these neurons are still unknown, electrophysiological recordings from the nervus corporis allatum I demonstrated that their axons propagate action potentials from the corpora allata to the brain. These neurons could in principle modulate the sensory-motor processing relevant for reproductive behaviors and potentially adjust their initiation thresholds to the activity state of the JH producing corpora allata.

In addition to neuronal regulation, brain functions may also be modulated by JH or other humoral factors associated with the regulation of reproductive states. JH titers have been demonstrated to regulate JH production by the corpora allata. As one example, reduced titers resulting from ablation of one corpus allatum or enhanced titers resulting from implantation of additional corpora allata are compensated by enhanced JH production of the remaining secretory organs of the host (Tobe and Stay, 1980). In locusts, this compensatory response requires intact brain to corpora allata connections, suggesting that JH mediates its regulatory effects on corpora allata activity indirectly via effects on brain neurons (Tobe et al., 1977; Cassier, 1979). JH is a membrane permeable messenger that binds to a large number of proteins including carriers, enzymes, membrane bound receptors, and nuclear receptors that may initiate long-term changes of cellular physiology through transcriptional regulation (reviewed by Wheeler and Nijhout, 2003). Direct actions on neuronal processing and the modulation of phonotactic mate localization have been documented in female crickets (Stout et al., 1991). By altering transcription, JH reduced the activation threshold of an ascending interneuron that carries auditory information from thoracic neuropils to the lateral protocerebrum, where neural filters for sound pattern recognition are localized.

Juvenile hormone stimulates maturation, vitellogenesis, and activity of insect ovaries (Loher, 1966; Sroka and Gilbert, 1971). In a stage-specific manner, ovaries produce and secrete peptidergic signals into the hemolymph that stimulate JH release from the CA (Elliott et al., 2006) and may exert additional physiological effects on other organs.

Though some factors that regulate reproductive readiness and the initiation of components of mating behavior have been characterized in acoustically communicating grasshoppers (and some other insect species) our understanding about the interplay of endogenous maturational and exogenous situation-specific signals that converge to generate a coherent state of readiness is still rudimentary. All regulatory components, including the central complex and the corpora allata are known to contain various additional players. The central complex is known to be innervated by neurons that release a huge variety of chemical signals and only a fraction of these have been tested for a potential contribution to the regulation of reproductive behaviors. The central complex in orthopteran insects has been shown to receive pre-processed sensory information of various modalities, including acoustic (this review), visual (Vitzthum et al., 2002), and tactile (Ritzmann et al., 2008). In addition, both acoustic and chemical signals have been demonstrated to initiate and promote courtship in *Drosophila melanogaster* (reviewed by Greenspan and Ferveur, 2000). Since males of some grasshopper species include rhythmic movements of head, antennae, and mouthparts in their courtship and assume particular postures during its progress (Elsner and Huber, 1969: *G. rufus*; Vedenina et al., 2007: *C. oshei*) and highly receptive female grasshoppers may stridulate upon seeing a male (Loher and Huber, 1964) it can be assumed that species and/or gender specific visual signals modulate the progress of courtship and hence must be integrated with auditory information. One study on *G. rufus* (Elsner and Huber, 1969) indicates that female-associated optical, acoustic, and tactile stimuli promote male courtship behavior while chemical input (touching male antennae with parts of a female) had no effect on the male. Although the promoting impact of specific visual, tactile, and chemical signals on grasshopper courtship is not well investigated, it is clear that acoustic, visual, and tactile stimuli can suppress or interrupt courtship behavior, indicating that their nervous representations are relayed to the central complex.

In addition to RFamide releasing neurons, grasshopper corpora allata are innervated by protocerebral proctolin containing neurons and allatostatin containing terminals of unknown source, suggesting that the local processing of information in the corpora allata is more complex than described above. Future studies will first investigate each factor individually for its role in the regulation of reproductive readiness and the selection of state-dependent actions and subsequently determine how individual factors converge on decisive nervous structures that activate adequate motor programs.

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Decision points: the factors influencing the decision to feed in the medicinal leech

Quentin Gaudry [1] ***and William B. Kristan Jr.*** [2] *******

[1] *Department of Neurobiology, Harvard Medical School, Boston, MA, USA*
[2] *Neurobiology Section, Division of Biological Sciences, University of California San Diego, La Jolla, CA, USA*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Pavel M. Itskov, Champalimaud Foundation, Portugal
Romuald Nargeot, Universités Bordeaux, France

****Correspondence:***
William B. Kristan Jr., Neurobiology Section 0357, Division of Biological Sciences, University of California San Diego, 3119 Pacific Hall, 9500 Gilman Drive, La Jolla, CA 92093-0357, USA.
e-mail: wkristan@ucsd.edu

The decision to feed is a complex task that requires making several small independent choices. Am I hungry? Where do I look for food? Is there something better I'd rather be doing? When should I stop? With all of these questions, it is no wonder that decisions about feeding depend on several sensory modalities and that the influences of these sensory systems would be evident throughout the nervous system. The leech is uniquely well suited for studying these complicated questions due to its relatively simple nervous system, its exceptionally well-characterized behaviors and neural circuits, and the ease with which one can employ semi-intact preparations to study the link between physiology and decision-making. We will begin this review by discussing the cellular substrates that govern the decision to initiate and to terminate a bout of feeding. We will then discuss how feeding temporarily blocks competing behaviors from being expressed while the animal continues to feed. Then we will review what is currently known about how feeding affects long-term behavioral choices of the leech. Finally, we conclude with a short discussion of the advantages of the leech's decision-making circuit's design and how this design might be applicable to all decision circuits.

Keywords: behavioral choice, decision making, distributed, feeding, leech, modular, sensory gating, serotonin

INTRODUCTION

To survive and obtain the necessary energy to fuel everyday life, animals must feed. This universal drive makes feeding an ideal system for studying decision-making processes within the nervous system. The decision to feed involves key decision points such as how to locate a food source, the decision to initiate a bout of feeding, whether or not to continue feeding given competing external stimuli, and when to terminate feeding. In this review, we will consider behavioral choice as a form of decision-making. In general, behavioral choice means that an animal has more than one stimulus or behavioral state to which it may respond. Second, and most importantly, the animal responds to just one of them (Sherrington, 1906; Kovac and Davis, 1977; Davis, 1979; Everett et al., 1982; Misell et al., 1998). In the experiments described within, leeches were presented with either multiple stimuli (such as food and tactile stimulation) or the same stimulus while in different behavioral states (such as satiated or hungry). In such cases, we will describe the ensuing motor pattern, or lack thereof, as the leech's behavioral "choice." The neuronal mechanisms leading up to this choice will be described as the "decision-making process."

The nervous system of the leech is an ideal system for studying the neuronal substrates of decision-making, particularly for feeding. First, the leech nervous system is relatively small with only about 400 unique neurons reiterated in its 21 segmental ganglia (Macagno, 1980). Second, most of these neurons are identifiable from preparation to preparation, which makes studying their role in decision-making far simpler than sampling from populations of neurons. Third, there already exists a wealth of knowledge about the feeding behavior of leeches and the circuits within their nervous system (Kristan et al., 2005). Fourth, there are many species of leeches that have evolved different feeding strategies, which makes this system an attractive model for studying how the neuronal circuits governing behaviors and decision-making processes evolve within a phylogenic clade (Lent, 1973; Keyser and Lent, 1977; Baltzley et al., 2010).

In this review, we will focus mainly on the European medicinal leech, *Hirudo verbana*, with occasional comparisons to other leech species. In general, when we refer to "the leech," we mean *H. verbana* with our apologies to the hundreds of other leech species. We will first describe some of the factors underlying their sensation of hunger and the sensory cues that influence the decision to initiate and terminate a feeding bout. Next, we will describe the more complex interactions within the leech nervous system that prevent competing behaviors from being expressed during a feeding bout and how feeding affects their long-term behavioral choice. Then we will conclude with a brief discussion of the advantages of the design of this circuit in the leech and what this research has taught us about decision-making as a general phenomenon.

NEURONAL MECHANISMS AND DECISION TO INITIATE AND TERMINATE FEEDING

The first of many decision points in feeding is the decision to initiate a feeding bout. To begin feeding requires two key elements: (1) the animal must be sufficiently motivated (i.e., it must be hungry), and (2) the proper appetitive stimuli must be present. Medicinal leeches may go a year or more between bouts of feeding (Lent and Dickinson, 1988) and serotonin levels are strongly correlated with the behavioral state of the leech (Lent et al., 1991). Well-fed

or satiated leeches are typically found in deeper water and do not respond to appetitive cues such as warm objects (Dickinson and Lent, 1984). Leeches in this state have up to 28% less serotonin in their nervous system compared to hungry leeches. Removing the ingested blood from sated animals returns their serotonin levels back to levels seen in hungry leeches, and feeding behaviors resume (Lent et al., 1991). Distention not only prevents serotonin levels from returning to the levels of hungry leeches, but artificial distention also blocks 5-HT neurons from responding to appetitive stimuli as they normally do in hungry animals (Lent and Dickinson, 1987, 1988). Furthermore, injection of the toxin 5-7 D-HT depletes serotonin from leech neurons and makes hungry leeches act as though they are satiated. Soaking these toxin-treated leeches in a bath containing serotonin restores appetitive behaviors (Lent and Dickinson, 1984). These studies clearly illustrate the strong influence of serotonin on a leech's decision to initiate feeding.

A hungry leech will feed if appropriate stimuli are present. Hungry *H. verbana* use both visual (Dickinson and Lent, 1984) and mechanical (Young et al., 1981) cues from water waves to determine whether prey is present and which direction to move. Chemical cues also promote swimming during foraging behavior (Brodfuehrer et al., 2006). Once contact is made with a potential host, both thermal and chemical cues govern the decision to feed. Leeches bite with a higher frequency to test stimuli at 38°C when either tested on a hot plate covered with parafilm® wax or when exposed to a warmed feeding apparatus (Lent and Dickinson, 1984). Alternative choice assays that expose leeches to two temperatures of mammalian blood show the same temperature preference (Q. Gaudry and W. B. Kristan, unpublished observations). Along with temperature, leeches also sample the chemical composition of a potential prey using chemosensory receptors located on their dorsal lip (Elliott, 1987). Studies evaluating the chemical cues required to carry out feeding behavior to completion have revealed that only NaCl and the amino acid arginine or NaCl plus simple sugars are required (Galun and Kindler, 1966; Elliott, 1986). An interesting correlate of the decision to feed can be found as early as these primary chemosensory neurons. When appetitive stimuli are presented to the dorsal lip of the animal, an increase in neuronal firing is observed in the cephalic nerves that connect the dorsal lip to the cephalic ganglion. These action potentials likely belong to the chemosensory neurons themselves (Groome et al., 1995; Perruccio and Kleinhaus, 1996). Combining aversive chemical agents to these appetitive stimuli suppresses the chemosensory activity in the cephalic nerves (Li et al., 2001). These data suggest that the integration of appetitive and non-appetitive cues may occur as early as the periphery and that the central nervous system may not have to weigh these conflicting chemical cues against each other. While this result is surprising, a similar observation occurs in the CO_2 olfactory receptor neurons (ORNs) of the fruit fly *Drosophila melanogaster*. CO_2 is highly aversive to this fly (Jones et al., 2007). However, CO_2 is also found in ripened fruit, a favorite food of fruit flies. Extracellular recordings from the CO_2 sensitive ORNs reveals that these receptors are inhibited when CO_2 is combined with odors that co-occur in ripening fruit (Turner and Ray, 2009). Thus CO_2 behavioral aversion is inhibited in the context of feeding. The decision to escape or to feed in this fly appears to be governed, at least in part, directly at the level of the sensory receptor.

The leech must not only decide when to start feeding, but also when to stop. There are at least two distinct sensory stimuli that are known to effectively terminate ingestion in leeches. The first is a change in the chemical quality of the food being ingested. In addition to the external chemosensory receptors mentioned above, the leech also possesses receptors that are located in its gut. These serve to continuously sample the quality of food being ingested (Kornreich and Kleinhaus, 1999). Switching feeding solutions to an aversive agent (such as quinine, denatonium, or water) quickly terminates a feeding bout. The same result is observed when these chemicals are injected into the gut of a feeding leech, thus reducing the likelihood that these chemicals came into contact with the external chemosensory neurons of the dorsal lip. The second well-described stimulus that terminates feeding is distention of the leech due to the large volume of the blood meal (Lent and Dickinson, 1987). The termination of feeding by distention is likely mediated by stretch receptors located either in the gut or the body wall of the animal. Removing the blood meal of a leech through cannulation will increase the duration of ingestion near indefinitely, thus ruling out fatigue as a meaningful cue to terminate feeding (Lent and Dickinson, 1987). Additionally, distending a leech with a saline solution is sufficient to disrupt ingestion and suggests that chemical cues may also not be necessary for signaling the leech to stop feeding. The role of distention in terminating a feeding bout is well documented among other animal groups as well, particularly in the insects (Chapman and de Boer, 1995) and mollusks (Kuslansky et al., 1987).

SHORT-TERM INHIBITION OF COMPETING BEHAVIORS

The decision to feed is generally not made in the context of appetitive stimuli alone, but also in the presence of competing non-appetitive stimuli. For sanguivorous leeches, this decision is highly predictable: when a hungry sanguivorous leech detects food-related chemical cues, feeding takes precedence over all other behaviors (Gaudry et al., 2010). These animals will even ignore noxious stimuli until they obtain a full meal. Tactile stimulation of the leech normally results in a number of behaviors (Kristan et al., 1982) that are mutually exclusive with the ingestion of a blood meal. These behaviors include the locomotory behaviors, such as swimming and crawling away from the source of stimulation, or shortening, which is a rapid withdrawal of the head. Just prior to and during feeding, these behaviors are robustly inhibited (Misell et al., 1998). Furthermore, largely dissected animals and reduced preparations will display robust feeding behavior despite the trauma imposed on them during surgery (Lent and Dickinson, 1987; Wilson et al., 1996; Wilson and Kleinhaus, 2000; Gaudry and Kristan, 2009). Leeches also appear to be insensitive to aversive chemical stimuli while feeding. While a non-feeding leech will retract and pull away when exposed to denatonium or quinine (Li et al., 2001), a feeding leech will ignore these chemicals when they are presented to their external chemoreceptors located on the dorsal lip (Kornreich and Kleinhaus, 1999).

Before discussing the cellular substrates that underlie the suppression of noxious stimuli while feeding in leeches, we feel compelled to first ask, "why does the medicinal leech behave in this

manner?" From our own experiences, we would avoid harm at the cost of a single meal. For the leech, however, a meal may come along only rarely and the leech makes the most of each opportunity by consuming a huge meal that can sustain it for up to a year. In fact, such dominance of feeding over escape responses may be a common feature among obligate sanguivores that feed at a low frequency. For instance, hard ticks (*Ixodidae*) place a similar premium on feeding and mated female ticks can gain an astounding 11,000% their body weight in a single meal and wait a full year between meals (Sonnenshine, 1991). And like medicinal leeches, they are capable of ignoring tremendous physical torment including burning and exposure to alcohol to keep feeding (Needham, 1985). Sanguivorous leeches reliably consume large meals that increase their weight by more than 800%, and mechanical stimulation of these leeches while feeding does not affect the duration of a meal or the weight gained (Gaudry et al., 2010). Among different species of leeches, diet is strongly correlated with the priority of feeding (**Figure 1**). Because sanguivory and carnivory have probably evolved independently several times within the leech lineage (**Figure 1A**; Borda and Siddall, 2004), the correlation between sanguivory and behavioral choice is more likely to be due to the diet of a species rather than its place in phylogeny. Canonical correspondence analysis (CCA) was recently used to study the relationship between leech species, feeding, and behavioral choice in detail (Gaudry et al., 2010). CCA is an analytical technique that was initially developed in the field of ecology but has also proven useful for studying the relationship between stimuli, manipulations, and behavior (Cornford et al., 2006). Similar to the more popular principle component analysis (PCA), CCA allows one to see trends in large multi-dimensional data sets by reducing the dimensionality of these data and producing biplots that highlight the relationship between important variables (Braak Ter, 1986). Unlike PCA, which is most appropriately applied to continuous and monotonic data, CCA is best applied to discrete data that can vary either linearly or unimodally. Six species of leeches (three carnivorous and three sanguivorous) were tested for their responses to tactile stimuli prior feeding. All six species responded similarly: they mostly shortened to touches at the anterior end, bended their bodies in a variety of ways when touched in the middle, and locomoted (swam or crawled) when touched at the posterior end (**Figure 1B**). The responses to the same stimuli were strongly curtailed by feeding in all three sanguivorous species tested, but were not changed in the carnivorous species (**Figure 1C**). It will be of great interest in the future to determine how the nervous systems of the carnivorous and sanguivorous leeches differ to gain a better understanding of how decision-making circuits may have evolved.

So how do sanguivorous leeches block out competing stimuli while feeding? To determine how the nervous system of a sanguivorous leech prevents mechanosensory stimuli from eliciting feeding-incompatible behaviors, we used a previously described semi-intact preparation (Wilson et al., 1996) that allows intracellular recordings to be made from the central nervous system while the rest of the animal is free to behave and most importantly, feed. These experiments revealed that the excitatory postsynaptic potentials (EPSPs) at the synapses between the pressure mechanosensory neurons (P cells) that encode touch stimuli and several of their targets is reduced (**Figure 2A**), some by more than

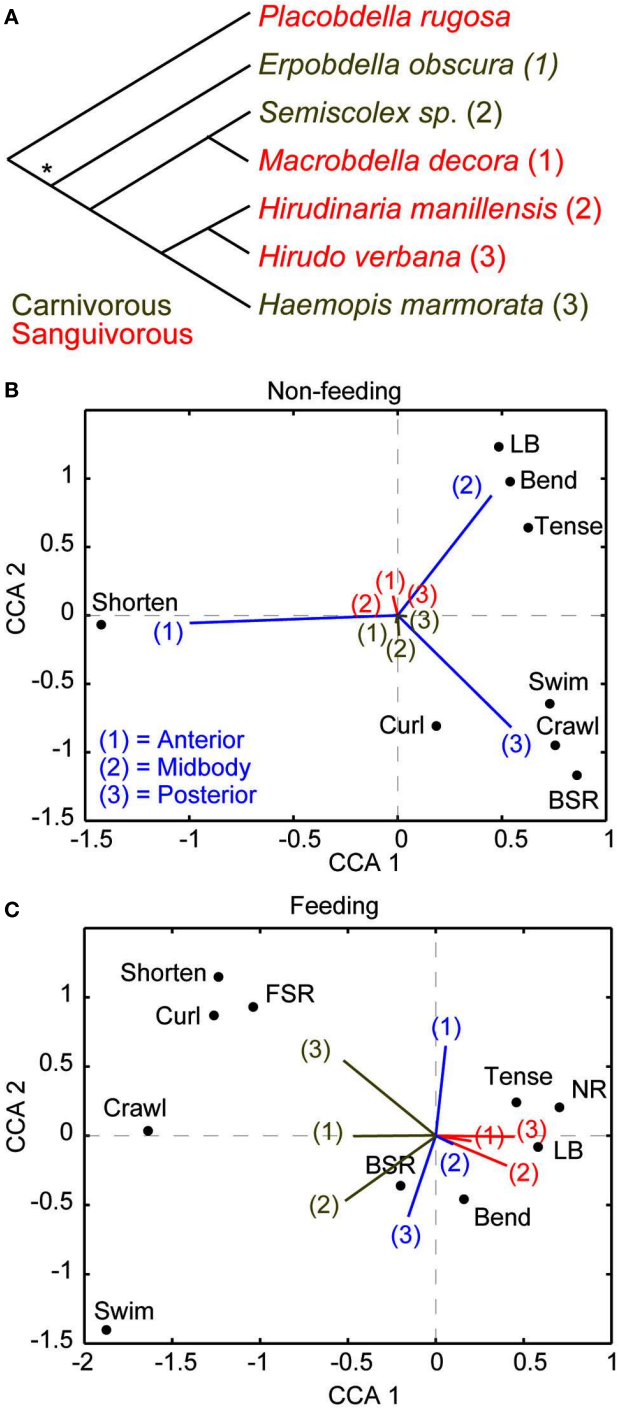

FIGURE 1 | Diet and not phylogeny determine leech behavioral choice. **(A)** The phylogenic relationship of leeches used for this study (Gaudry et al., 2010) is based on a comparison of morphological and molecular features (Borda and Siddall, 2004). An asterisk implies an ancestral state of unknown feeding preference. The most parsimonious explanation of these relationships is that the sanguivorous feeding strategy evolved three different times among these species from a carnivorous ancestor. The numbers following each species is used to reference that species in **(B,C)**. **(B)** Canonical Correspondence Analysis (CCA) biplot showing the

(Continued)

FIGURE 1 | Continued
relationship between species, stimulus location, and behavioral output in the non-feeding state. Species and stimulus location serve as predictors and the magnitude of their vectors denotes the influence they have on the raw variables (behavioral outputs). The predictor vectors point toward the behaviors they are most strongly correlated with. The clustering of all species at the middle means that all species responded to all stimuli in similar ways. Thus species has little predictive power over the resulting behavior while stimulus location is a good indicator of which behavior will be elicited in response to stimulation in the non-feeding state. Coloring and numberings as in **(A)** where brown refers to carnivorous species and red refers sanguivorous species. **(C)** CCA results for the same group of leeches as in **(B)** but during the feeding state. The carnivorous leech vectors shown in brown point toward active behaviors [shortening, swimming, crawling, and back sucker release (BSR)] whereas the sanguivorous leech vectors shown in red point in the direction of local responses [Bend, Tense, local bend (LB)] or no response (NR). The results indicate that the diet of the leeches (regardless of their phylogenetic relationship) is the best predictor of stimulus response during feeding. (More details about CCA are found in Gaudry et al., 2010).

50% (**Figure 2C**; Gaudry and Kristan, 2009). Paired pulse ratios (PPRs) are a useful tool to assess whether a change in synaptic strength has a pre-synaptic component (Schulz et al., 1995). If the synaptic depression observed during feeding is the result of a post-synaptic mechanism, such as glutamate receptor modulation, we would expect each pulse in a paired pulse protocol to diminish by the same amount. Thus the ratio of the first to second pulse would stay the same before and during feeding (regardless of the absolute value of that ratio). If the depression during feeding occurs because less neurotransmitter is released pre-synaptically, more neurotransmitter should be available for release on the second pulse compared to the pre-feeding condition. This will cause the PPR to increase. Because a decrease in EPSP amplitude is observed along with an increase in the PPR at P cell synapses, the locus of plasticity is thought to be the pre-synaptic terminals of the P cell. Although, no change is observed in the intrinsic properties of the P cells in the midbody ganglia, a hyperpolarization of ∼4 mV is observed in the P cells of the leech head brain when a synthetic feeding solution is applied to the lip of a semi-intact preparation (**Figures 2B,C**). The hyperpolarization observed in the P cells of the cephalic ganglion was absent in midbody ganglia, probably because the cephalic P cells have a much more compressed dendritic arbor and may thus be electrotonically more compact (Yau, 1976).

Additional experiments suggest that the pre-synaptic inhibition of the tactile sensory neurons is the *only* site targeted by the ingestion phase of feeding to suppress competing behaviors. We found that stimulating downstream command-like neurons during feeding can still elicit swimming – which would normally be a behavior incompatible with feeding. Cell 204 is a potent initiator of swimming (Weeks and Kristan, 1978) and is situated only two synapses downstream from the P cells in the leech swim circuit (Brodfuehrer and Friesen, 1986). Using a semi-intact preparation capable of feeding (**Figure 2D**), we injected current into this neuron which elicited bouts of swimming in the posterior end of the leech (**Figure 2E**) while the anterior portion of the animal continued to feed (Gaudry and Kristan, 2009). These bouts of swimming were characteristic of normal leech swimming including a distinctive anterior to posterior progression (**Figure 2F**). This indicates that the neuronal circuit from the command-like neurons through the central pattern generator circuit to the motor neuronal firing is not affected by the inhibition generated by feeding. This pre-synaptic inhibition of sensory input functions as a form of sensory gating that diminishes the ability of mechanosensory stimuli from eliciting incompatible behaviors such as shortening, swimming, or crawling during feeding. There are two distinct advantages to this mechanism. First, it can abolish all mechanically elicited behaviors through a single target (the P cells), and second, it leaves interneurons unmodified in case they are needed to play a role in some aspect of feeding, because many leech neurons are multifunctional (Briggman and Kristan, 2006, 2008). Interestingly, the local bend reflex which would not seem to compromise the feeding movements, is nevertheless greatly diminished during feeding (Misell et al., 1998; Gaudry et al., 2010) as a consequence of this general mechanism. This decrease in local bending may be a collateral, neutral loss of a function or it may be an indication that the local bend interneurons are used as part of some component of feeding; the resolution of these possibilities awaits further study.

The inhibition of the P cells is thought to be mediated by the release of serotonin onto the P cell axon terminals (Gaudry and Kristan, 2009). Exogenous serotonin mimics the decrease observed in EPSP amplitudes and the increase in the PPR measured in the postsynaptic targets of the P cells. The reduction in excitatory drive is also observed at the level of motor output from the isolated leech ganglion. Stimulating P cells in the isolated ganglion elicits a burst of motor activity that corresponds to a local contraction in the intact animal (Lockery and Kristan, 1990a,b) and serotonin decreases this activity. Additionally, the serotonin antagonist mianserin reversed these effects both in the reduced isolated ganglion preparation as well as in the semi-intact feeding leech (Gaudry and Kristan, 2009).

Although all serotonin containing neurons within the leech ganglion have been putatively identified (Lent and Frazer, 1977; Lent and Dickinson, 1987), the source of serotonin that mediates this pre-synaptic inhibition remains a mystery. Serotonin has been shown to work in the leech nervous system in either a hormonal manner or as a common neurotransmitter (Kristan and Nusbaum, 1982), and it is not clear which mode of action causes the inhibition of the P cell terminals. Stimulation of the neurohormonal Retzius cells, the largest serotonin neurons in the leech CNS, does not mimic the depression of P cell synapses (Q. Gaudry, personal observation), but none of the remaining serotonin cells have been tested. The source and nature of this serotonin action may be complex, because serotonin has a wide variety of effects – even contradictory ones – on leech circuits and behavior. For example, serotonin has been shown to both promote and inhibit swimming behavior. This diversity in serotonin action can in some cases be explained by whether serotonin is applied to the brain or within specific regions within the segmental ganglia (Crisp and Mesce, 2003; Calviño and Szczupak, 2008). Additionally, we do not know which sensory neurons activate these serotonergic neurons, although it is likely that the lip chemoreceptors (Elliott, 1986, 1987) are a major source because the suppression of other behaviors is observed during the exploration of a potential food item even before the leech begins to feed (Gaudry and Kristan, 2009) and when full strength artificial blood is presented at ambient

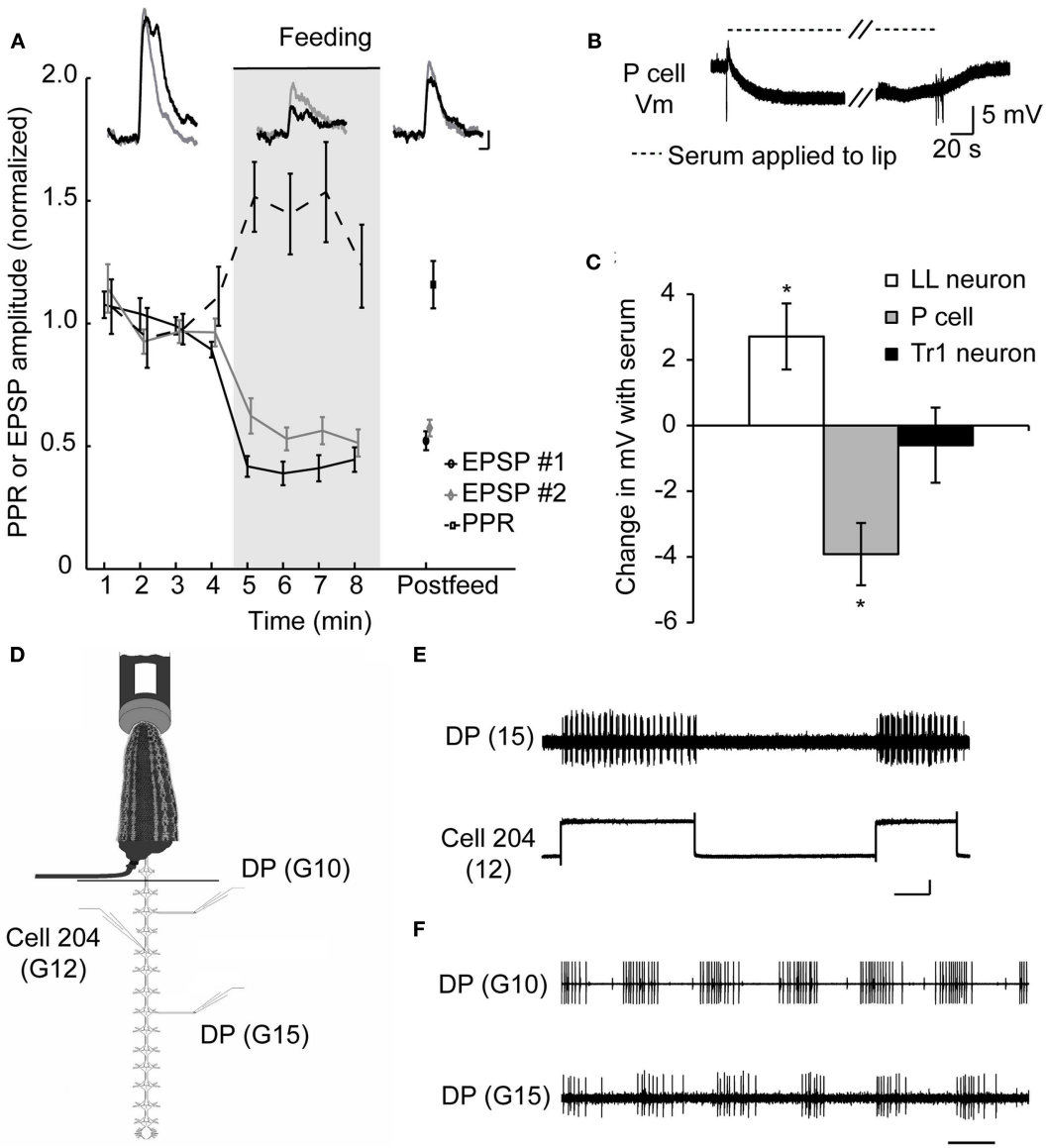

FIGURE 2 | Evidence for presynaptic inhibition of pressure mechanosensory cells. (A) Effects of feeding on EPSP amplitudes and PPR at the P cell-to-AP neuron synapse. Inserts show overlapping pairs of traces from one such experiment, sampled from pre-feeding, feeding, and post-feeding times. In each pair, the black trace is the first EPSP and the gray trace is the second EPSP in response to a P cell spike triggered 500 ms after the first one. Scale bars represent 2 mV and 50 ms (from Gaudry and Kristan, 2009). **(B)** Recording from a P cell in the leech head brain while blood serum was applied to the isolated lip of a semi-intact preparation similar to Groome et al. (1995). Diagonal dashes denote a break in the sample trace corresponding to ∼3 min. Fast vertical deflections in voltage trace are artifacts of switching the solution on at the lip of the preparation. **(C)** P cells of the cephalic or head brain are hyperpolarized when blood serum is applied. As controls we show that neurons capable of triggering swimming (Tr1) remain unaffected while the serotonergic motor effector LL cell depolarizes as described previously by others. $*p < 0.05$, $N = 5$ leeches. **(D)** A schematic diagram of semi-intact feeding preparation showing the sites of intracellular stimulation and extracellular recordings. Leeches were fed on warmed bovine serum. Dorsal posterior nerves (DP) contain the axon of a dorsal excitor motor neuron (DE-3) and spiking indicated the dorsal contraction phase of each swim cycle. **(E)** Depolarization of cell 204 with a 2-nA current elicited a swim pattern in the DP recorded in ganglion 15. Traces were recorded while the anterior end of the leech was feeding from the serum tube. The vertical scale bar represents 50 mV and the horizontal scale bar represents 5 s. Cell 204 spikes are small (∼5 mV) and are obscured by the relatively large depolarization caused by an inability to completely offset the electrodes resistance while passing large currents. **(F)** DP nerve recordings made anterior and posterior to the impaled 204 cell in **(E)**. Scale bar represents 500 ms. (Data from Gaudry and Kristan, 2009).

temperature to the lip of head-intact isolated nerve cord preparations (Brodfuehrer et al., 2006). Furthermore, chemosensory stimulation is known to activate some of the serotonergic neurons (Groome et al., 1995; Zhang et al., 2000). The neurons providing this modulation and their inputs need to be identified and studied directly.

FEEDING INDUCES LONG-TERM CHANGES IN BEHAVIOR CHOICE

The effects of feeding on the leech's behavioral choice extends far beyond the ingestion period. One clear result of feeding is the massive weight gain and distention that the animal experiences. Feeding strongly biases leeches away from swimming and toward crawling for at least 1 h following a meal (Misell et al., 1998), and unpublished data show that this period of suppression lasts for several days (S. Copado, Q. Gaudry, W. Kristan, Unpublished data). This bias toward crawling could be caused by one or more candidate cues: thermal, chemical, and distention. A series of experiments using semi-intact preparations point to stretch receptors likely located in the body wall of the animal as the likely decision point for biasing the animal away from swimming (Gaudry and Kristan, 2010). After severing the connections between the anterior brain and the rest of the nervous system, leeches will feed and their gut fills with the ingested fluid without any descending neuronal information. Stimulating the posterior end of such a leech reliably induces swimming behavior. As the feeding episode continues and the amount of body distention increases, swimming decreases. Removing the blood meal from the crop of the distended leech restores pre-feeding levels of swimming. Artificially distending semi-intact animals with a saline solution rapidly (in a few seconds) and reversibly inhibits swimming (**Figures 3A,B**). This inhibition scales logarithmically with distention (**Figure 3C**). Thus it is likely that distention, along with the inhibition of P cell synaptic release described above, help to inhibit swimming during ingestion. However, because some swimming episodes can be elicited even during distention, it is unlikely that distention is inhibiting the P cells in the same manner as ingestion. Rather, distention is thought to target the maintenance of swimming rather than its initiation. Surgical removal of either the leech body wall plus gut tissue or gut tissue alone, suggests that the stretch receptors sensitive to feeding-induced distention are likely to be located in the body wall. Probable candidates for these receptors are the previously described stretch receptors (Blackshaw and Thompson, 1988; Cang et al., 2001; Friesen and Kristan, 2007) that help entrain the leech swim central pattern generator (Blackshaw and Thompson, 1988; Cang et al., 2001; Friesen and Kristan, 2007).

Although the above-described study (Misell et al., 1998) found little to no swimming within an hour following a full blood meal, a more recent report demonstrated that swimming can be induced post-feeding in some conditions (Claflin et al., 2009). Why the difference? One possibility is procedural differences: Misell et al. stimulated electrically with a train of pulses with fixed duration and amplitude, whereas Clafin et al. used mechanical stimulation. Additionally, the weight gain reported by Claflin et al. is substantially smaller (∼500%) than the ∼900% reported in other studies (Lent, 1985; Gaudry et al., 2010); the smaller distention might allow some maintained expression of swimming. Finally, Misell et al. compared swimming and crawling probabilities, whereas Claflin et al. focused solely on swimming. Regardless of this discrepancy, Claflin et al. found that distention through feeding profoundly affected the mechanics of leech swimming (**Figure 3D**). Immediately following ingestion, the speed of swimming was reduced by 25% and did not return to pre-feeding levels until the 10th day post-feeding. This decrease in swim speed was accompanied by decreases in the cycle frequency and the stride length (defined as the distance traveled in one swim cycle) of a swim cycle. Together all the data obtained from recently fed leeches suggests that swimming performance is negatively altered for an extended duration, biasing the leech's behavior toward crawling rather than swimming.

Feeding affects not only locomotion in the leech but also the animal's temperature preferences (Petersen et al., 2011). Prior to feeding, leeches acclimated to 21°C will settle into cooler waters below 15°C when placed in a temperature gradient (**Figure 3E**). Feeding shifts the leeches' preference toward warming temperatures up to 24°C 1 day after feeding and elevated temperature preferences persist for up to 10 days. This phenomenon, termed post-prandial thermophily, is thought to aid animals in the digestion of their meal and has been extensively studied in reptiles (Sievert et al., 2005; Tsai and Tu, 2005; Bontrager et al., 2006; Stuginski et al., 2011). The study by Peterson et al. is likely the first to report such behavior in an invertebrate and it would be highly interesting to see if other obligate sanguivores such as the tick (*Ixodidae*) described above show similar behavior.

CONCLUSION

Decades of research on the feeding behavior of the medicinal leech have revealed the complex interactions between neuromodulators, sensory receptors, and the downstream targets that influence how the medicinal leech controls feeding behavior (**Figure 4A**). Why are so many different mechanisms used just to perform one behavioral act? The research described in this review clearly illustrates just how complex decision-making processes are and how even the most mundane task requires several "check points" to ensure that the proper behavior is being performed and that competing behaviors are blocked out (**Figure 4B**). First, the right cues need to be detected. The leech relies on its keen thermal and chemoreception for this. Appetitive stimuli elicit feeding behaviors and aversive stimuli do not. However, once feeding has initiated, the leech now relies on a second check point to ensure that it has not made a mistake. These are the internal chemoreceptors located in its gut. This theme of multiple check points and circuits that can be recruited independently occurs throughout the decision to feed. During ingestion chemoreceptors drive serotonergic neurons that ultimately inhibit P cells and mechanosensory input into the leech ganglion. This prevents the initiation of behaviors like swimming. As the leech ingests blood, distention activates stretch receptors in the body wall decrease activity in the circuitry that maintains swimming, presumably in the system that activates the central pattern generator (Gaudry and Kristan, 2010). This design allows the nervous system to shut down all competing mechanosensory behaviors while the leech is feeding and allows most behaviors to come back online post-ingestion. However, because distention-mediated suppression of swimming can be recruited independently of ingestion, swimming remains inhibited long after the feeding bout has terminated.

How is decision-making in the leech similar to what is observed in mammalian nervous systems? Studying how a leech chooses to feed rather than respond to mechanosensory stimuli has revealed three majors principles that are also found in mammalian systems;

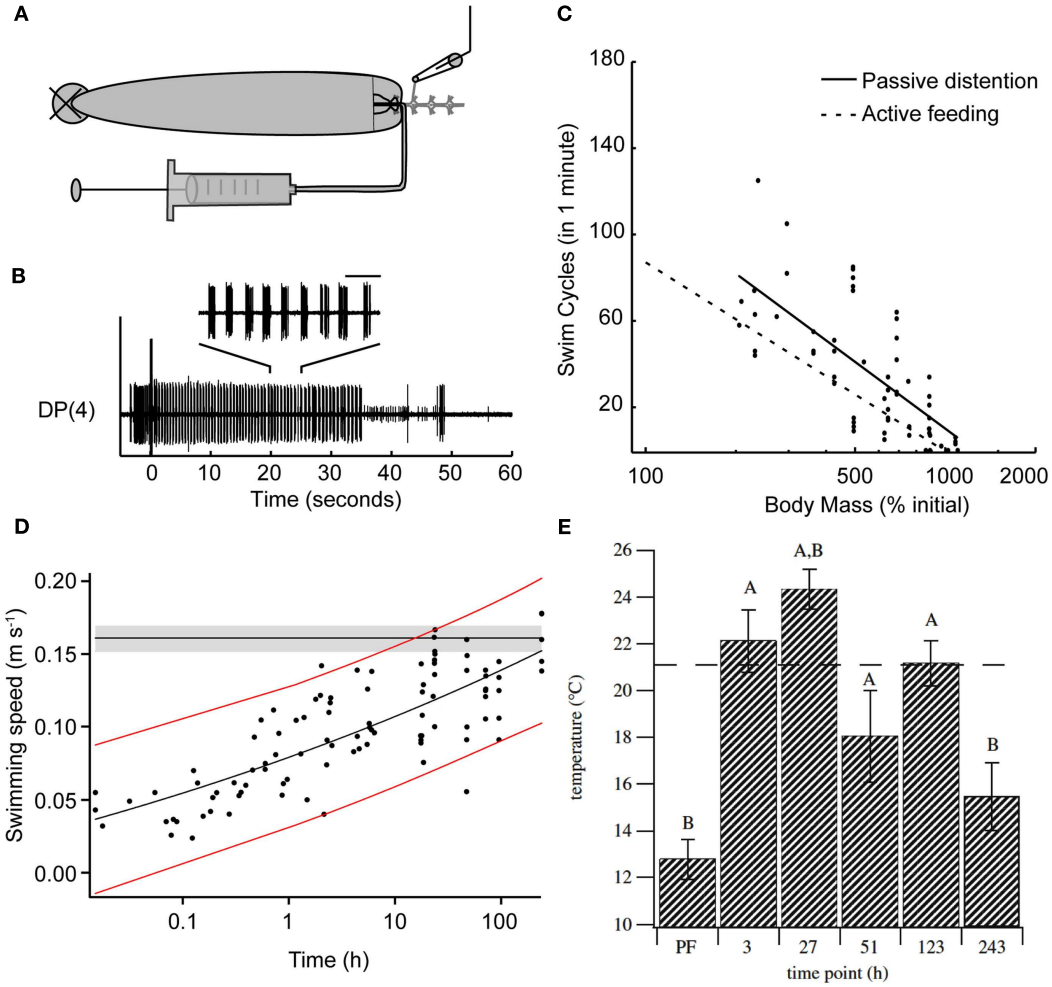

FIGURE 3 | Feeding has long term effects on other leech behaviors. (A) Schematic diagram of the semi-intact preparation used to test the impact of distention on swimming. The nerve cord was severed between ganglia 2 and 3, ganglia 3 through 5 were dissected free of the body, and extracellular recordings were made from the DP nerves of ganglion 3 or 4. Saline solution was injected via a syringe into the gut to vary the amount of distention in the intact part of the animal. **(B)** Sample trace of a DP nerve recording showing the motor neuron bursts that define swimming. The horizontal bar above inset trace corresponds to 1 s. Between each pair of bursts, the intact portion of the leech swam one complete cycle. The large stimulus artifact at time zero shows when we stimulated the body wall electrically. Motor activity that precedes the stimulus is from contact made from the stimulating electrode onto the body wall before the electrical stimulus was delivered. The inset shows an expanded view of the swim bursts between 20 and 25 s within the swim episode. **(C)** The effect of induced distention on the number of swim cycles observed within 1 min of stimulation. The *x*-axis is a logarithmic scale because this relationship appeared to be exponential. The black line is the linear regression for these data points. The dashed gray line shows best fit derived from intact active feeding preparations. [**(AC)** from Gaudry and Kristan, 2010.] **(D)** Swimming speed measured following a bout of feeding. Leeches were fed and then stimulated to swim. The speed of each swim episode was calculated and leeches were tested for up to 10 days following feeding. Red lines represent the 95% confidence interval for post-feeding data. The horizontal black line and gray shaded area show the mean pre-feeding values and 95% confidence interval of the mean. (Modified from Claflin et al., 2009.) **(E)** Preferred temperature of leeches before and up to 10 days following feeding. Leeches were acclimated to 21°C, fed, and then tested on subsequent days. The dashed line indicates the acclimation temperature (T_a). **(A)** is significantly different from the pre-feeding (PF) preferred temperature (ANCOVA, planned contrasts, Dunnett's procedure, $p < 0.05$; $n = 7$ for PF, 3, 27 and 51 h, $n = 6$ for 123 and 243 h); **(B)** is significantly different from T_a (one-sample, two-tailed, *t*-test, $p < 0.05$ after applying Dunnett's correction). Error bars indicate 1 SEM. (Data from Petersen et al., 2011.)

sensory gating of information, distributed targets of decision circuits, and decision modules that can be recruited independently across tasks.

SENSORY GATING

The mechanism used by a feeding leech to turn off all mechanosensory-induced behaviors (by serotonin-mediated pre-synaptic inhibition of the mechanosensory afferent terminals) is also found during modulation of pain in the mammalian nervous system. All pain afferents that enter the spinal cord are pre-synaptically inhibited by 5-HT and norepinephrine (Yoshimura and Furue, 2006) and this analgesic effect is prolonged by the action of endogenous opioids in the same pre-synaptic terminals, under a variety of behavioral conditions (Fields, 2007). For

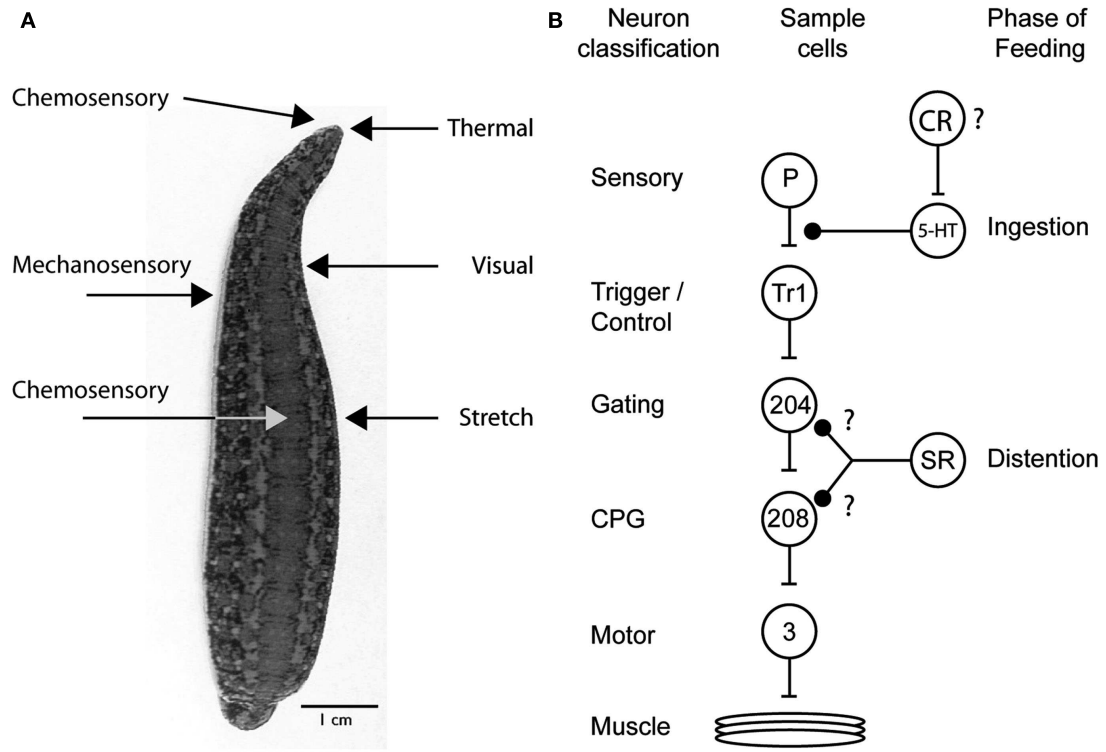

FIGURE 4 | ensory receptors and targets involved in the leech's decision to feed. (A) Sensory receptors implicated in feeding based on behavioral experimentation. Chemosensory and thermal receptors on the dorsal lip are used to determine whether to attempt to feed on a potential food source. Additional chemosensory receptors sample the food in the gut and determine whether feeding will continue or cease. Visual and mechanosensory receptors located in the body wall allow the leech to orient into water waves to find their point of origin and thus likely prey. Stretch receptors in the gut of the leech serve to terminate feeding once a full meal has been ingested. **(B)** Diagram summarizing the multiple ways that leech feeding is known to inhibit the swimming circuit. The circles represent cell populations; the letters and numbers inside the circles indicate one identified neuron from that population type. The lines ending in bars represent excitatory connections, and those ending in solid black circles represent inhibitory connections. The diagram shows the excitatory, feedforward nature of the circuit but does not show the inhibitory interactions among the CPG neurons and between particular motor neurons. The inhibition from ingestion arises from an unknown source, probably chemical sensory pathways; it inhibits the P cell terminals via an unidentified serotonergic neuron. The actions of distention likely originate from stretch receptors in the body wall and target either the gating neurons or CPG neurons. The inhibition of cell 204 is speculative but consistent with an increase in swim period and a cessation of swimming behavior. Because leech stretch receptors hyperpolarize during stretch, the excitation of cell 208 may reflect the removal of inhibition rather than direct excitation. The swim circuit connections have been identified previously (Kristan et al., 2005). P, pressure mechanosensory P cell; Tr1, trigger neuron 1; 204, gating neuron 204; 208, CPG neuron 208; 3, dorsal longitudinal muscle excitatory motor neuron 3; SR, stretch receptors; CR, chemosensory receptor; speculated to encode distention; ?, potential connections.

instance, in mice, just the presence of a cat evokes an analgesic effect (Kavaliers and Colwell, 1991). In general, when a mammal is performing a biologically important behavior (e.g., hiding, fighting, copulating, feeding), it often completes that behavior while ignoring stimuli that are painful or even injurious (Fields, 2007). This "gating out" of painful inputs is a mechanism for deciding "do not respond" to a sensory stimulation. The greater complexity in the mammal (i.e., three transmitters to "gate out" the pain, rather than a single one in the leech) may reflect a wider diversity of behaviors that modulate sensory inputs in the mammal, or it may mean that there will be additional modulatory substances and pathways to be found in the leech nervous system. In addition, this gating mechanism is not unique to mechanosensory inputs: similar examples of sensory gating have been found in the auditory (Krause et al., 2003) and olfactory (Murakami et al., 2005) systems of mammals.

DISTRIBUTED TARGETS

In both leeches and vertebrates, decision-making is distributed across various regions of their brain. In mammalian pain modulation, for instance, the μ-opioid receptor responsible for pain suppression is expressed at every known supraspinal component of the pain modulating pathway, including the insular cortex, amygdala, hypothalamus, periaqueductal gray, dorsolateral pontine tegmentum, rostral ventromedial medulla, and the spinal cord dorsal horn (Fields, 2004). Thus pain is likely to be inhibited at several loci, analogous to how swimming is inhibited by satiety signals at multiple points in the leech.

DECISION MODULES

Like those in the leech, vertebrate decision-making circuits are modularized, with particular tasks performed by different brain regions that can be recruited independently. For instance, when a

monkey compares two successive vibrating tactile stimuli, its brain encodes the sensation, stores the information, compares the two stimuli, and reports the decision. This complex series of actions are performed by different circuits for each component (Romo and Salinas, 2003): primary somatosensory cortex (S1) encodes the sensory stimuli; the prefrontal cortex (PFC) and secondary somatosensory cortex (S2) hold the signal in working-memory; at least part of the comparison between the two stimuli occurs in S2; and, finally, motor movements are initiated in the primary motor cortex (M1). The components of this highly distributed decision process can be recruited for other tasks; for example, the PFC is also used in making visual discriminations (Miller et al., 1996; Romo et al., 1999).

The similarities between decision-making circuits in leeches and mammals demonstrates the general usefulness of these broad concepts and illustrates how highly evolved invertebrate and vertebrate brains can use similar mechanisms to perform similar tasks.

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Bursting neurons and ultrasound avoidance in crickets

Gary Marsat [1,2] **** and Gerald S. Pollack*** [3]

[1] *Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada*
[2] *Center for Neural Dynamics, University of Ottawa, Ottawa, ON, Canada*
[3] *Department of Biology, McGill University, Montreal, QC, Canada*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Philippe N. Tobler, University of Zurich, Switzerland
Shunsuke Kobayashi, Fukushima Medical University, Japan

****Correspondence:***
Gary Marsat, Department of Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Canada, K1H8M5.
e-mail: gmarsat@gmail.com

Decision making in invertebrates often relies on simple neural circuits composed of only a few identified neurons. The relative simplicity of these circuits makes it possible to identify the key computation and neural properties underlying decisions. In this review, we summarize recent research on the neural basis of ultrasound avoidance in crickets, a response that allows escape from echolocating bats. The key neural property shaping behavioral output is high-frequency bursting of an identified interneuron, AN2, which carries information about ultrasound stimuli from receptor neurons to the brain. AN2's spike train consists of clusters of spikes – bursts – that may be interspersed with isolated, non-burst spikes. AN2 firing is necessary and sufficient to trigger avoidance steering but only high-rate firing, such as occurs in bursts, evokes this response. AN2 bursts are therefore at the core of the computation involved in deciding whether or not to steer away from ultrasound. Bursts in AN2 are triggered by synaptic input from nearly synchronous bursts in ultrasound receptors. Thus the population response at the very first stage of sensory processing – the auditory receptor – already differentiates the features of the stimulus that will trigger a behavioral response from those that will not. Adaptation, both intrinsic to AN2 and within ultrasound receptors, scales the burst-generating features according to the stimulus statistics, thus filtering out background noise and ensuring that bursts occur selectively in response to salient peaks in ultrasound intensity. Furthermore AN2's sensitivity to ultrasound varies adaptively with predation pressure, through both developmental and evolutionary mechanisms. We discuss how this key relationship between bursting and the triggering of avoidance behavior is also observed in other invertebrate systems such as the avoidance of looming visual stimuli in locusts or heat avoidance in beetles.

Keywords: burst, feature extraction, insect flight, phonotaxis, predator avoidance, sensory coding

BURSTING AS A NEURAL CODE

Behavior, including decision making, is an inherently temporal process so it goes without saying that the underlying neural activity is also temporally patterned. The study of the activity patterns of neurons has revealed clear links between spiking patterns and functions of neurons (Gerstner et al., 1997; Rieke, 1997; Decharms and Zador, 2000). As one of the most easily recognizable spiking patterns, bursting has been suggested to play a crucial role in many systems (Connors and Gutnick, 1990; Gabbiani et al., 1996; Martinez-Conde et al., 2002; Marsat and Pollack, 2006; Schwartz et al., 2007a; Lin and Nicolelis, 2008; Marsat et al., 2009). Bursts are clusters of two or more spikes occurring in quick succession separated from other spikes by longer inter-spike intervals (ISI). Several techniques can be used to determine objectively if a cell is bursting and which spikes are parts of bursts (Cocatre-Zilgien and Delcomyn, 1992; Bastian and Nguyenkim, 2001). Such analyses are particularly important to differentiate cells that are simply firing at high rates or responses with clustered spikes caused by a pulsatile stimulus from truly bursting cells in the narrow sense of the term. The simplest methods, based on ISIs histograms or spike-train autocorrelation functions, identify the range of ISIs typical of bursts (**Figure 1**). As shown in **Figure 1**, bursting neurons have specific spiking patterns that reflect the presence of a non-linear transformation between input and output (Chacron et al., 2004). We will not discuss the cellular mechanisms underlying bursting as several reviews are available on the subject (Krahe and Gabbiani, 2004; Izhikevich, 2006, 2007).

Studying spiking patterns became crucial once it was realized that information was contained not only in the mean firing rate of a neuron but also in the detailed temporal structure of its spike train (Gerstner et al., 1997; Rieke, 1997; Eggermont, 1998; Borst and Theunissen, 1999). The instantaneous firing rate of a neuron can sometimes be linearly related to the input signal but the spiking pattern of bursting neurons relates non-linearly to the input signal (Chacron et al., 2004; Marsat and Pollack, 2004; Lesica et al., 2006). Studies in several sensory systems showed that bursting neurons act as feature detectors, where the occurrence of specific patterns of stimulation results in bursts (Gabbiani et al., 1996; Lesica et al., 2006; Marsat and Pollack, 2006). Sensory bursts are often described as units of neuronal information that exhibit several advantages over single spikes (i.e., spikes that are not part of a burst) from the point of view of information coding and neural dynamics (see last section).

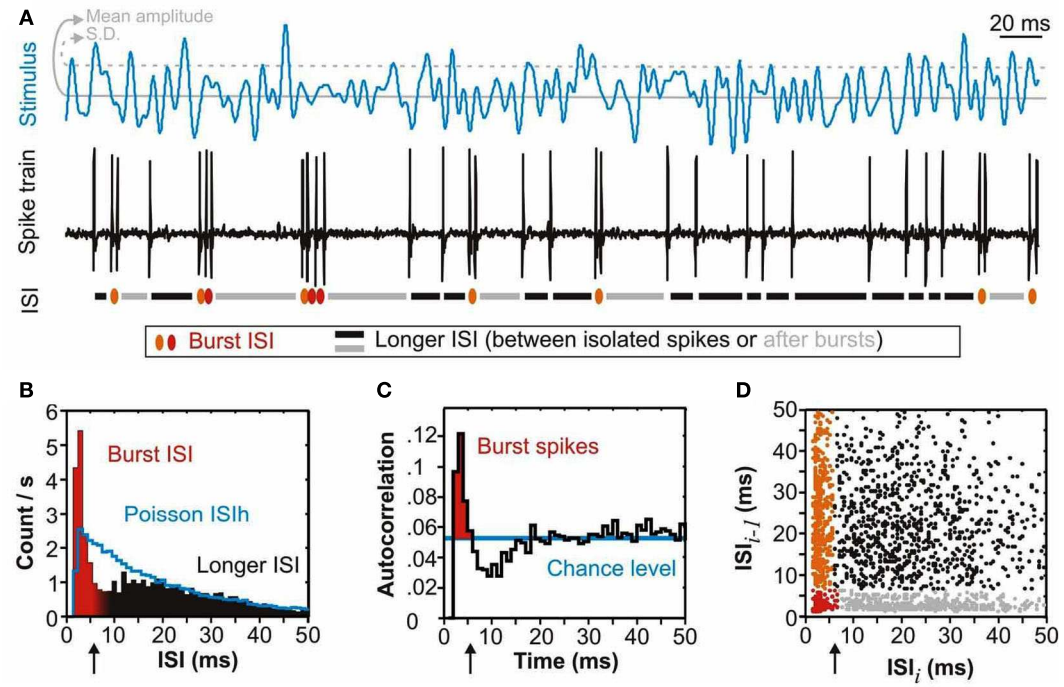

FIGURE 1 | Bursting neural responses. Bursting properties of a neuron can be revealed by stimulating it with a Gaussian input and characterizing its output. We show here an example of a response of the AN2 neuron of crickets which displays a characteristic bursting response to ultrasound stimuli. The lower panels are calculated from 45 s of recording; arrows indicate the threshold of the intra-burst-ISI. **(A)** Stimulus and response. The stimulus is an ultrasound tone (30 kHz) randomly modulated in amplitude. We display here only the amplitude modulation envelope: a low-pass (<200 Hz) filtered Gaussian with a mean amplitude of 90 dB SPL and an SD of 5 dB. The neuron responds with bursts of spikes to the largest peaks in ultrasound amplitude. The short inter-spike intervals (ISI) of spikes within bursts can clearly be identified (red). The ISI distribution is quantified in **(BD)** . **(B)** ISI distribution histogram (ISIh). The ISIhs of bursting neurons have a characteristic bimodal distribution with a peak (shown in red) at short intervals – the intervals of spikes within bursts – and broad tail of longer intervals (black). The bimodal nature of the ISIh – the output – highlights the non-linearity of the input-output transformation since the input – the stimulus – has a Gaussian distribution. A linear transformation would transform a Gaussian input into a Poisson point process. The ISIh of a model Poisson neuron with identical mean firing rate and refractory period is shown in blue. **(C)** Autocorrelation function. The autocorrelation function gives the probability of having a spike at different time intervals following each spike in the response. A random process would lead to a probability per bin of (Firing rate) × (bin width), in this case 52 Hz × 0.001 s, for all intervals (blue line). The peak at short intervals (red) quantifies the higher-than-chance probability of having spikes separated by short intervals in bursting neurons. **(D)** ISI return map. The return map plots the ISI that precedes each spike as a function of the ISI that follows it. Several clusters can be observed: a cluster along the *y*-axis consisting of the first ISIs of each burst (orange), in the bottom left corner the subsequent intra-burst ISIs (red), a cluster along the *x*-axis for the ISIs that follow bursts (gray) and a more scattered cluster at longer intervals for ISIs between isolated spikes (black). The ISI return map is a way of visually characterizing the spike-train structure and revealing the bursting tendency of the neuron, as non-bursting neurons will not display clusters along the axes at short intervals.

Despite numerous studies on bursting in vertebrate and invertebrate sensory systems and the clear understanding of the type of information carried by bursts to higher brain centers, it is only recently that the links between bursts, the information they carry, and behavioral output were firmly established. We review below recent findings about bursting and ultrasound avoidance in crickets that show how the bursting properties of a few sensory neurons determine the behavioral output.

ULTRASOUND AVOIDANCE IN CRICKETS

Sound perception and production in crickets co-evolved as a communication tool (Stumpner and Von Helversen, 2001). Male crickets produce songs with relatively low sound frequencies (3–6 kHz) to communicate with conspecifics. Crickets are also sensitive to ultrasound, which allows detection of hunting bats. Ultrasound avoidance is widespread among nocturnally flying insects (Hoy et al., 1989; Hoy, 1994). In crickets, the use of hearing for detecting insectivorous bats is thought to have evolved more recently than conspecific communication; the pre-existing hearing range was most probably widened to encompass the ultrasound of echolocating bats (Hoy, 1992; Yager, 1999). Ultrasound stimuli trigger a stereotyped, short-latency (30–40 ms), reflex-like steering response in tethered flying crickets that, in free flight, would direct them away from the sound source (Moiseff et al., 1978; Nolen and Hoy, 1986). Echolocating bats produce sound pulses that vary in duration (1 to >20 ms) and repetition rates (1–200 Hz) depending on the species of bat and the task they perform (for reviews see Schnitzler and Kalko, 2001; Schnitzler et al., 2003). Typically, bats will increase the repetition rate and decrease the sound-pulse duration when they require better temporal resolution in their auditory representation of space. For example, while searching for insects in open spaces, the bats emit pulses at low rates (a few Hertz) but increase the rate as they approach an insect for capture. In the terminal phases of the bat-insect pursuit, pulses are

often only 3–5 ms apart. The crickets must therefore be sensitive to ultrasound stimuli with a wide variety of temporal patterns, and experiments with tethered crickets show that this is indeed the case (Pollack and Elfeghaly, 1993).

Bats and insects are engaged in an evolutionary "arms race" of auditory sensitivity, in which each combatant would benefit from long-distance detection of the other. Bat echolocation is a relatively short-range modality; although echolocation calls can be very intense as they leave the bat (∼120 dB SPL; Surlykke and Kalko, 2008), the amplitude of returning echoes is limited by the small reflective surface of an insect's body, and by frictional loss to the air. As a result, many bats can detect their prey at an average distance of only ∼5–10 m depending on the species of bat and size of the prey (Kick, 1982; Holderied and Von Helversen, 2003; Surlykke and Kalko, 2008). By contrast, ultrasound sensitivity of crickets is such that they should be able to detect a bat at a distance of 10–18 m (Nolen and Hoy, 1986). The best strategy for an insect that detects a distant bat, then, is to steer away from the sound source to escape detection, and indeed that is what both moths and crickets do in response to ultrasound stimuli of low-to-moderate intensity (Hoy, 1994). Once the bat detects its potential prey, however, a chase may ensue. Video recordings show that such "dog fights" between bats and beetles can last for many seconds, during which the beetle continues to exhibit evasive maneuvers (Simmons, 2005). The neural and behavioral responses during close range interactions are not well defined. We know that, in some insects, very loud sound pulses (>95 dB SPL for crickets) will cause a different kind of evasive maneuver that is non-directional, a strategy that may serve to prevent the bat, which can out-fly the insect, from predicting the trajectory of its prey (Roeder, 1967; Nolen and Hoy, 1986). However, considering that bats typically reduce the intensity of their vocalization as they approach their prey (Schnitzler and Kalko, 2001; Fullard et al., 2003; Schnitzler et al., 2003), it is unknown how commonly these evasive maneuvers are triggered. Also, experiments with playbacks of recorded echolocation pulses to crickets and moths (Fullard et al., 2003, 2005) indicate that the temporal pattern and the frequency of the vocalizations of some bats during the final attack phase might not stimulate the neurons very strongly. These experiments however were done in a fixed spatial configuration. The spatial dynamics of a bat-insect interaction can obviously cause large modulations in the intensity of ultrasound as it reaches each ear beyond those present in the pulse train that the bat produces. The consequence of this spatial dynamic on sensory processing remains unexplored and would be an interesting subject for future studies.

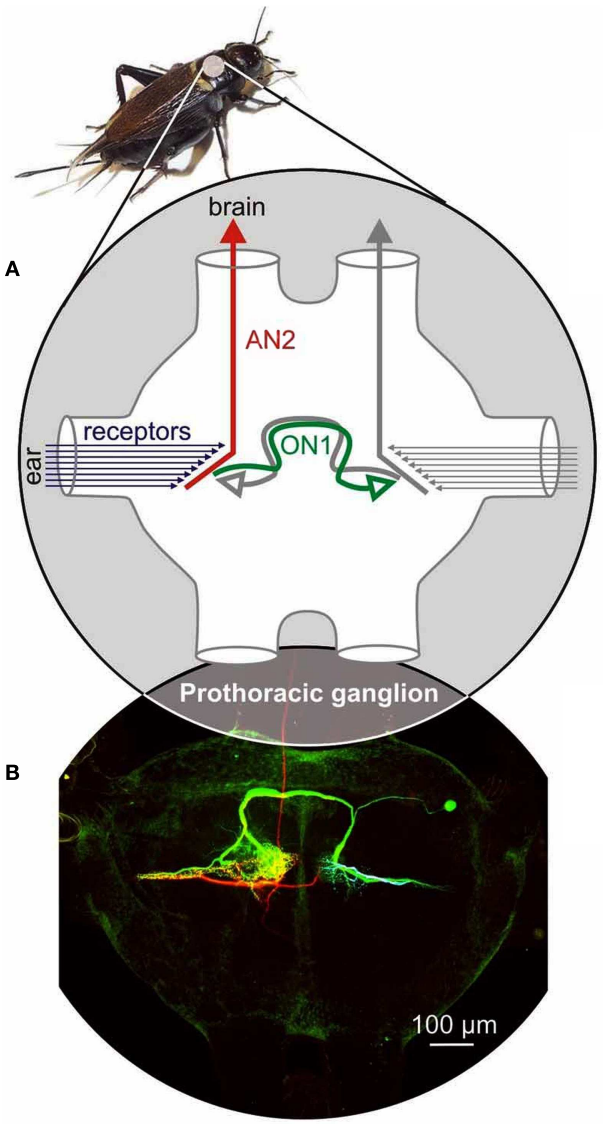

FIGURE 2 | Auditory neurons of the prothorax of crickets. (A) Auditory receptors project from the ears located on the tibiae of the forelegs to the prothoracic ganglion. A population of approximately 15 ultrasound receptors on each side provides inputs to the Ascending Neuron 2 (AN2) which carries the information to the brain, and to the Omega Neuron 1 (ON1), which inhibits the contralateral interneurons (both AN2 and ON1). **(B)** Morphologies of AN2 (red), ON1 (green), and an ultrasound receptor (blue) filled with fluorescent dyes. Note that while the AN2 and ON1 neurons were imaged together, the receptor has been superimposed *post hoc*.

ULTRASOUND CODING IN CRICKETS

The cricket's ears are located on the tibiae of the fore legs. Auditory information is carried from the ear to the prothoracic ganglion by auditory receptors, and is then relayed to the brain by ascending interneurons (**Figure 2**). The population of about 60–70 auditory receptors is composed of distinct types of cells based on their anatomy and tuning to sound frequency (Imaizumi and Pollack, 1999, 2001, 2005). About one-fourth of the receptors respond most strongly to high frequencies, including ultrasound. The remaining three-fourth are tuned to the low frequencies used during conspecific communication. The tuning of receptors to either high or low frequencies forms the basis for categorical perception of sound frequency. This was demonstrated by using an habituation-dishabituation paradigm to show that stimuli with various carrier frequencies were categorized either as attractive or repulsive, with a sharp transition between the two at around 16 kHz (Wyttenbach et al., 1996). At this transition frequency, responses depended on the stimulus temporal pattern; non-cricket-like (2 pulses/s) stimuli consistently elicited avoidance steering, but a calling-song

model with 15 kHz carrier frequency typically elicited biphasic responses, in which initial steering away from the sound was replaced after a few seconds by steering toward the sound, suggesting inhibitory interactions between song-recognition and predator-avoidance systems (Pollack et al., 1984).

Auditory information is processed initially by bilaterally paired, identified interneurons that receive monaural excitatory inputs from receptors (Hennig, 1988; Hardt and Watson, 1994). Two well studied and particularly important bilaterally paired neurons for the processing of ultrasound are Ascending Neuron 2 (AN2) and Omega Neuron 1 (ON1; **Figure 2**). AN2 is the main conduit by which information about ultrasound reaches the brain, and can thus be seen as a bottleneck in the ultrasound processing pathway. ON1 is a local interneuron; it receives input from the receptors of one ear and provides contralateral inhibition to several interneurons that receive input from the other ear, including AN2 and the contralateral ON1 (Selverston et al., 1985), thereby enhancing bilateral differences used to localize sound.

Several high-frequency-sensitive brain neurons have been described, some of which, based on their anatomical and physiological characteristics, are candidates for receiving input directly from AN2 (Boyan, 1980, 1981; Schildberger, 1984; Brodfuehrer and Hoy, 1990); however paired simultaneous recordings from AN2 and its potential targets have not yet been performed. Such experiments are necessary not only to confirm putative connectivity, but also to study the effects of AN2 bursts on its targets. One type of local brain neuron, which arborizes on both sides of the brain, is excited by ipsilateral sound pulses and inhibited by contralateral pulses and thus could, in principle, act as a bilateral comparator. Such a bilateral comparison could be involved in processing the spatial information of the sound necessary to direct behavior away from the source but nothing is known about this process.

Another group of brain neurons, which are probably at least two synapses removed from AN2, carry the output of brain circuits to lower ganglia where motor responses are generated (Boyan and Williams, 1981; Brodfuhrer and Hoy, 1989; Staudacher, 2001). The most interesting of these, with respect to avoidance responses, is a subset the activity of which is modulated by flight (Brodfuhrer and Hoy, 1989). Negative phonotaxis to ultrasound occurs only when crickets are actively flying (or, much more weakly, while walking: Pollack et al., 1984); quiescent animals do not respond to ultrasound, no matter how intense the stimulus. Thus, information about the sound stimulus and about behavioral state must be integrated somewhere along the processing chain. Flight has no effect on responses of AN2, ruling out early processing as the locus of this integration, but responses of several descending brain neurons are both stronger and more rapid during flight than while animals are quiescent, pointing to the brain as the site where auditory and behavioral-state information are combined. Although responses are enhanced during flight, the neurons do respond even while the animal is quiescent. Indeed, response magnitude of quiescent animals to intense stimuli can be larger than that of flying animals to stimuli that, though less intense, are nevertheless above typical threshold for eliciting negative phonotaxis. Thus, the role of these descending neurons in triggering or controlling flight-steering responses remains unclear.

Nevertheless, key elements of the network generating the ultrasound avoidance behavior are understood. AN2 in particular has a deterministic role in triggering avoidance steering even though it is on the sensory side of the pathway. The causal relationship between AN2's activity and avoidance steering was first demonstrated by Nolen and Hoy (1984, 1986). They performed intracellular recording of AN2 in a tethered cricket while also monitoring the activity of muscles used for steering during flight (see **Figure 3**). They showed that hyperpolarizing AN2 abolished the motor response to ultrasound, and that depolarizing AN2 evoked a motor response even though no sound was presented, thereby demonstrating AN2's necessity and sufficiency. Thus, an interneuron directly post-synaptic to sensory receptors is the decisive trigger for ultrasound avoidance. Most interestingly, one property of AN2 underlies the decision to trigger avoidance steering or not: bursting.

The temporal coding properties of AN2 were investigated by Marsat and Pollack (2005, 2006, 2007, 2010) using ultrasound stimuli with random amplitude modulations (**Figure 1A**), rather than using more naturalistic pulsed stimuli. The use of a "white noise" amplitude envelope is convenient because it allows one to quickly characterize the temporal tuning properties of the cell, and also reveals some of the coding strategies. Two key findings came from comparing the input – the pattern of amplitude modulation to be encoded – to the output spike train. First, AN2 is able to encode a wide range of amplitude modulation rates that encompass those typical of bat echolocation signals (Marsat and Pollack, 2005). Second, the neuron encodes the signal in a non-linear manner. Indeed, the input stimulus has a Gaussian amplitude distribution but the output spike train has a bimodal distribution of ISI; the short ISIs of spikes within bursts form the first peak in the distribution, and the longer ISIs between isolated spikes, or between bursts, form the second, broader, peak (**Figure 1**). Because ISI is the inverse of instantaneous firing rate, the distribution of the latter is also bimodal. No linear transformation can turn a Gaussian distribution signal into a bimodal distribution, and information-theoretic quantification of AN2's coding of stimuli with white noise amplitude envelopes showed that the majority of the information is encoded non-linearly (Marsat and Pollack, 2005). Similar to bursting neurons in other sensory systems, AN2 bursts act as feature detectors (Marsat and Pollack, 2006), signaling the occurrence of specific patterns of amplitude modulation. Specifically, AN2 bursts are triggered by peaks in amplitude larger than the standard deviation of the stimulus, in other words, salient peaks in amplitude (**Figure 1A**). Bursts are very reliable in their timing and thus carry accurate information about the occurrence of these features. These response characteristics presumably allow differentiating background noise from those peaks in ultrasound amplitude that are most likely to originate from a hunting bat.

The bursting characteristics of AN2 have a direct impact on behavior. Nolen and Hoy (1984, 1986) showed that AN2 evokes avoidance steering only if it fires at 180 spikes/s or more. This threshold corresponds to the longest ISIs that occur in AN2's bursts (∼6 ms; **Figure 1**; Marsat and Pollack, 2005, 2006). Marsat and Pollack examined explicitly the relationships between bursts or isolated spikes and behavior (see **Figure 3**). They showed that avoidance steering was triggered only by bursts whereas spikes that

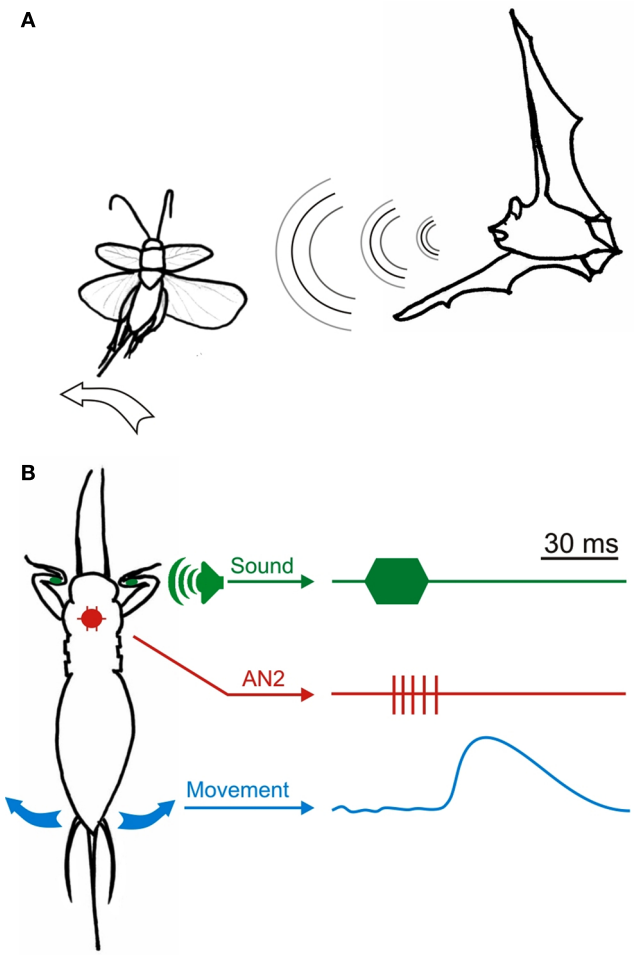

FIGURE 3 | tudying the neural basis of ultrasound avoidance. (A) Ultrasound pulses such as the ones produced by foraging insectivorous bats cause avoidance steering in flying crickets directed away from the sound source. The steering movements consist of changes in the wingbeat pattern but also of movements of the abdomen, hindlegs and antennae in the direction of the intended turn. **(B)** Tethered crickets continue to produce the motor pattern of flight and can thus be stimulated with ultrasound while the neural and/or motor output is recorded (Nolen and Hoy, 1984, 1986; Marsat and Pollack, 2006, 2010).

were not part of a burst had no detectable influence on avoidance behavior (Marsat and Pollack, 2006).

Although bursts are often considered as all-or-none units, they may vary in the number of spikes they contain or in the intra-burst firing rate. The role of burst structure in temporal coding has been discussed in several systems (Kepecs et al., 2002; Oswald et al., 2007; Butts et al., 2010), because this can carry fine-scale information about the feature that the bursts encode. For example, the intra-burst firing rate of AN2 varies with the duration of the peak in ultrasound amplitude, and number of spikes per burst is correlated with the intensity of the ultrasound peak. Marsat and Pollack (2010) examined the influence of these parameters on behavior. They found that the amplitude of steering responses varied with the number of spikes per burst, but that the intra-burst firing rate had no effect on behavior. These results make the point that even though information about a stimulus can be retrieved by the experimenter by analyzing a specific aspect of the spike train, this does not necessarily mean that the nervous system uses that information.

Together these results show that the bursting properties of AN2 have a key impact on ultrasound avoidance behavior. A stimulus that does not trigger spiking in AN2, or that triggers only non-burst spikes, will not cause ultrasound avoidance. Therefore the bursting mechanism is a key component of the decision mechanism. Although it is not yet known whether AN2's intrinsic properties contribute to bursting, Sabourin and Pollack (2009) showed that ultrasound receptors also burst and that these bursts have a high probability of causing a burst in AN2.

NEURAL DYNAMIC UNDERLYING ULTRASOUND AVOIDANCE

When neurons are part of a population, as is the case for the 15 or so ultrasound-sensitive receptors, the relationship between the spike trains of the different neurons shapes the population response. Ultrasound receptors tend to burst synchronously, causing a large input to AN2 (Sabourin and Pollack, 2009). Redundancy in response patterns across a population has the well known consequence of limiting the amount of information carried by the population, since different neurons do not carry entirely different information (Sompolinsky et al., 2001; Puchalla et al., 2005; Sabourin and Pollack, 2010). A useful comparison can be established with the population of low-frequency receptors, the spike trains of which show much less redundancy than those of ultrasound receptors. Consequently the accuracy of stimulus coding is greatly improved by pooling the information from many of these low-frequency receptors. It is tempting to relate these two coding strategies to behavior: low-frequency communication sounds need to be encoded with much detail to permit, for example, the fine discrimination of male quality during mate choice, whereas bat-like ultrasound need only be detected to trigger a stereotyped avoidance response.

Interestingly, it is not sufficient that the population response of ultrasound receptors be composed of many synchronous spikes to elicit bursting in AN2; rather, individual receptors must burst (Sabourin and Pollack, 2009). A "distributed burst" of eight spikes (eight single spikes coming in close temporal proximity from eight different receptors) has much less chance of triggering a burst in AN2 than eight spikes produced by four cells, each producing a two-spike burst. These results suggest that synaptic properties contribute to processing the signal, specifically, that facilitation might enhance the response to true bursts, acting as a filter to prevent responses to coincident non-burst spikes in different receptors. This mechanism helps to maintain the separation between burst-encoded information and that encoded by isolated spikes.

Recent studies have also revealed that adaptation in AN2 can lead to filtering out weaker signals (Wimmer et al., 2008; Hildebrandt et al., 2011). Based on experiments combining current injection and pharmacological manipulation Hildebrandt et al. (2011) suggested that both cell-intrinsic currents and pre-synaptic inhibition contribute to adaptation of AN2. The former mechanism has a subtractive effect on the input-output relationship whereas the latter has a divisive effect. Both mechanisms influence

the response to ultrasound and presumably help to scale AN2's response so that only peaks in ultrasound amplitude larger than the recent average amplitude elicit bursts. Another factor contributing to adaptation of AN2 is the very pronounced adaptation of ultrasound-tuned receptors (Sabourin and Pollack, 2009).

Bursts appear as a distinct medium of information transmission and the computations performed by network interactions can operate selectively on the burst-component of the response. This is apparent, as described above, when only bursts in receptors can cause bursts in AN2. It is also apparent in the synaptic interaction between ON1 and AN2. ON1 provides contralateral inhibition to AN2, and its response pattern to ultrasound – including bursting – is very similar to that of AN2. Interestingly, ON1 does not produce bursts when stimulated with low, cricket-like sound frequencies (Marsat and Pollack, 2004, 2005). By using dichotic stimulation and judicious choice of stimuli, it was shown that ON1's coding properties – in particular its bursting tendency – play an important role in shaping the influence of ON1 on AN2. Specifically, bursts in AN2 are inhibited most efficiently by coincident bursts in ON1 (Marsat and Pollack, 2005, 2007). The role of ON1 is to enhance the bilateral contrast that serves as a basis for sound localization. As a consequence, stimuli that trigger bursts in AN2 and ON1 will be represented with larger bilateral contrast, and thus sound localization will be selectively enhanced for these stimuli. These observations support the idea that neural codes and the architecture of neural computations are mutually determined.

MODULATION OF NEURAL PROPERTIES

Crickets are most at risk of predation by bats while flying. Although some bats are able to glean prey from the tops of vegetation, this is unlikely to be a problem for crickets, which spend their time on the ground in burrows, under the leaf litter, etc. Not all crickets can fly. In many species, individuals may develop either with stunted wings and weak flight muscles, or with full-length wings and strong muscles; the former cannot fly, and the latter can. As crickets age their flight muscles undergo histolysis, so that even though the wings remain long, the flight muscles can no longer generate sufficient force for flight. Thus, there are three flight-classes in the same species; individuals that could never fly, those that can, and those that could at one time but can no longer. Interestingly, sensitivity to ultrasound varies with flight capability: the minimum sound level required to elicit avoidance steering is lower in flight-capable than in flight-incapable individuals; and this is paralleled by a lower threshold of AN2 for ultrasound stimuli (Pollack and Martins, 2007) and by an increased rate of bursting in AN2 compared with that of flight-incapable crickets (Pollack, unpublished observations). These flight-related changes in sensitivity are specific to ultrasound; neither behavioral threshold – in this case for attraction to a model of the species' communication signal – nor threshold sound level to stimulate AN2, differs between flight-classes for low-frequency, cricket-like sounds (Pollack and Martins, 2007).

The "decision" to develop with short or long wings may be mediated in part by the titer of Juvenile Hormone (JH) during late larval stages (Zera and Tiebel, 1988), with high JH-level leading to short-winged phenotype. An increase in JH-level during adulthood also triggers wing-muscle histolysis (Shiga et al., 1991). Experiments suggest that JH may also be involved in setting sensitivity to ultrasound. Treatment with a JH analog of late-stage larvae of a species in which all individuals are normally long-winged and flight-capable, results in adults with poor sensitivity to ultrasound. As in wing-dimorphic species, the loss of sensitivity is specific to high sound frequencies (Narbonne and Pollack, 2008). The possible role of JH in the loss of ultrasound sensitivity that accompanies wing-muscle histolysis remains to be tested.

Another factor affecting the risk of predation by bats is the presence of bats themselves. Bat-free islands in French Polynesia have been invaded by a cricket species that also occurs in bat-rich areas, including Australia. Behavioral threshold for ultrasound avoidance, and AN2 threshold for ultrasound stimulation, are both higher for crickets in the bat-free environment (Fullard et al., 2010), suggesting that sensitivity to ultrasound has considerable evolutionary, as well as developmental, plasticity. The fact that this plasticity influences the sensitivity of sensory neurons and is reflected in the behavioral output argues for the importance of these neurons' properties in the decision process.

BURSTS, A CODE FOR RAPID SENSORY PROCESSING OF IMPORTANT SIGNALS?

The preceding paragraphs describe the relationship between the bursting properties of the cricket auditory neurons and ultrasound processing. The results summarized in these paragraphs also show that beyond determining the information available to the nervous system, the response properties of the sensory neurons relate to the motor output. Specifically, the encoding of different parts of the ultrasound stimulus is multiplexed onto two channels within the neuron's response: some features of the stimulus are encoded by bursts and others by isolated spike; only the "burst channel" influences avoidance behavior. Consequently, the neuronal properties that generate bursts in response to specific stimulus features also determine when avoidance steering is triggered and can therefore be considered part of the decision mechanism. Although some aspects of ultrasound avoidance, such as its reliability and short-latency, are reflex-like in nature, the fact that responses occur only following specific patterns of sensory input is indicative that a decision has been made. More specifically, the neural network generating the response must decide whether an ultrasound stimulus is sufficiently intense, relative to recent experience, to represent a potential threat and, if so, in which direction to steer.

The influence of a sensory neuron's coding properties on behavioral decisions has been demonstrated in many systems and is particularly clear in reflex-like behaviors such as the escape behavior of fish triggered by the activity of Mauthner cells (Korn and Faber, 2005), the crayfish escape response (Edwards et al., 1999), or the avoidance of looming stimuli in locust (Fotowat and Gabbiani, 2011). The influence of sensory neuron properties on decisions has also been examined in much more complex behaviors. For example, the decisions of a monkey asked to perform a discrimination task seem to be correlated with the response patterns of populations of sensory neurons, although the causal nature of the relationship is difficult to establish (Nienborg and Cumming, 2010).

Indeed, a clear, causal link between the bursting properties of sensory neurons and behavior has not yet been demonstrated in other systems. Nevertheless, the central role of burst codes

becomes obvious in a growing number of cases. In locusts, the presence of a looming stimulus is detected through sophisticated computation carried out at the level of the sensory neuron LGMD (Fotowat and Gabbiani, 2011) which triggers an escape response (Fotowat et al., 2011). LGMD exhibits intrinsic bursting properties and strong spike-frequency adaptation. LGMD is pre-synaptic to the descending neuron DCMD, which provides the motor command. DCMD mirrors LGMD's spiking pattern; thus LGMD's bursting properties are likely to have a crucial influence on behavior. Heat sensitive neurons of the beetle provide another example of a probable link between sensory bursts and behavior in invertebrates (Must et al., 2010). In this case burst timing does not relate to the temporal features of the stimulus; rather, burst rate indicates the presence of dangerously high temperatures that the beetle tries to avoid.

Examples of the relevance of sensory bursts for behavior also abound in vertebrate model systems. Synchronized bursts in a subset of cells in a sensory processing area signal the occurrence of aggressive communication signals in electric fish, whereas in a different subset of cells, bursts code for prey (Gabbiani et al., 1996; Oswald et al., 2004; Marsat et al., 2009; Marsat and Maler, 2010, 2012). In the retina and the lateral geniculate nucleus of mammals, bursts also reliably signal the occurrence of specific behaviorally relevant features of visual inputs such as a sharp contrast or motion reversal (Lesica and Stanley, 2004; Schwartz et al., 2007b). Thus in many model systems and modalities, sensory bursts seem to serve similar purposes: extracting from the sensory environment features that are of particular relevance to the organism and signaling their occurrence reliably to post-synaptic networks.

Burst coding is well suited to this task. Bursts can be seen as self-contained units of information because they capture, with high reliability, the timing and the identity of specific stimulus features, making this information readily available to decoding circuits. This property is particularly advantageous when a signal must trigger a response very quickly, as in escape behaviors. Also, the fact that bursts consist of several closely spaced spikes suggests that they will have strong effects on post-synaptic cells, where a simple threshold mechanism could separate relevant signals encoded by bursts from background noise. Moreover, frequency-dependent mechanisms, such as facilitation, depression, or resonance, can allow post-synaptic cells to respond differently to bursts and to non-burst spikes (Izhikevich et al., 2003). It is known for example that burst-induced facilitation can lead to an increase in the reliability of synaptic transmission (Lisman, 1997; see also: Harvey-Girard et al., 2010; Bol et al., 2011). Therefore bursts can carry reliable information quickly and efficiently to the next stage of processing. On the other hand bursts might be limited in their capacity to carry information in their internal structure about fine-scale variations in the feature that they represent. In particular we note that because intra-burst ISIs are small, a decoder would have to be sensitive to minute differences to detect variations and might thus be more sensitive to noise (Avila-Akerberg and Chacron, 2011). Furthermore, the internal structure of bursts is often determined by neuron-intrinsic burst-generating mechanisms rather than by subtle stimulus features, thus limiting their coding capacity.

In conclusion we suggest that, because of the properties outlined above, bursts are frequently observed in networks involved in behaviors requiring quick stereotyped responses. In crickets, the use of bursts as an indicator of impending danger allows the decision to take evasive action to be made very early in the sensory system, promoting reliable and rapid responses. We argue that the same strategy may be used by other systems as a way to reduce the need for temporally costly decision networks.

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A core circuit module for cost/benefit decision

Keiko Hirayama[1], Marianne Catanho[2], Jeffrey W. Brown[3] and Rhanor Gillette[1,3,4]*

[1] The Neuroscience Program, University of Illinois, Urbana, IL, USA
[2] Electrical and Computer Engineering, University of Illinois, Urbana, IL, USA
[3] Biophysics and Computational Biology, University of Illinois, Urbana, IL, USA
[4] Department of Molecular and Integrative Physiology, University of Illinois, Urbana, IL, USA

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
James Alan Murray, California State University East Bay, USA
Bjoern Ch. Ludwar, Mt. Sinai School of Medicine, USA

****Correspondence:***
Rhanor Gillette, Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, 414 Burrill Hall, 407 S. Goodwin Avenue, Urbana, IL 61801, USA.
e-mail: rhanor@illinois.edu

A simple circuit for cost-benefit decision derived from behavioral and neural studies of the predatory sea-slug *Pleurobranchaea* may closely resemble that upon which the more complex valuation and decision processes of the social vertebrates are built. The neuronal natures of the pathways in the connectionist model comprise classic central pattern generators, bipolar switch mechanisms, and neuromodulatory state regulation. Marked potential exists for exploring more complex neuroeconomic behavior by appending appropriate circuitry *in simulo*.

Keywords: approach avoidance, central pattern generator, neuroeconomics, neuronal switch, *Pleurobranchaea*, simulation, decision making

INTRODUCTION

Organisms are designed to engage three basic functions: resource acquisition, defense against accident (e.g., predation and disease), and reproduction. Their lifestyles represent behavioral economic strategies, and range in complexity from very simple solitary foraging to the complicated, multi-layered economies of the social vertebrates. The complexities of valuation, decision-making, and lifestyles are parallel over this range. What are the gradations of complexity in behavioral economy, and how are they traversed in evolution? Let's begin by examining the simpler systems.

In simple terms, goal-directed decision is regulated by an animal's appetitive state, which is the summation of sensation, internal state, and learning. Operationally, the appetitive state itself is the likelihood that an animal will perform any of a repertory of goal-directed, homeostatic behaviors. The basic premise of behavioral economics is that decisions so made will, on average, optimize success in foraging and reproduction, and minimize accompanying risk. How these computations are effected at levels of neural networks and nerve cells is basic to understanding the genesis of behavioral economics in nervous system function.

For foraging animals, a most critical and simple behavioral decision regulated by appetitive state is that for approach or avoidance of a stimulus. The neuronal nature of appetitive state, and how it toggles decision, are problems that have been investigated in the predatory sea-slug, *Pleurobranchaea californica*.

THE ECONOMIC LANDSCAPE OF A SIMPLER PREDATOR

To put the decision mechanism in natural context, it is useful to describe *Pleurobranchaea*'s simple economy of lifestyle. The sea-slug (**Figure 1**) is an opportunistic predator with simple behavior and nervous system, and it makes value-based decisions that balance need for resource against personal risk (Gillette et al., 2000). Very hungry animals not only have very low thresholds for feeding stimuli, but will even attack mildly noxious stimuli such as acidic seawater. Satiated animals actually actively avoid food stimuli, and partly satiated animals may avoid weak appetitive stimuli but attack stronger stimuli. Thus, level of effort is related to need (hunger), and the perceived value of a resource is weighed against the potential risk of an attack, such as in prey defenses and possible attraction of another predator (like a cannibal conspecific), and probable cost of energy outlay in an attack. The ability to associate specific odors with the positive or negative consequences of an attack on potential prey (Davis et al., 1980; Mpitsos and Cohan, 1986a,b) lends the predator another important skill for optimizing foraging success.

Thus, a simple cannibal predator like *Pleurobranchaea* operates at an extremely simple neuroeconomic level, one in which the three basic organismal functions are satisfied in an uncluttered manner. The model for decision, discussed below, is so simple that it may represent a basic core type of circuit whose relations are common to most foragers, and one onto which the more complex circuits for value and risk in social vertebrates are built in evolution.

BACKGROUND TO THE PRESENT

Dr. Rimmon Fay, a notable biological supplier of southern California, was a key figure in the history of neuroethological research for several molluscan preparations, including *Aplysia*, *Navanax*, *Bulla*, and *Pleurobranchaea*. Without his supply side efforts in the 1960s to the 1980s, it is unlikely that much of the present progress in molluscan neuroethology would have been made. The strenuousness of his efforts was made clear to those of us lucky enough to accompany him on a collecting cruise. He noted a population boom of *Pleurobranchaea* and sent several specimens to researchers at Stanford University. Davis and Mpitsos (1971) realized the marked potential for a model system for study of behavioral choice, and the following decades saw novel reports on

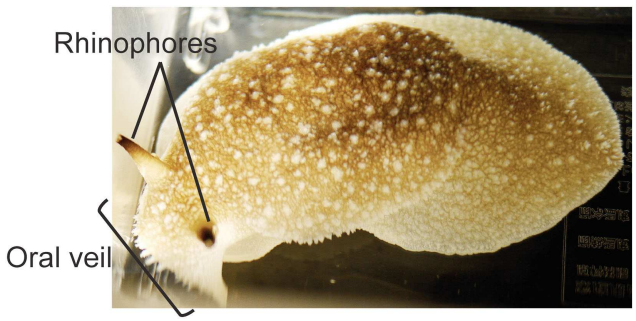

FIGURE 1 | A *Pleurobranchaea* with chemotactile oral veil and rhinophores indicated.

odor learning abilities for food avoidance (Mpitsos and Collins, 1978; Davis et al., 1980; Mpitsos and Cohan, 1986a,b) and demonstrations of diverse and actual neuronal mechanisms of choice involving network interactions for feeding vs. withdrawal to touch (Kovac and Davis, 1980a,b), escape vs. feeding (Jing and Gillette, 1995), escape vs. turning (Jing and Gillette, 2003). Behavioral studies showed that animals could integrate hunger state, taste, and pain to decide between approach and avoidance of appetitive stimuli, consistent with a cost-benefit decision mechanism rooted in appetitive state (Gillette et al., 2000). Study of this phenomenon was given a great boost by findings that the isolated CNS conserved the appetitive state of the intact donor (Hirayama and Gillette, 2012). Thus, spontaneous activity in the feeding network in CNS isolated from hungry animals was higher than for those less hungry. In fact, the spontaneous activity recorded in feeding motor nerves of isolated CNSs was proportionate to the feeding thresholds of CNS donors (**Figure 2**), in a remarkably linear log–log relationship.

A second helpful finding was that isolated CNSs display fictive turns, recorded in motor nerves following unilateral stimulation of sensory nerves that innervate the chemotactile oral veil (Jing and Gillette, 2003). The third finding was that the appetitive state of isolated CNS also controlled the direction of the fictive turn: turn direction was contralateral to the stimulated nerve in CNS from less hungry donors, but ipsilateral in those from hungry animals (Hirayama and Gillette, 2012). It was found that increasing the excitation state of the feeding network – either by driving an identified feeding command neuron or by stimulating a sensory nerve innervating the buccal cavity – could reversibly change fictive decision from an avoidance to an orienting turn. This observation had two implications: first, the turn network was probably organized by default for avoidance, and second, that corollary outputs from the feeding network must somehow switch sensory input from one side of the turn network to the other. This resembles control of vertebrate spinal reflexes, whose default circuits are redirected to other, even oppositely directed, behaviors by descending voluntary control (Sherrington, 1906; Stuart, 2002).

THE CORE MODULE FOR COST-BENEFIT DECISION

The model of **Figure 3** emerged from the studies of the isolated CNS. It takes into account that the feeding motor network is basically a homeostatic neural network that economically

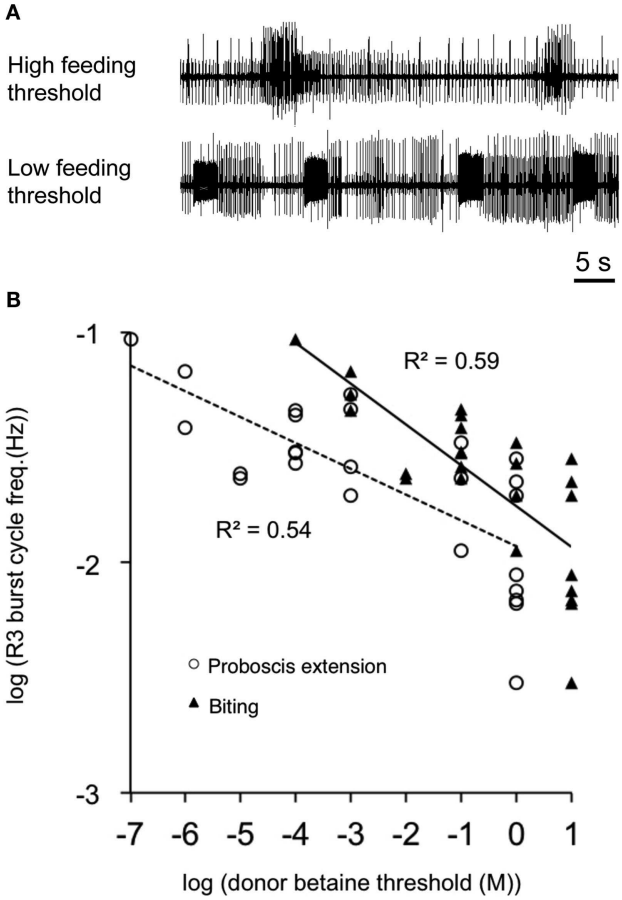

FIGURE 2 | Conservation of appetitive state in the isolated CN . Spontaneous feeding nerve burst frequency correlated with donors' sensory feeding thresholds to the appetitive stimulant betaine (trimethylglycine). **(A)** Spontaneous burst frequency recorded from buccal motor nerve *R3* of isolated CNSs was less from high-threshold donors than from low-threshold donors. **(B)** *R3* burst frequency was an approximately linear function of donor feeding thresholds on a log–log plot ($n = 25$; $R^2 = 0.54$ and 0.59 for proboscis extension and biting, respectively). Line fits were by least squares. Three high-threshold donor CNSs did not show burst patterns in *R3* and were excluded from the figure. From Hirayama and Gillette (2012).

combines representation of appetitive state with central motor pattern generation. To summarize the model: the excitation state of the feeding network embodies appetitive state as a sum of intrinsic excitability, stimulus salience, and effects of memory. Corollary outputs from the network toggle the directional response of the turn network to change avoidance to orienting. Adding known relations of sensory pathways and interactions among neuronal networks in *Pleurobranchaea* fills out a complete, simple model for decision. The model represents a basic cost-benefit decision module for foraging, encoding appetitive state in a homeostatic neuronal network that controls decision for approach/avoidance to appetitive and noxious stimuli.

The model is built on an empirical approach to a general theory of cost-benefit decision. It is accessible to hypothesis testing

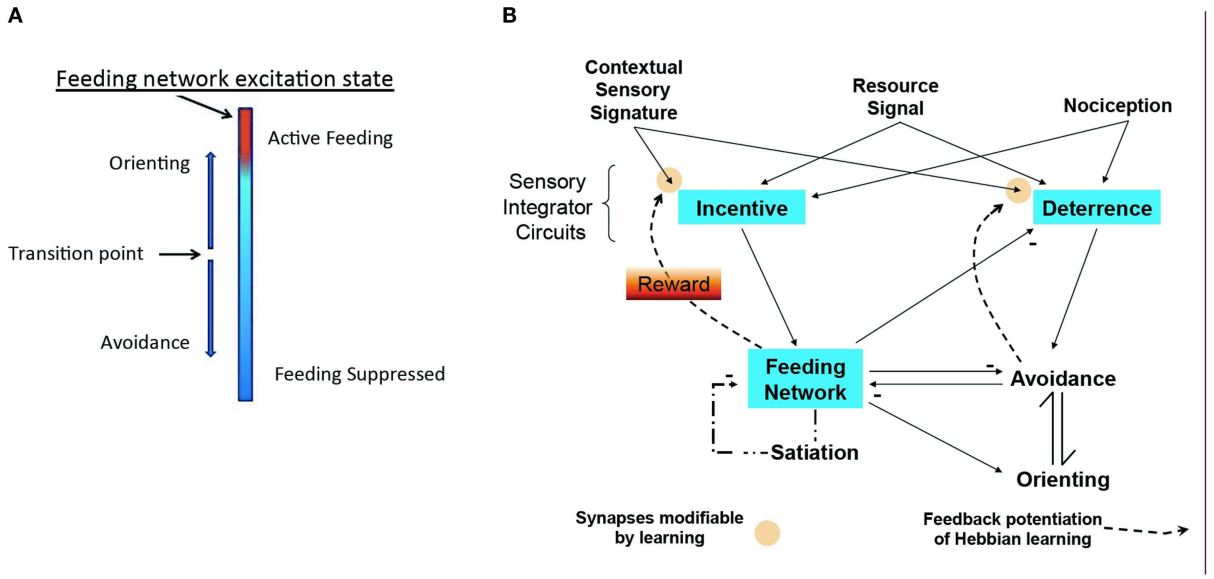

FIGURE 3 | Modeling homeostatic decision. (A) Excitation state of the homeostatic feeding network switches avoidance to approach. **(B)** Sensory inputs for resource quality, sensory signatures, and nociception access sensory networks for *Incentive* and *Deterrence*, which promote excitation of feeding and avoidance turns, respectively. Excitation in the homeostatic network suppresses avoidance and promotes orienting turns (approach). Active avoidance and satiation inhibit the homeostatic network, while homeostatic network activity suppresses *Deterrence*. Modulatory feedback pathways from the *Feeding* and *Avoidance* networks potentiate learning of sensory signatures, mediating reward. Modified from Hirayama and Gillette (2012).

and modifiable from the results of those tests. It represents three types of neuronal networks interconnected in feed-forward and feedback loops: (1) The goal-directed feeding network is regulated internally by satiation state, and externally by sensory inputs that include effects of odor memory (Davis and Gillette, 1978). The feeding network makes coordinating connections with agonistic and antagonistic networks. (2) A premotor network for directional responses that mediates approach-avoidance output and thereby expresses decision; and (3) Two sets of sensory processing networks, *Incentive* and *Deterrence*, are predicted to integrate afferent sensory inputs and pass them on to the first two networks. Odor learning is presumed to occur in these sensory networks through modulatory feedback (*Reward*) from the activated motor networks. Thus, the network interconnections modify appetitive state, mediate reward, and direct behavioral choice.

The feeding motor network itself in *Pleurobranchaea* is a major homeostatic, core processor of foraging decision, manifesting appetitive state in the extent and configuration of its excitation (cf. **Figure 2**), and thus setting feeding thresholds and assigning stimulus values on the basis of need and incentive. Most recurrent circuit models for categorical choice incorporate leaky integrator modules and recurrent inhibition (cf. Wang, 2008). These qualities are reprised in the dynamic circuitry of the feeding network, its sensory inputs, and its interactions with turning and escape swim networks (Gillette et al., 1982; Jing and Gillette, 1995, 2000, 2003). Its corollary outputs control the approach/avoidance output of the turn network. Sensory inputs, effects of learned odors, and hunger state sum in the excitation state of the feeding network, directly targeting critical identified interneurons, and indirectly excite or inhibit feeding command neurons (Gillette et al., 1982; London and Gillette, 1986; cf. also Gillette, 2008).

Satiation (internal state) sums into appetitive state with the effects of sensation and learning. Both satiation and general arousal mechanisms entail serotonin (5-HT), a modulator of the feeding network (Palovcik et al., 1982; Jing and Gillette, 2003). 5-HT from interneurons in the feeding network regulates excitation state and arousal, much like orexin in mammals (Gillette, 2006). 5-HT content in those neurons varies over fourfold with satiation, which is likely reflected in 5-HT output and consequent regulation of feeding network excitability (Hatcher et al., 2008). This may go a long way toward explaining the conservation of donor appetitive state in the isolated CNS.

The turn motor network computes inputs from the feeding network and sensory inputs from the body, and expresses approach/avoidance decision in the direction and amplitude of its output. It appears by default to be configured for avoidance bhavior to unilateral inputs, but is redirected to orienting by feeding network input. It is expected that sensory inputs determine the computation for turn angle, but orienting turn direction is controlled by the feeding network. When excitation state of the feeding network is low, as in satiated animals, or in absence of appetitive sensory input or when the network is suppressed through learned food avoidance, sensory inputs to the turn network cause avoidance motor output. A simple switch mechanism is shown in **Figure 4** that could re-direct sensory input from one side of the turn network to the other, converting avoidance to orienting. This is a hypothesis awaiting test.

Sensory integration in prey tracking is to a large extent performed at the animal's oral (**Figure 1**), the anterior chemotactile

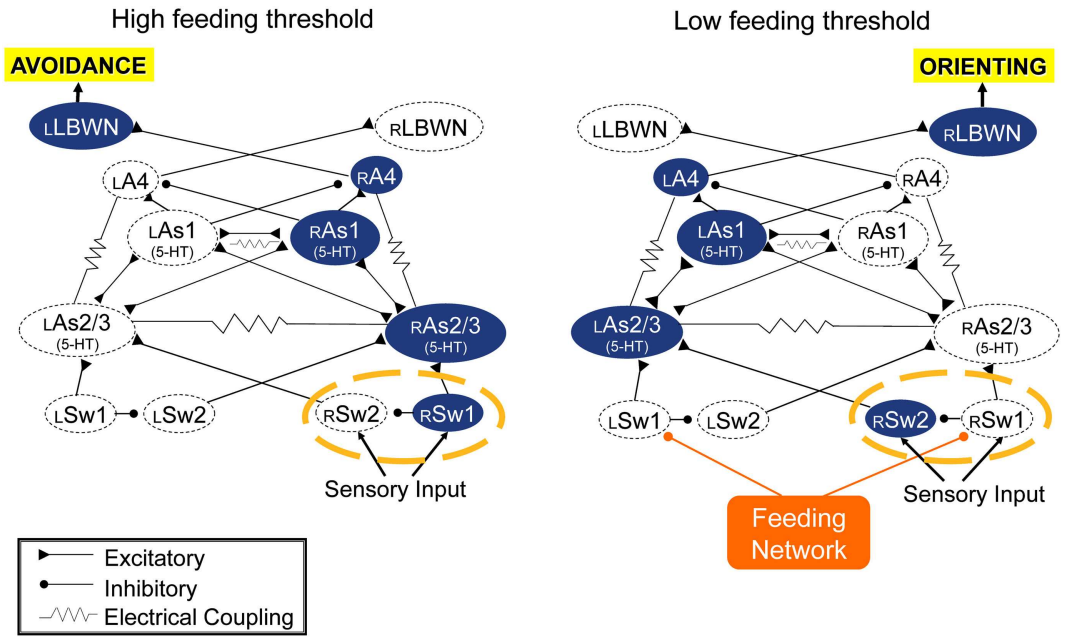

FIGURE 4 | imulation suggests that the turn network is default organi ed for avoidance responses to unilateral sensory inputs (left), and that inputs from the feeding network could reverse responses to orienting (right) via a hypothetical dyad of switch neurons (circled with a broken orange line). Double-headed connections indicate reciprocal excitatory connections.

structure where sensory afferents feed into peripheral ganglia (Bicker et al., 1982a,b). Those in turn send integrated information to the CNS in the sensory Large Oral Veil (LOVN) and Tentacle nerves (TN), respectively. Functionally, the oral veil is a composite of mammalian gustatory and olfactory system, with receptors for amino acids (but not sweet or bitter chemicals) to assess nutritive content, and others that must encode odors for associative learning. Turning behavior has been described quantitatively. In prey tracking, *Pleurobranchaea* averages chemotactile stimuli at multiple sites on the oral veil into precise angles of turn, and also uses a simple working memory to optimize the chase (Yafremava et al., 2007). Two relevant findings are: (1) turn direction is affected by appetitive state, so non-hungry animals actively avoid appetitive stimuli; and (2) the angles of avoidance turns induced by noxious stimuli are computed similarly to orienting, only differing in direction. Chemotactile stimuli at the oral veil are encoded for site and amplitude in peripheral ganglia via putative lateral inhibition (Yafremava and Gillette, 2011). The information is transmitted by LOVN and TN to CNS, to be integrated for computing turn angle.

The peripheral ganglia of the oral veil are thought to integrate primary afferent information regarding both stimulus nutritive content and nociception. Specific odor signatures are so far not detected in LOVN and TN activity (unpublished). Thus, it is presently considered that peripheral ganglia may encode the memory of odors for transmission to the CNS in terms of secondary appetence and nociception in the relatively few sensory interneurons (Yafremava and Gillette, 2011) recordable in the nerve responses.

NETWORK CONNECTIVITY

Corollary outputs from feeding to turning network reverse the direction of the turn response to unilateral inputs from oral veil through an as yet undetermined switch mechanism (cf. **Figure 4**). Enhanced activity in the feeding network suppresses the motor output of withdrawal to touch by inhibiting sensory input to withdrawal motor neurons (Kovac and Davis, 1980a). Raising the excitation state of the feeding motor network also suppresses the avoidance turn and promotes the orienting turn (**Figure 5**). A reciprocal inhibitory pathway from avoidance to feeding, predicted by Kovac and Davis (1980b), has also appeared (unpublished).

It is necessary to postulate existence of reinforcement pathways (*Reward* in **Figure 3**) to the sensory integrating networks, *Incentive* and *Deterrence*, driven from the goal-directed feeding network and from avoidance to account for odor learning. These would serve to potentiate learning mechanisms for odors and thereby assign them positive or negative values, depending on their association with nutrient reward or punishment in an attack on prey. Perhaps likely mediators are serotonergic neurons, the anterior cerebral cluster (Moroz et al., 1997), embedded in the feeding network and innervating the oral veil, and/or putative dopaminergic neurons histochemically demonstrable in the oral veil (in preparation). Both serotonin and dopamine have functions in molluscan learning (Brembs et al., 2002; Marinesco et al., 2004; Gillette, 2006). Other configurations are possible.

However, the general form of the model is clear. Its advantage is that it may well apply broadly across species. Details of neuropharmacology and internal circuitry of the networks might be expected to vary considerably within the major structure of

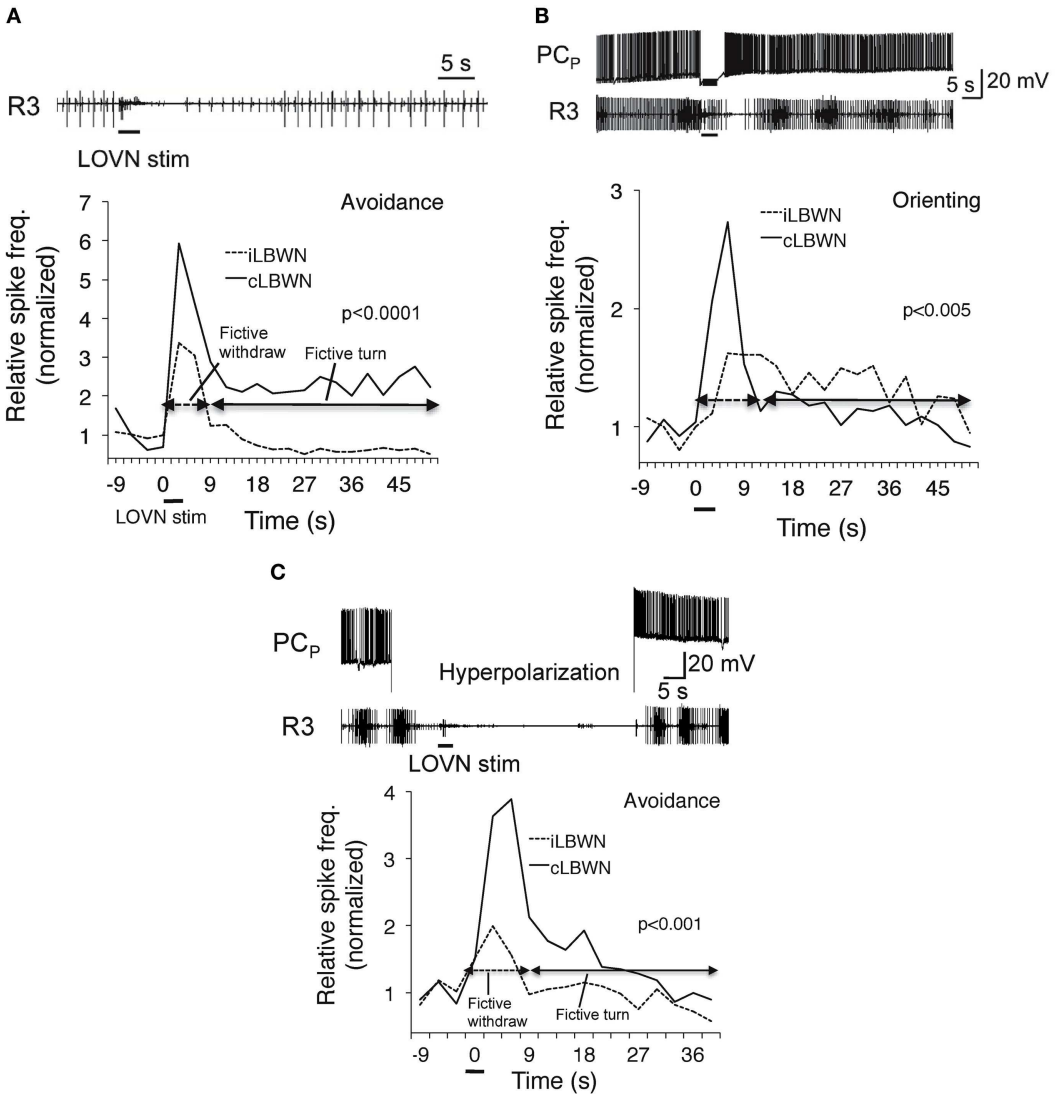

FIGURE 5 | Dependence of approach avoidance decision on the excitation state of the feeding motor network. Fictive avoidance **(A)** was switched to orienting **(B)** following penetration of a feeding command neuron (PCp; Gillette et al., 1982) whose firing induced rhythmic bursting in a feeding nerve (*R*3). Hyperpolarization of PCN **(C)** suppressed fictive feeding and restored avoidance. From Hirayama and Gillette (2012).

the model. For instance, mammals, insects, and molluscs differ markedly in roles of dopamine, octopamine, and serotonin in mediating reward (Schwaerzel et al., 2003), but reward mechanisms play similar roles across taxa. The utility of the comparative approach here is not dependent on exact correspondence of neurotransmitter involvement. Similarly, the complicated choices and valuations made by social vertebrates are likely to arise from circuitry concatenated upon the basic neuronal module of cost-benefit decision visible in the simplest model systems.

A preliminary test of the cost-benefit decision model has been done in a computational simulation of foraging and prey choice. The logical relations of the model of **Figure 3** are implemented in simple equations representing sensation, appetitive state, orienting and avoidance, and odor learning, and the resulting predator/prey simulation successfully reproduces state- and learning-dependent cost-benefit decisions of the real sea-slug predator. Cyberslug 2.0 is presently available for preview and interactive play at http://www.life.illinois.edu/slugcity/Cyberslug21.html.

FUTURE DIRECTIONS

What actual benefit is offered by the model of **Figure 3** and the resulting simulation? We expect that this type of model forms a basis for a bottom-up approach to cognitive processes higher than *Pleurobranchaea* could ever achieve. The sea-slug is streamlined for simplicity in predation and reproduction, and completely lacking in any of the social graces that normally attend cognitive processes in mammals; its only social behaviors are copulation and (arguably) cannibalism, and its larvae are left to find their own luck with myriad other plankton. Thus, the barebones model of the decision process is markedly amenable to *in simulo* experiment with modifications and add-ons that could bring the artificial entities toward social interactions characteristic

of functional cognition and consciousness. Some logical add-ons that might be implemented in evolutionarily plausible ways to achieve social characters of higher vertebrates could be territoriality, social hierarchy formation, and altruistic partnering. Here, the building-from-the-bottom-up approach is a potent complement to the top-down, in which the truly intelligent animals are taken apart like one might analyze a complex electronic instrument. Modern computers and communication devices themselves were developed over time from very simple electronic circuits. Indeed, the training of our technicians begins with and builds on those simple circuits. The analogy is obvious for the comparative approach to understanding higher function. We look forward to future efforts in neuroscience and engineering aimed at moving back the borders of this frontier.

Interactions of appetitive state with neural bases of reward, learning, and cognition are also only beginning to be appreciated at the neuronal level (e.g., Tindell et al., 2009) although they have been long regarded as basic to decision. Hebb and Thompson (1954) noted over 60 years ago that "*. . .cortical or cognitive components in motivation are clearest when we compare the behavior of higher and lower species. Application of a genuine comparative method is essential in the field of motivation as well as of intellectual functions.*" It is timely that the comparative method finds fuller fruition now in parallel studies of self-awareness, cognition, and value assessment in species ranging across nematodes, rotifers, sea-slugs, cephalopods, leeches, arthropods, fish, frogs, birds, rodents, carnivores, and the varied species of primates.

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Deciding which way to go: how do insects alter movements to negotiate barriers?

Roy E. Ritzmann[1]*, Cynthia M. Harley[2], Kathryn A. Daltorio[3], Brian R. Tietz[3], Alan J. Pollack[1], John A. Bender[1], Peiyuan Guo[1], Audra L. Horomanski[1], Nicholas D. Kathman[1], Claudia Nieuwoudt[1], Amy E. Brown[1] and Roger D. Quinn[3]

[1] *Department of Biology, Case Western Reserve University, Cleveland, OH, USA*
[2] *Division of Biology, California Institute of Technology, Pasadena, CA, USA*
[3] *Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH, USA*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Ansgar Buschges, University of Cologne, Germany
Philip Newland, University of Southampton, UK
Volker Dürr, Bielefeld University, Germany

****Correspondence:***
Roy E. Ritzmann, Department of Biology, Case Western Reserve University, 10900 Euclid Avenue, Cleveland, OH, USA.
e-mail: roy.ritzmann@case.edu

Animals must routinely deal with barriers as they move through their natural environment. These challenges require directed changes in leg movements and posture performed in the context of ever changing internal and external conditions. In particular, cockroaches use a combination of tactile and visual information to evaluate objects in their path in order to effectively guide their movements in complex terrain. When encountering a large block, the insect uses its antennae to evaluate the object's height then rears upward accordingly before climbing. A shelf presents a choice between climbing and tunneling that depends on how the antennae strike the shelf; tapping from above yields climbing, while tapping from below causes tunneling. However, ambient light conditions detected by the ocelli can bias that decision. Similarly, in a T-maze turning is determined by antennal contact but influenced by visual cues. These multi-sensory behaviors led us to look at the central complex as a center for sensori-motor integration within the insect brain. Visual and antennal tactile cues are processed within the central complex and, in tethered preparations, several central complex units changed firing rates in tandem with or prior to altered step frequency or turning, while stimulation through the implanted electrodes evoked these same behavioral changes. To further test for a central complex role in these decisions, we examined behavioral effects of brain lesions. Electrolytic lesions in restricted regions of the central complex generated site specific behavioral deficits. Similar changes were also found in reversible effects of procaine injections in the brain. Finally, we are examining these kinds of decisions made in a large arena that more closely matches the conditions under which cockroaches forage. Overall, our studies suggest that CC circuits may indeed influence the descending commands associated with navigational decisions, thereby making them more context dependent.

Keywords: barriers, central complex, electrolytic lesion, foraging in arena, insect brain, multi channel recording, procaine injection, tethered walking

INTRODUCTION

As animals move through their environments, they must negotiate barriers that block their paths toward goals or away from threats. These challenges require changes in leg movements and posture as they execute appropriate maneuvers in the context of ever changing conditions. Thus, an animal must integrate both internal and external cues in order to appropriately alter local systems that re-direct movement. How do insects deal with these complex situations?

Contrary to the notion that insects are simple animals, they actually have at their disposal numerous sensory systems that monitor their own limb movements and their surroundings as well as a central nervous system that includes a sophisticated brain with several large and complex processing regions (Gupta, 1987; Strausfeld, 2012). Numerous studies indicate that insects use the information gained from visual (Pick and Strauss, 2005; Budick et al., 2007; Jeanson and Deneubourg, 2007; Duistermars et al., 2012), tactile (Blaesing and Cruse, 2004; Staudacher et al., 2005; Harley et al., 2009; Schutz and Dürr, 2011), auditory (Pollack and Pourde, 1982; Nolen and Hoy, 1986; Hedwig and Poulet, 2005; Poulet and Hedwig, 2005), and olfactory cues (Carde and Willis, 2008; Martin et al., 2011) to guide their movements in a context dependent fashion (Huston and Jayaraman, 2011). Moreover, aspects of physiological state may impact such decisions as certain goals may be more attractive to a hungry or thirsty insect than one that is satiated (Bell, 1990; Browne, 1993). In this review, we describe a top down strategy of behavioral and electrophysiological observations that begins to address this question. Our strategy relies on behavioral observations to document movements and generate neurobiological hypotheses. We then test those hypotheses using a range of electrophysiological recording methods. Finally, we return to behavioral studies in which various brain regions are lesioned or reversibly silenced and look for predictable behavioral deficits.

MOVEMENT AROUND BARRIERS

As anyone who has had the misfortune of co-habiting with them knows all too well, cockroaches are very agile insects. In particular, they are adept at navigating all manner of barriers including blocks, shelves, holes, and walls in their attempts to reach goals or escape threats. Our first step toward understanding how they deal with these situations was to perform behavioral studies that quantified the cockroaches' behavioral choices.

To accomplish this goal, we placed cockroaches in narrow tracks that contained individual barriers that had to be negotiated if the insect was to pass by. These could be blocks that had to be climbed over, shelves that could either be climbed over or tunneled under and bends that forced turning movements. Movement over blocks was examined in great detail (Harley et al., 2009). This behavior could be divided into a series of choices. For example, as the cockroach approached the block and touched the surface with its antenna, it could have stopped moving, turned around, or initiated a climb over the block (**Figure 1**). After looking at numerous trials, we assigned probabilities to each possible outcome. By following this process through the entire behavior until the cockroach was over the block, we generated ethograms that described the entire behavioral sequence in quantitative detail. The ethograms of these, and other behaviors, were critical to further studies. Without them, we would not have known whether the perceived changes that we recorded after a lesion or other procedure were part of the inherent variability of the behavior or a consequence of the manipulation. For block climbing, the ethograms showed that cockroaches approached the block and palpated it with their antennae. They then rotated their middle and front legs so that extension now pushed the body up and over the barrier (Watson et al., 2002). These climbing movements typically commenced well before any leg contacted the block and the degree to which the insect reared up was dictated by the height of the block. Thus, an intact cockroach moving at normal walking speed appeared to evaluate the barrier with sensors on its head and then acted accordingly rather than relying on reflexes generated by bumping into the object. The importance of the antennae in this behavior was clearly demonstrated by either shortening or removing them (Harley et al., 2009). Cockroaches with shortened antennae delayed climbing onset until the remaining antennal segments made contact, whereas individuals with ablated antennae reverted to simpler and less controlled strategies such as an elevator reflex that lifted the front legs ever higher each time they contacted the front of the object or even more simply by bulling forward until the head was forced over the object.

To create a more complex decision making paradigm, we replaced the block with a shelf (Harley et al., 2009). Now the cockroach had a choice. It could either climb over or tunnel under the object. Again, the resulting ethograms supported the central role of antennae in this decision making process (**Figure 2**). When the antennae contacted the shelf from above, the cockroach almost always climbed over it. When they contacted the shelf from below, the cockroach invariably tunneled under it. However, an interesting bias was detected in these data. About three quarters of the trials resulted in tunneling (**Figure 2D**). What could cause this bias? The cockroach's inherent avoidance of light suggested an answer. Our initial observations were performed under bright lights. When we repeated them under low infrared lighting, the bias was no longer significant (Harley et al., 2009). Furthermore, under the original lighting conditions the bias could be eliminated by covering the ocelli but not by covering the larger compound eyes. Taken as a whole, these data clearly suggest that cockroaches use their antennae to negotiate objects in their path but in the context of ambient light, where bright lighting conditions bias the insect toward tunneling.

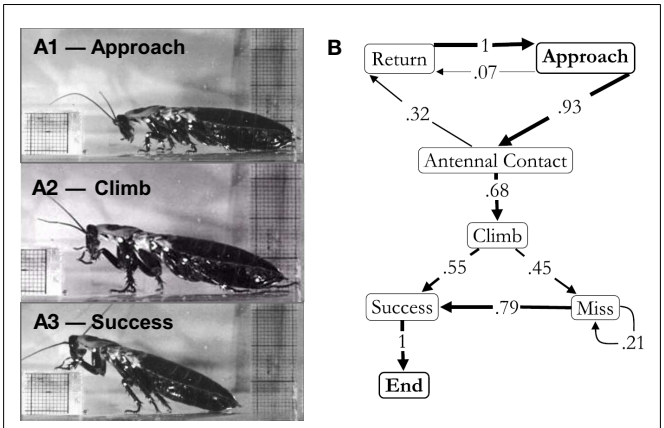

FIGURE 1 | (A) Block climbing behavior: approaching the block **(1)**, swinging the leg to climb **(2)**, and climbing success **(3)**. **(B)** Ethogram of block climbing in the light. Arrows represent a direct transition from one behavior to the next. The number on the arrow and its thickness represent the frequency of that transition. This was calculated by dividing the number of times a specific transition was made by the total number of transitions exiting a specific element. All behavioral sequences began with the cockroach approaching the block (approach). It could then turn around and walk away from the obstacle (return) before or after antennal contact (antennal contact). The cockroaches would then enter a climbing sequence (climb), which could either be successful, with their foot reaching the top of the obstacle (success), or not be successful (miss). In the event that the cockroach missed, it would then produce another climbing motion, which again could either be successful or not. The end of the behavioral sequence occurred when the cockroach climbed the block. The beginning and end of the sequence must be "approach" and "end," respectively. For this reason these elements are represented in bold. This sequence represents the responses of 58 individuals (one trial per individual). From Harley et al. (2009).

It is important to point out that while the ethogram studies that we describe above point to an important role for antennae in directing movement around, over or under barriers, they do not implicate specific sensory structures on the antenna. Antennae are very complex sense organs that contain numerous sensors including campaniform sensilla on the flagellum, hair plates at the base, and chordotonal organs as well as the Johnston's organ (Staudacher et al., 2005). At this point, we cannot distinguish exactly which specific sensory receptors triggered transitions between individual stages of the behaviors described in these ethograms.

The behavioral importance of tactile cues detected by antennae is consistent with several other observations in both cockroach and stick insect. Okada and Toh (2000, 2006) have examined the role of antennal contact in the American cockroach as they navigate poles placed in their surroundings. Blinded cockroaches moving

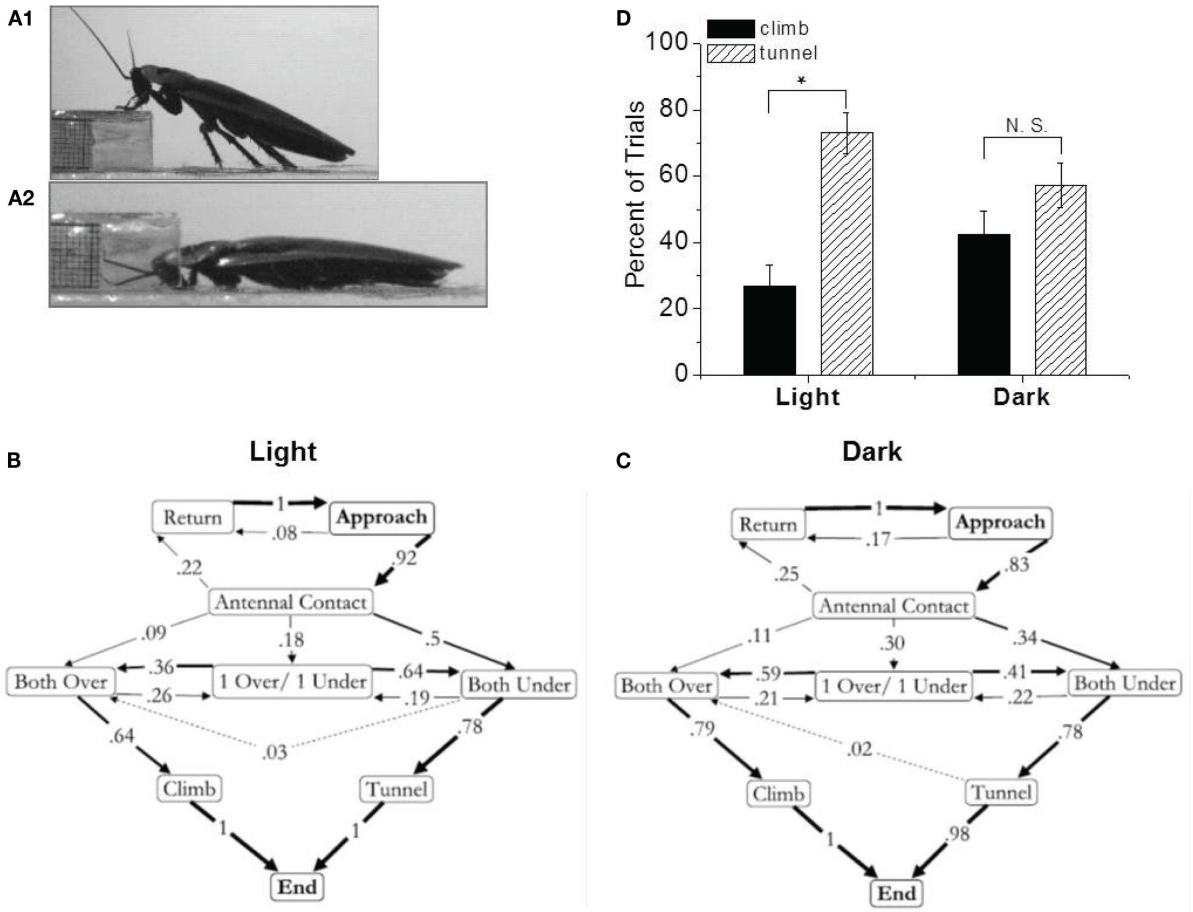

FIGURE 2 | helf climbing and tunneling is related to antennal contact. **(A)** Pictures of climbing **(1)** and tunneling **(2)** behavior. Ethograms of shelf behavior in the light **(B)**, and dark **(C)**. Arrows represent a direct transition from one behavior to the next. The number on the arrow and its thickness represent the frequency of that transition. Dotted lines were used when two or fewer individuals preformed a specific transition. Antennal position relative to the shelf was determined as being both over the shelf (over/over), both under the shelf (under/under) or if one antenna contacted the top of the shelf and the other contacted the underside the pattern was recorded as (over/under). **(D)** Climbing and tunneling in insects presented with a shelf under different ambient lighting conditions. Naïve cockroaches were placed in the experimental arena with an obstacle they could climb over or tunnel under. The light condition represented 56 trials (14 climbs and 42 tunnels). The dark condition represents 61 trials (26 climbs, 35 tunnels). The error bars represent the ±standard deviation (±SD; calculated using methods for binomial data). In the light, the climbing and tunneling percentages are significantly different ($p < 0.01$, χ^2 test). In the dark, this difference is not significant ($p > 0.5$, χ^2 test). From Harley et al. (2009).

freely in an arena or tethered over a Styrofoam ball occasionally touch an object. They then approach it and often climb onto it (Okada and Toh, 2000). The scapal hair plates at the base of each antenna appear to be critical to this behavior, since shaving them increased the time to approach the object in unrestrained insects and impaired turning under tethered conditions. These researchers further described the active sensing movements of the antennae under tethered walking and showed that the position of a wooden dole relative to the body axis was correlated with the turn angle (Okada and Toh, 2006). Antennal sensing has also been shown to be important in gap crossing in stick insects (Blaesing and Cruse, 2004) in combination with tactile information from the front legs. More recently, the active sensing movements of stick insect antennae associated with climbing have been described in detail showing that leg movements are re-targeted as a result of tactual antennal information (Schutz and Dürr, 2011).

What about other sensory cues? In order to test competing directional signals, we set up another task. The insect was placed in a T-maze and we examined where it ended up (Kathman et al., 2011). In this experiment a transparent acrylic T-maze was constructed and placed over a mirror. The entry track was 12 cm long and connected to the middle of a cross track that was 20 cm long. The mirror allowed us to record the cockroach's movements with a video camera at 60 fps. We then simply scored the number of times the cockroach ended up in the right or left arm of the T-maze then related those results to behavioral events such as the manner in which each antenna contacted the back wall. In some trials, computer generated moving black and white stripes were displayed on an LCD monitor placed behind the cross arm of the T-maze. Direction of the stripes was randomized. We then tested whether the visual pattern altered the number of times the cockroach followed antennal based turning rules.

In each trial, the cockroach walked down the entry corridor, touched the back wall with one antenna and 84% of the time moved to the opposite arm of the maze. That is, if the right antenna contacted the wall first, the cockroach ended up in the left arm and vice versa. The subject acted against this "touch-and-turn" rule in only 16% of trials, and the 84:16 ratio of turns away from the side of initial antennal contact is significantly different from chance ($p < 0.01$, Chi Square). With no other factors present, 50% of the time the cockroach ended up in the right arm and 50% in the left indicating that there was no inherent bias in the maze.

When we added the pattern of moving stripes to the back of the T-maze, the ratio of turning according to antennal contact was altered. If the stripes moved in the same direction as that dictated by the antenna touch-and-turn rule, there was little difference, the cockroach still predominantly turned away from the side where the antenna first contacted the back wall. If, however, the stripes moved in the opposite direction (e.g., stripes moved from right to left and the left antenna touched the back wall first) the tendency to turn with the antenna rule was reduced significantly ($p < 0.05$). Now only 60% of subjects turned with the antenna rule (down from 84%) and the incidence of turning against the touch-and-turn rule increased to 40% (up from 16%). This result suggests that an optomotor response generated by a pattern of moving stripes in the cockroach's visual field can countermand the antennal touch-and-turn rule on some trials.

In light of the topic of this volume on invertebrate decision making, it is important to ask whether these actions are really decisions or are simply reflex driven behaviors. Most if not all of the behaviors we have discussed to this point could be explained by relatively simple reflexes. Even where two outcomes are possible (movement over or under a shelf), the "decision" is based primarily on the manner in which sensory structures, in this case antennae, contact the object. Greater insight into the distinction between reflex driven behavior and real decision making may come from examination of this entire volume. However, our sense is that unquestionable "decision" processes will be uncovered as we consider behaviors in more realistic situations, where the insect is free to choose among several possibilities and those choices are affected by environmental and internal conditions. We will discuss this notion further at the end of this review in the context of observations of cockroach behavior in a larger arena. We believe that the results of the more constrained behaviors, described above, will be components of those decisions, but this remains to be resolved through future experimentation.

SENSORY INTEGRATION IN THE BRAIN

These behavioral observations demonstrate that multiple sensory factors are used to guide the insect's movement strategy (climb vs. tunnel) and direction (left vs. right turns). Clearly this requires a level of multi-sensory integration that must occur somewhere in the central nervous system. Given the use of head based sensors (antennae and eyes), the brain is a likely site for this process. Moreover, a considerable body of neurogenetic and electrophysiological data suggest that the central complex (CC) might play a role in this process (Huber, 1960; Strauss, 2002; Pick and Strauss, 2005; Ritzmann et al., 2008; Bender et al., 2010).

The CC is a set of interconnected neuropils situated in the midline region of the protocerebrum of virtually all insects (**Figure 3**; Homberg, 1987;Strausfeld, 1999, 2012; Wessnitzer and Webb, 2006). It includes the fan-shaped body (FB), ellipsoid body (EB), paired nodules and the protocerebral bridge (PB), which is dorsal and posterior to the FB, and links the two halves of the protocerebrum. Note: some laboratories use the notation of Central Body Upper (CBU) and Lower (CBL) divisions for FB and EB respectively. The FB and EB are believed to receive afferent fibers from multimodal sensory interneurons that in turn receive inputs from the various sensory neuropils of the brain. Fiber tracts link the EB and FB to the lateral accessory lobes (LAL), where contact is made with interneurons that descend to thoracic ganglia (Homberg, 1987, 2004) known to contain the local motor control circuits for walking and flying (Reichert and Rowell, 1985; Rowell, 1988; Burrows, 1996; Büschges and Gruhn, 2008; Büschges et al., 2008).

The CC is highly structured. In cockroach, the EB and FB each contain 16 columns, as does the PB (8 on each side of the midline). In histological sections, a very regular pattern of fibers can clearly be seen to connect the FB and PB of some species (Homberg, 1987; Strausfeld, 1999). Each pair of adjacent FB columns appears to receive inputs from two locations in the PB (Müller et al., 1997).

Based upon intracellular physiological and morphological data, Homberg and his colleagues developed a model that describes the flow of polarized light information from the FB or EB to the PB, and then out to the LAL and nodules of the locust (Heinze and Homberg, 2008, 2009; Heinze et al., 2009). Under this scheme, sensory information enters the FB and EB via tangential cells such as TL2 and TL3. Within the EB and FB are numerous polarized light sensitive columnar cells that form the columns and also project between neuropils (Vitzthum et al., 2002). In addition, tangential, amacrine, and pontine neurons cross columns horizontally within each neuropil (Müller et al., 1997; Heinze and Homberg, 2008). One horizontal class of polarized light neurons, called TB1's, has dendrites that are arranged topographically within the PB columns according to the E-vectors to which they are sensitive, suggesting a topographic map of polarized light (Heinze and Homberg, 2007).

Considerable amounts of data also suggest that the CC plays an important role in supervising locomotion (Strausfeld, 1999). Earlier electrical stimulation studies implicated the CC in motor control. Huber (1960) examined movements of crickets walking on a ball and found that stimulation of the mushroom bodies via fine copper wires inhibited locomotion, while stimulation in the CC generated increased forward movement and turning. Our own studies, which will be described below, are consistent with these observations. Genetic manipulations also pointed to a role for the CC in locomotion control, in that several mutants that disrupt one or more CC neuropils have locomotor deficits. A *Drosophila* mutant called *no-bridge (nob)* has gaps in the PB and shows decreased frequency of walking bouts (Strauss et al., 1992; Strauss, 2002). Furthermore, when these flies do walk, steps are smaller and changes in step frequency do not occur as precisely as in wild type individuals. During turning, they may stumble rather than making smooth turns. Two additional mutant phenotypes that affect the PB in *Drosophila* initiate locomotion at normal rates, but for shorter durations (Martin et al., 1999). More

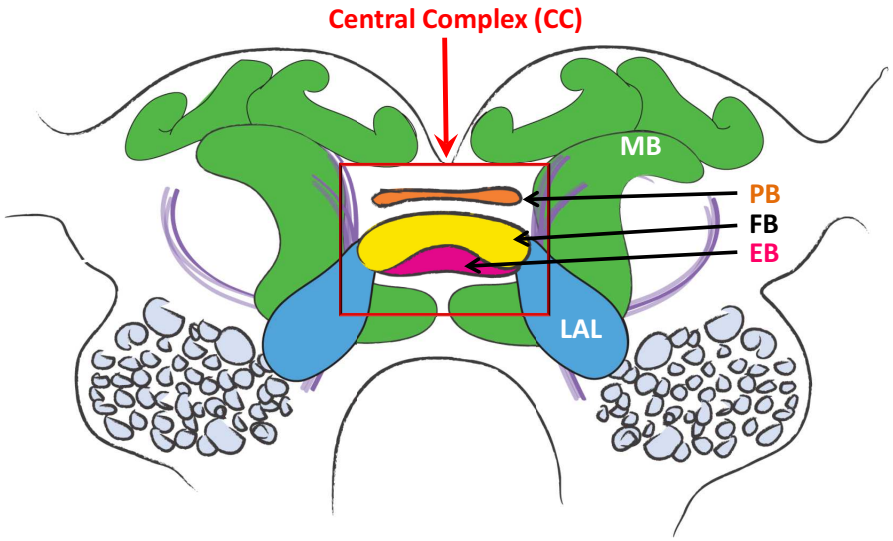

FIGURE 3 | Diagram of the cockroach brain. Central Complex is within red rectangle. PB, protocerebral bridge; FB, fan-shaped body; EB, ellipsoid body; LAL, lateral accessory lobes; MB, mushroom body.

recent neurogenetic studies indicate that a small subset of neurons in the EB, GABAergic ring neurons, are necessary for orientation memory in flies (Neuser et al., 2008) and that peptidergic neuromodulators can alter movements of flies within an arena (Kahsai et al., 2010).

With this background, we hypothesized that the antennal and visual cues that were critical to our barrier responses affect neural circuits within the cockroach CC that then alter movement through descending pathways. To test this hypothesis, we first had to establish that visual and mechanical sensory information reaches the CC. Because of the size of these structures (the combined EB and FB are ∼400 μm × 200 μm in *Blaberus discoidalis*), we chose to address this very basic question with an extracellular multi-channel recording technique (McNaughton et al., 1983; Ritzmann et al., 2008). This technique has proven to be very useful, in that it allows us to record from numerous neurons simultaneously for long periods of time. Indeed, with some modification, we can even maintain our recordings while the insect is moving on a tether. However, as with any extracellular method, multi-channel recording does not provide the specific identity of the recorded neuron that intracellular techniques yield. We are limited to knowing simply where the extracellular electrodes were located at the time of the recording. Thus, a full assessment of the electrical properties of CC neurons will eventually require both intracellular and multi-channel electrical methods.

Since our initial goal in these studies was to establish whether visual and antennal information even reached the CC, we began with a restrained preparation. The insect was placed in a tube with its head stabilized by a wax covered plate. Each antenna was threaded through a hook that was connected to a servo motor. The servos were controlled by a custom computer program which, in turn, was controlled by user defined scripts. These scripts instructed the servos, for example, to first move one antenna medially for a predefined distance and velocity, then pause 5 s and return it laterally, then repeat that sequence 10 times. It then reiterated this process for the other antenna and finally repeated the entire routine one more time for a total of 20 movements of each antenna in each direction.

As with our behavioral studies, we did not attempt to determine exactly which sensory receptors on each antenna were stimulated. Since the entire antenna was pulled, the basal segments moved back and forth. This movement would certainly activate the hair plates that were shown to be important in orientation studies on the American cockroach (Okada and Toh, 2000). However, we cannot rule out that other sensory structures were also affected. In an attempt to isolate the stimulus onto the strain sensors of the flagellar segments, we placed a second hook just below the one that was attached to the servo. This bent the flagellum at one of three locations. Responses that were recorded at each site were similar to those recorded when the whole antenna was moved.

A time mark was saved each time the servo was commanded to move so that electrical records could be lined up accordingly (**Figure 4**). In these experiments, recordings were made by inserting a 16 channel silicon iridium probe into the brain in the region of the CC. The 16 channels on these probes were arranged in four tetrodes that sampled axons simultaneously. The shapes of the action potentials recorded at the four electrodes within a tetrode allowed us to separate responses from individual units off-line using cluster cutting software such as MClust. More details on this procedure can be found in the supplemental text of Bender et al. (2010). Typically we can record activity for 5–6 h from 5–6 units at each tetrode for a total of 20–24 units. After the experiment, the location of the probe was identified histologically.

The multi-channel recordings clearly demonstrated that both antennal and visual information do in fact reach the CC neuropils (Ritzmann et al., 2008). Most of the units recorded in either the FB or the EB responded to lateral movements of one or both antennae (**Figure 4**). Of these, the majority responded to either antenna

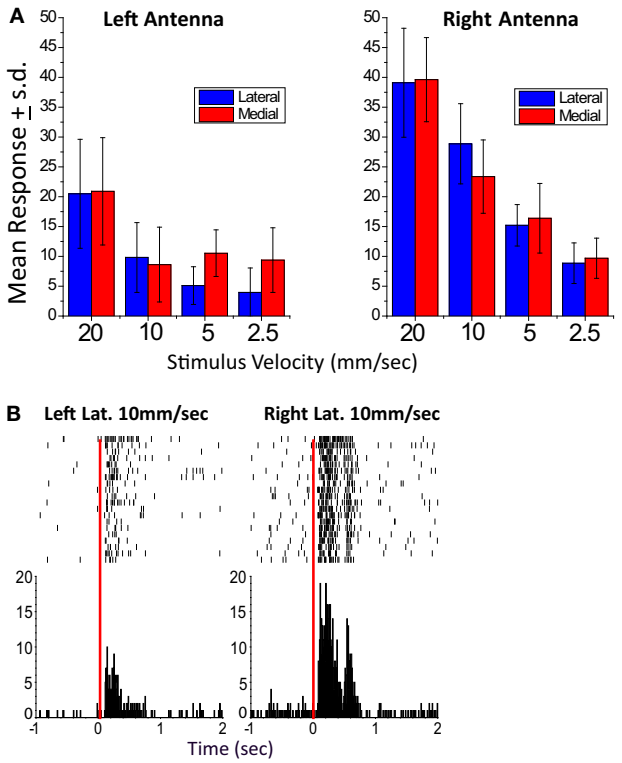

FIGURE 4 | A left right biased unit recorded in the EB. (A) Mean response graphs of lateral and medial stimulation of the left and right antenna. For this unit, all response values for stimulus paradigms of the left antenna were tested against the comparable paradigms of the right antenna (Student's *t*-test). All were significantly greater for the right antennae ($p < 0.01$) except right and left medial 2.5 mm/s which was significant at $p < 0.05$. **(B)**. Sample left and right lateral raster display (10 mm/s stimulus velocity). In both records, the raster plots are of 20 responses of Left antenna moved laterally at 10 mm/s (left) and Right antenna moved laterally at 10 mm/s (right). In each case the top row is the first trial and the bottom the last. The time marks indicate the time that an action potential in this unit occurred. All records were lined up on the time at which the stimulation servo was activated (time 0). The histograms underneath the raster plots show the sum of all action potentials in 10 ms bins. From Ritzmann et al. (2008).

with about one third showing a bias toward stronger activation from one or the other. We are confident that this bias was not the result of asymmetries in our stimulus system, because other units that were recorded simultaneously showed no bias or a bias to the other antenna. With these stimuli, we noted no spatial relationships between recording sites and response properties. The responses were typically velocity or acceleration sensitive. Velocity and acceleration sensitivity of CC units is important to the actual behavior, because when the cockroach touches objects with its antennae, it typically uses much lighter movements than those which we used in these initial experiments. Most antennal sensitive units were multi-sensory in that they also responded to changes in ambient light level. The majority of the visual responses were phasic, turning on with either increased or decreased light. Some were tonic units that showed much greater activity when the light was on than when it was off or vice versa.

More recently, we examined how neurons recorded in CC neuropils responded to moving stripe patterns (similar to those used in the T-maze experiments) that were projected onto a screen above the cockroach's head (Kathman et al., 2011). These experiments utilized the same recording and analysis techniques as those that were employed for recording antennal responses in CC units. Several units recorded in the CC responded to moving stripes and a small subset of those units were sensitive to the direction of movement. Since many of these units were also sensitive to imposed antennal movement, we were concerned that the responses that we attributed to antennal movement might actually have been responding visually to movement of the antennal stimulation hook as it passed over the insect's eyes. Indeed, for some units, responses persisted albeit at lower levels when the antenna was removed from the stimulation hook (Ritzmann et al., 2008). In these cases, the response was eliminated when ambient light was extinguished supporting the notion of a visual component. However, when antennal stimulation was conducted under these same dark conditions, the antennal response was typically unchanged. Thus, units within the CC appear to respond to *either* mechanical antennal stimulation or to visual cues with often little if any summation between them.

In the experiments described above, stimulation was imposed upon the antennae by the experimenter. In many systems sensory responses generated by the animal's own active tactile movements produce very different responses than imposed stimuli (Prescott et al., 2011). We have, therefore, begun to examine responses in the CC to antennal stimulation produced by the cockroach's own antennal movements toward an object (Guo et al., 2011). The recording techniques in these experiments employed two fine wire bundles that each formed a tetrode. These wire bundles are similar to those used in our locomotion studies described below and in Bender et al. (2010). Here, the cockroach is tethered in such a way as to permit normal walking and promote antennal searching movements toward a bar placed near its head. We used high-speed video to note when one of the insect's antennae touched the bar. We then compared the responses of these self-generated contacts with those that occurred in the same unit when the antenna was tapped by the experimenter. Many units did not respond to self-generated antennal contact even though they may have responded to imposed stimulation; however others responded to both classes of stimuli. Where there was a response to self-generated contact, it typically contained fewer spikes than the responses to imposed stimulation. This difference is to be expected given the velocity dependence that is described above (Ritzmann et al., 2008). It may well be that the sparse nature of the self-imposed stimuli reflects a more realistic pattern that, over the entire CC population, provides more spatial information than that implied by the results from our stronger imposed trials.

CC INFLUENCE ON LOCOMOTION

With the exception of the active sensing trials described above, our sensory studies were conducted in restrained preparations. In order to determine the relationship between CC activity and locomotion, we turned to a preparation in which the cockroach was tethered over a lightly oiled glass plate with a flexible plastic strip that allowed minimal up and down movement (Bender

et al., 2010). Under these conditions, the cockroach walked in place with normal leg kinematics (Tryba and Ritzmann, 2000). High-speed video recording then allowed us to determine when the cockroach walked and, furthermore, to document changes in step frequency. Although the probes we used in the sensory studies were too delicate for these experiments, we could achieve similar multi-channel recordings from fine wire bundles implanted in the brain in a tetrode arrangement.

We found several units that did change their response properties when the cockroach began to walk. Some of these units maintained their elevated firing level regardless of step frequency. However, other units in this class altered their firing rate along with step frequency (Bender et al., 2010). In order to examine this relationship more closely, we plotted two functions with time; the firing rates of each unit and the insect's step frequency (**Figure 5**). As the step frequency changed spontaneously during the course of a recording session, the neural firing and step frequency curves paralleled each other remarkably well, maintaining high correlation coefficients (**Figure 5A**). Indeed, for a few of these units, the correlation coefficients increased when the firing frequency curves were shifted forward several hundred milliseconds relative to the step frequency curve (**Figure 5B**). This observation implied that the changes in firing frequency in these units occurred *prior* to changes in stepping frequency and could be part of the descending commands that act upon thoracic local control circuits to evoke increased step frequency. To establish a causal link, we then stimulated through the same electrodes that had been used previously to record neural activity (Bender et al., 2010). This stimulation typically increased the step frequency dramatically often with delays that were similar to the shift in spike frequency that produced the maximum correlation coefficients.

More recently we examined CC activity recorded in response to self-generated antennal stimulation (Guo et al., 2011). In this case, we used a different tether in which the cockroach walked on an air-suspended Styrofoam ball. By monitoring the movement of the ball, we could relate firing rate to forward walking movement and speed as well as to left and right turning. A bar was placed near the cockroach's head where its antenna would occasionally tap it, causing the cockroach to turn. This paradigm simulates the touch-and-turn rule that we observed in the T-maze.

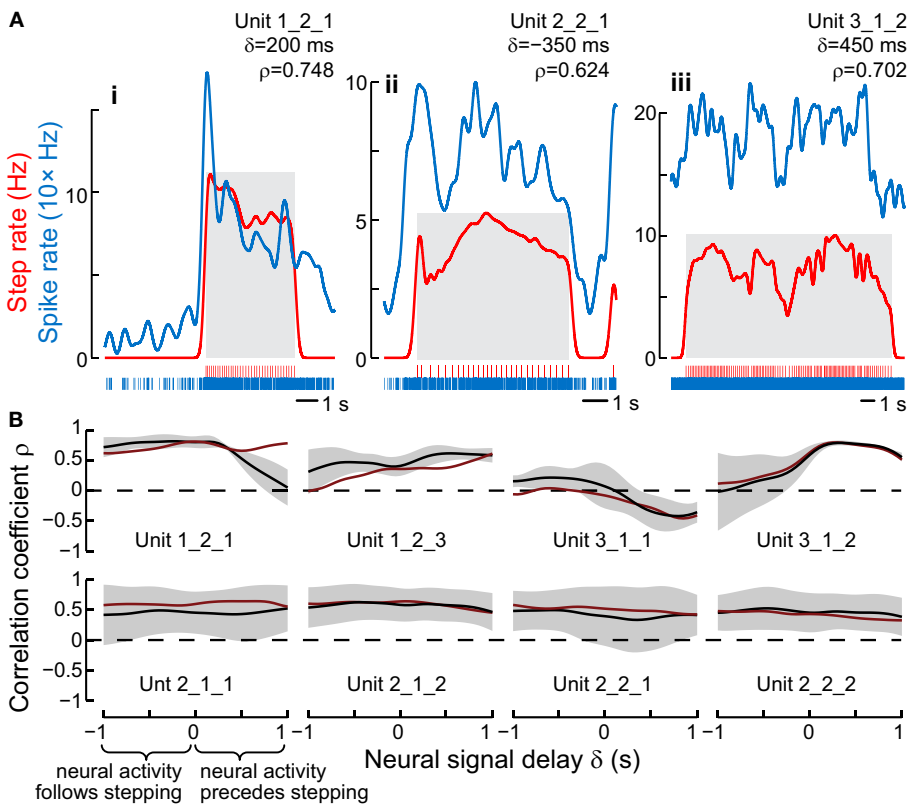

FIGURE 5 | Instantaneous stepping rate and neural firing rate were correlated in some units. (A) The spike and step rasters were convolved with a Gaussian kernel (SD = 150 ms) to calculate instantaneous frequencies. The firing rate (blue) was shifted to the right by δ and cross-correlated with the step rate (red), leading to the listed maximum value of ρ (correlation coefficient) for each walking bout (gray boxes). Some walking bouts were elicited by a tap to the animal's antenna, which evoked an additional response in some units **(i,ii)**. **(iii)** shows steps from an entire 16 s video in which the cockroach was walking before the recording started and continued after the camera's memory was filled. **(B)** The correlation coefficient ρ changed as the spike rate curve was shifted relative to the step rate curve. The black line shows the mean and SD envelope for ρ at each value of δ. These 8 units were the only ones with a peak average absolute value of the correlation coefficient (|ρ|) of at least 0.4. The top row shows units with a peak |ρ| at $\delta > 0$, meaning that changes in spike rate usually preceded changes in step rate. The units in the bottom row had flat curves with peaks at $\delta < 0$. The red lines show the mean ρ calculated after removing the first 1 s of each walking bout to eliminate possible artifacts of antennal stimulation. From Bender et al. (2010).

The recording procedures were identical to those used in Bender et al. (2010).

Here we found CC activity that preceded turning or changes in forward walking with some consistent spatial relationships within the CC. Units recorded on the midline of the EB or FB tended to increase firing rate prior to forward walking or turning in either the right or left direction. However, units recorded in lateral regions of the EB and FB only increased firing rate prior to turns to one direction or to that direction and forward movement. Turns in the opposite direction were not coupled to significantly elevated activity in these units. As with walking speed, stimulation in these regions consistently evoked turning movements.

It should be noted that any changes in motor activity caused by the influence of CC neural circuits must act through the local circuitry that exists in the thoracic ganglia. This factor brings another level of complexity to this hierarchical arrangement of the central nervous system. Space does not allow us to discuss the properties of thoracic circuits other than to comment that in stick insect inter-joint reflexes may reverse when that insect walks backward (Akay et al., 2007) or turns (Hellekes et al., 2012). Similar reflex reversals can be generated by severing both of the neck connectives, thereby, eliminating communication with all brain regions (Mu and Ritzmann, 2008). The reader is referred to the following reviews on local circuits that control leg movements in insects for further information (Ritzmann and Büschges, 2007; Büschges and Gruhn, 2008; Büschges et al., 2008).

IMPACT OF CC LESIONS ON BEHAVIOR

Our recordings within the CC suggest that the tactile and visual information that we identified as important decision making factors in negotiating objects in the animal's path are indeed processed within CC neuropils. Moreover, the walking preparations suggested that units recorded in these same neuropils can alter firing rate prior to changes in stepping frequency or directional movements, while activation of these units can evoke similar locomotory changes. With that information, we felt that it was necessary to return to our behavioral studies and ask whether manipulation of the CC neuropils could alter the various responses to barriers. Our results using electrolytic lesions and more recently reversible pharmacological silencing of neural activity complement and are consistent with *Drosophila* studies that use neurogenetic techniques to alter CC function (Pick and Strauss, 2005; Kahsai et al., 2010; Triphan et al., 2010).

We first examined the behavioral consequences of large mechanical lesions generated by inserting a foil lance into the CC or making sagittal cuts along the midline (Ridgel et al., 2007). These manipulations clearly showed major behavioral deficits associated with large scale damage within the CC. However, we felt that more discrete lesions were required to determine whether controls of individual behaviors are restricted to specific regions of the CC.

To accomplish this goal, we developed an electrolytic lesioning technique which could generate smaller lesions in discrete areas of the CC neuropils (Harley and Ritzmann, 2010). We examined the behavioral effect of a large number of these electrolytic lesions generated both within CC neuropils and elsewhere in the brain. Several behaviors were studied including climbing over blocks, climbing over or tunneling under a shelf, walking up a vertical wall then transitioning to a horizontal surface, and walking in a U-shaped track that required the animal to execute two turns typically generated by antennal contact. Each animal was tested before the lesion (pre-test) and after recovery from the surgery. Then the site of the lesion was identified histologically. The differences in each behavior were quantitatively scored and related to the lesion site.

Most of the behavioral effects that we recorded in these experiments were restricted to the CC and for some behaviors, to specific regions of the CC. Lesions outside the CC tended to have little or no behavioral consequences in our tests. Controls in which the lesioning probe was inserted into the CC but current was not applied could generate some deficit but always at much lower levels than the electrolytic lesions. The spatial effects within the neuropil were demonstrated particularly well for turning behaviors associated with lesions within the FB (Harley and Ritzmann, 2010). Here 11 lesion sites were generated in separate animals. Seven of these sites were in lateral regions of the FB, while four were near the midline (**Figure 6**). The lateral lesions produced a significant increase in mistakes as the cockroaches navigated the U-shaped track. That is, when the cockroach encountered a section of wall that bent to the right, it should have turned in that direction to follow along the wall. However, a significant number of individuals with lesions in the lateral FB turned in the wrong direction (e.g., a bend to the right resulted in a turn to the left and into the wall). In contrast, the midline lesions produced no turning mistakes, but did result in errors in bilaterally symmetrical behaviors such as climbing over blocks or dealing with the shelf.

These electrolytic lesions demonstrated that CC neuropils play a role in controlling changes in locomotion. Although our controls supported the specificity of the lesions to CC neuropils, they came with the caveat that the probe may have damaged some tissue as it was inserted into the brain. We, therefore, sought to find a technique for *reversibly* silencing regions of the brain. We reasoned that reversible deficits could not have been caused by any permanent damage done during surgery or as probes were inserted into the brain.

We turned to a procaine injection technique that had been used by other laboratories to generate reversible deficits in other regions of the brain (Devaud et al., 2007; Gal and Libersat, 2010). Procaine is a voltage gated Na^+ and K^+channel blocker that reversibly silences action potentials. We pressure injected 20%procaine mixed with fluorescein dextran into the CC neuropils while recording with our multi-channel electrodes and found that it silenced all units in the immediate region of the injection site for about 30 min (Pollack et al., 2011). The fluorescein allowed us to identify the injection site histologically (**Figure 7C**). In some cases the effect was restricted to units recorded at one pair of tetrodes. In locust, glial sheaths surround the CC and also project into CC neuropils (Boyan et al., 2011, 2012). We observed similar structures in cockroach which could have blocked migration of the drug to the other tetrodes After 30 min, activity returned to what appeared to be normal levels.

The behavioral consequences of procaine injection in the CC were profound (Pollack et al., 2011). For behavioral studies, we injected 20% procaine in saline with single 120–150 ms pressure

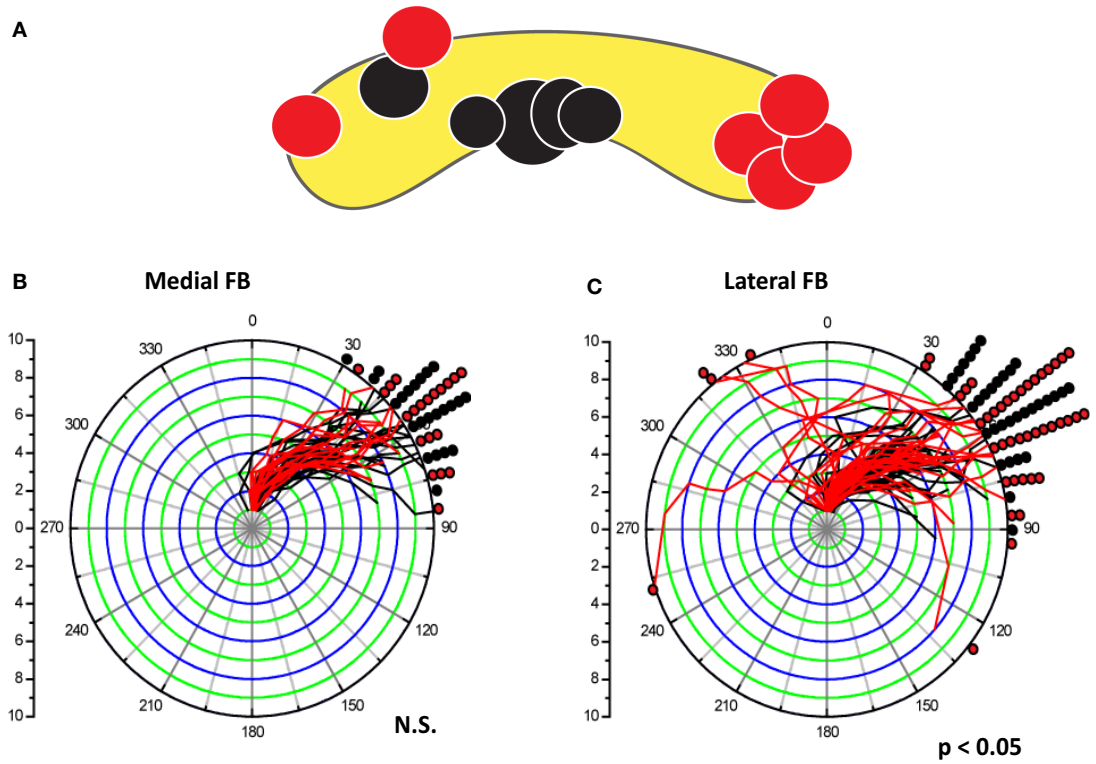

FIGURE 6 | Lateral lesions in the FB affected turning behavior in a U shaped track, but medial lesions in the FB did not. (A). Location of lesions within the FB. Black dots showed no significant change in turning behavior between pre-tests and post-lesion trials. Red dots indicate lesions that produced 2 SD changes in the behavior between pre-test and post-lesion trials. **(B,C)** compare turning behavior of all pre- and post-lesion trials. For each trial, the change in turning angle of the cockroach body was measured relative to the position at antennal contact (plotted at the center of the polar plot) and at each subsequent step (annuli) made by the middle legs. Regardless of the direction of movement, curves that bend to the right indicate turns in the expected direction (away from the side-wall). Curves that move to the left would be cases where the insect turned into the wall. Any lines moving from the origin to the 0 angle would indicate no turn at all (no trials in these cases). The pre-lesion traces (black) were plotted under the post-lesion (red) curves. The final position data were divided into 10° bins. Black and red dots were used to mark the frequency of each final position. All pre-lesion trials turned in the expected direction. **(B)** All post-lesion curves for medial FB lesion were similar to pre-lesion curves. In contrast, post-lesion traces (red) in individuals with lesions in the lateral FB **(C)** showed increased variability after the lesion with several turns into the wall or turns that start in the wrong direction well before correcting later in to the trial. Data from Harley and Ritzmann (2010).

pulses or multiple pulses of 30–40 ms. Within 10 min of the injection, the cockroach's walking and navigational behaviors were dramatically reduced. In the T-maze, insects failed to turn when their antennae contacted the wall (**Figure 7A**). Rather, they tended to walk along the wall with their heads pressed against it, something that normal intact cockroaches rarely, if ever, do. At 30 min after injection, the animals showed some recovery and by 60 min, they were close to normal behavior. On the ball tether, procaine injected cockroaches either failed to walk at all or walked straight ahead even as moving stripe patterns that routinely generate continuous turning movements were projected onto a screen in their visual field (**Figure 7B**). Again, within an hour, the cockroaches had begun to show signs of recovery. In both of these paradigms, injection of saline in the same manner had no effects.

WHAT IS THE CC'S ROLE IN NAVIGATING COMPLEX TERRAIN?

Our data point to a role for CC neuropils in decisions made by cockroaches as they navigate complex terrain. Our behavioral data demonstrate that mechanical antennal information along with visual cues guide these decisions as the cockroach walks through a track and encounters a barrier. These types of sensory cues are processed in CC neuropils where units are found whose activity appears to influence locomotory changes. Moreover, both permanent and reversible lesions in the CC have dramatically altered these same behaviors.

These results strongly suggest that activity descending from the CC interacts with local control circuits in the thoracic ganglia to re-direct leg movements. They are consistent with neurogenetic reports regarding the role of CC neuropils in *Drosophila* locomotion (Strauss, 2002; Pick and Strauss, 2005). But the question still remains, what is the precise role of the CC in this command structure? Certainly, simple orientation movements such as escape or wall-following could take place with much simpler reflex circuits. Most of the circuitry that controls escape turns in the American cockroach, *Periplaneta americana*, resides in the thoracic and abdominal ganglia (Ritzmann and Pollack, 1988, 1990; Ritzmann, 1993; Ritzmann and Eaton, 1997). Although activity descending

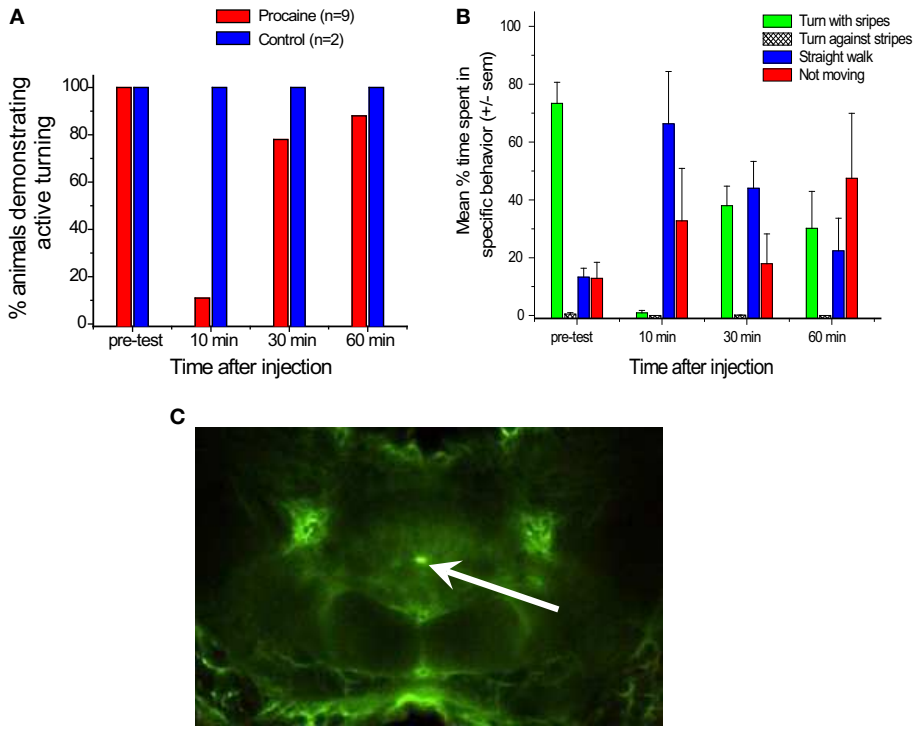

FIGURE 7 | Injection of procaine into the central complex alters turning behaviors. (A). Nine cockroaches were tested for active turning movements in the T-maze before and after injection of 20% procaine (red bars) or saline (blue bars). "Active turns" were defined as cases where the cockroach rotated its body after touching the wall with its antenna but before its head hit the wall. These movements contrasted with passive actions, where the head hit the wall and moved along the wall's surface as the cockroach appeared to try to walk forward. Some passive turning movements occurred while these cockroaches continued to force themselves forward, but were easily distinguished from active movements. Note that prior to injection, all trials resulted in active turning, but 10 min post-injection the percentage of active turning was greatly reduced. Recovery started at 30 min and was nearly complete at 60 min. Two controls in which saline was injected into the central complex in the same way showed no changes after the injections. **(B)**. Responses of five cockroaches walking on an air-suspended ball to a moving stripe pattern in their visual field. Bars indicate average percentage of time spent turning with the stripes (green), turning against the stripes (hatched), walking straight ahead (blue) or not moving at all (red). Note that in the pre-test most trials showed the cockroach walking with the moving stripes, while 10 min after injection of 20% procaine few trials followed the stripes and many more showed straight or no walking. At 30 and 60 min, there was some recovery, but not completely back to normal. **(C)**. Confocal image of a brain injected with procaine mixed with fluorescein dextran for six pulses at 40 ms and 30 ψ. The bright fluorescein dot at the center of the central complex between the fan-shaped body and ellipsoid body shows the injection site.

from the head ganglia does affect escape responses (Fouad et al., 1994; Schaefer and Ritzmann, 2001; Libersat et al., 2009), the turn direction arises primarily from the direct influence of directionally sensitive ventral giant interneurons on thoracic ganglion circuits (Levi and Camhi, 2000a,b). Antennal responses to tactile stimulation activate descending interneurons that evoke similar escape turns (Burdohan and Comer, 1996; Comer et al., 2003; Ye et al., 2003), but again, these interneurons appear to act through fairly simple direct activation of the thoracic interneurons that generate the escape turns. Similar antennal circuits could be envisioned to control rapid turns made by cockroaches as they run while maintaining contact with a wall (Camhi and Johnson, 1999; Cowan et al., 2006). If these rapid turning movements could be controlled by simple almost reflexive connections from sensors to local motor circuits that control legs, why does the cockroach, or any other insect, need something as large and intricately structured as the CC?

The answer to this question could reside in the multi-sensory nature of the behaviors that are disrupted by CC manipulation (Harley et al., 2009; Harley and Ritzmann, 2010). But here again, the escape system also is multi-sensory. The cockroach can escape equally well from tactile stimuli as from wind directed at the cerci (Comer et al., 1994; Schaefer et al., 1994; Stierle et al., 1994), and again this capability stems from convergence of tactile interneurons with the same thoracic interneurons that receive input from the wind sensitive ventral giant interneurons (Pollack et al., 1995).

The difference between the behaviors that are associated with direct sensori-motor connections in the thoracic ganglia and the kinds of behaviors that the CC is involved in may be temporal. Control of escape movements or even rapid wall-following must occur in the millisecond range. But foraging decisions can occur over much longer time frames. A study of walking speeds in a large arena demonstrated that the cockroach spends most of its time walking relatively slowly around in its environment exploring with its antennae while taking in tactile, visual, and olfactory cues, and then moving accordingly (Bender et al., 2011). Indeed, walking in that arena clustered around two speeds (**Figure 8**); a slow ambling gait (<10 cm/s) and a less common faster trotting

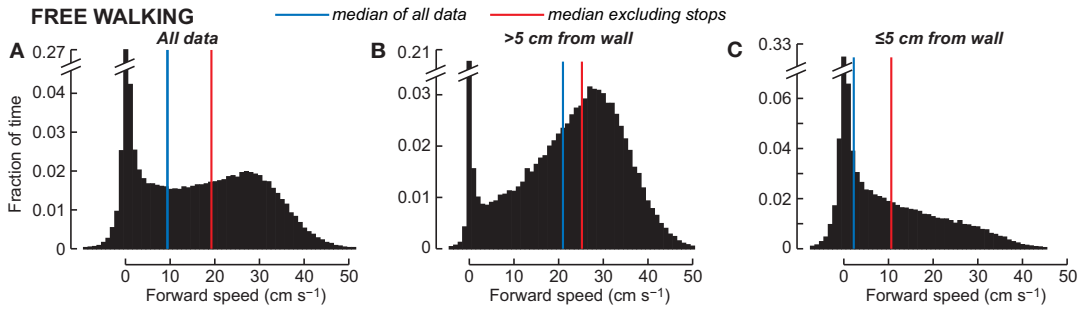

FIGURE 8 | Forward walking speed in the empty arena. (A) Pooled histogram from 44 animals, ∼1 min of walking each at 20 samples s^{-1}. **(B)** Speeds when the animal was more than 5 cm from a wall, approximately the radius of antennal contact ($n = 31,200$ samples). **(C)** Speeds when the animal was less than 5 cm from the nearest wall [**(A,B)**; $n = 28,464$ samples]. The blue vertical lines indicate the median walking speed in each histogram; the red lines show the median speed when data with an absolute value less than 1 cm/s are excluded. From Bender et al. (2011).

gait (∼30 cm/s). Even these trotting gaits occurred at speeds well below the median escape velocities recorded in the open arena (41.1 cm/s). A breakdown of the time that the cockroaches spent moving at these different speeds showed that when they were near the wall of the arena (their preferred location), most of their movements were in the slowest range. When they were in the middle of the arena, they sometimes changed to the faster trotting gait, possibly to get back near a wall. Thus, the cockroach spends most of its time moving slowly through its environment examining objects and reacting accordingly. The rapid movements associated with escape are, in fact, rare occurrences that happen only in the face of an imminent threat. So, while there is no question that these rapid behaviors are critical to the cockroach's survival and are very useful experimentally in working out reduced neural circuits and biomechanical properties (Jindrich and Full, 2002; Koditschek et al., 2004), we need to appreciate the relatively small part they play in the insect's behavioral life. Indeed, even in escape responses, it may only be the initiation of movement that is evoked by these relatively simple connections. More recent analysis of cockroach escape demonstrates that after the initial turn, movements become unpredictable (Domenici et al., 2008). Moreover, in *Drosophila* leaning movements directed away from a visual threat before the escape is triggered appear to incorporate some degree of motor planning that may involve the CC (Card and Dickinson, 2008).

When we begin to consider the slower and more complex decisions that occur during normal foraging, new hypotheses begin to arise for the role of the CC and other brain structures. In contrast to relatively simple escape circuits and behaviors, the CC seems to be positioned to take in massive amounts of information about an individual's surroundings and possibly also its internal state, then use this information to influence movement so as to match locomotory changes to current environmental and internal context. It is possible that CC circuits do not, on their own, evoke any changes in movement. Cockroaches with lesions within the lateral regions of FB, that were associated with most of the turning errors we scored, still showed many correct turns (Harley and Ritzmann, 2010). Thus, even these lesions did not completely disrupt the insect's ability to turn properly. Moreover, the neurons that exit the CC do not typically connect directly to the thoracic ganglion but rather project to areas such as the LAL where they encounter more direct descending neurons (Heinze and Homberg, 2008). This pattern suggests that CC circuits *influence* descending commands rather than evoke them directly. This model is consistent with Strausfeld's (2012) notion that the CC serves as a "brain within the brain" to integrate information about what is currently occurring and then fine tune behavior to current conditions. He points to observations in the wasp sting story as support. The jewel wasp, *Ampulex compressa,* stings an adult cockroach first in the prothoracic ganglion and then in the brain (Fouad et al., 1994). The first sting briefly paralyzes the cockroach, but the more long-term second sting prevents volitional or escape movement rendering the cockroach a virtual automaton. In this state, the wasp will pull on the cockroach's antennae and the cockroach will then follow the wasp into its nest where it will be entombed with the wasp's egg to serve as food for the new, developing wasp. Studies using radioactive tracers demonstrate that this second sting does in fact inject venom near the CC and mushroom bodies (Haspel et al., 2003).

If the CC plays a role in matching movement to external and internal states, one would expect that modulatory substances would influence the states of CC circuits and that appears to be the case. Numerous neuromodulators and their receptors have been found within the CC's of various insects (Homberg, 1991; Nässel and Homberg, 2006). One study used genetic tools to manipulate the transmission of *Drosophila* tachykinin from interneurons that innervate the CC (Kahsai et al., 2010). The affected flies significantly reduced their tendency to avoid the central zone of a test arena. The authors concluded that ". . .peptidergic pathways in the CC have specific roles in the fine tuning of locomotor activity in adult *Drosophila*." Consistent with these neuromodulatory effects, we noted that response patterns of units recorded in the CC during our multi-channel studies often varied dramatically over the course of a 5 h recording session (Ritzmann et al., 2010). These changes were typically not consistent from one unit to another even when they were recorded simultaneously. Rather some units increased sensitivity to antennal stimulation while others decreased or stayed the same. This finding could suggest that the CC moves through various different states in response to physiological transients (e.g., hunger, thirst, fatigue, aggression or attention) which could be associated with release of neuromodulators

or hormones. Similar state changes are well-known in more thoroughly studied structures such as the stomatogastric ganglion of crustacea (Harris-Warrick et al., 1992; Meyrand et al., 1994). Thus, it is possible that circuits within the CC monitor sensory cues surrounding the insect as well as internal physiological state and then modify descending commands to match decisions to current context.

If the principal role of the CC is to fine tune foraging movements in complex environments, we may have to move to more complex behavioral paradigms to understand it. The behaviors that we have examined so far required the cockroach to make only limited choices. We designed those experiments so that we could deal with manageable variables in our behavior and lesion studies, however, they may not have taxed the cockroach enough to reveal the CC's primary function.

Movement in an Arena: For this reason, we began to observe cockroach behavior in a more enriched situation. We allowed them to seek a darkened shelter in a well-lit 90 cm × 90 cm arena. Most cockroaches (including *Blaberus*) are extremely photonegative (Meyer et al., 1981; Okada and Toh, 1998; Canonge et al., 2009), and thus the dark shelter attracted the animals in about half the time than would be expected based on control trials in an empty (no shelter) arena (Daltorio et al., 2012). However, as noted above, they are also known for thigmotaxis (Okada and Toh, 2006; Halloy et al., 2007; Nishiyama et al., 2007), spending much of their time along the walls of such arenas while relying on antennal contact to maintain a constant wall proximity (Camhi and Johnson, 1999; Cowan et al., 2006). Thigmotaxis would dictate staying on the wall while photonegative tendencies would have the cockroach leave the wall and move directly to the shelter. How are these seemingly conflicting behaviors resolved? Is an environmental map required? Does the cockroach plan the best paths? Alternatively, can the total behavior occur as a result of the insect continually updating the direction of its movements based upon some relatively simple rules?

When the cockroaches encountered a wall in the arena, they generally followed it according to the touch-and-turn rule observed in the U-track and T-maze. Occasionally, they changed direction along the wall or departed it to explore the interior of the arena (**Figure 9A**), however, these actions did not appear to be specifically directed toward the shelter as might be expected if they were using an internal map or some other long-term strategy. Rather, they seemed to be continuously updating their situation relative to the competing goals of wall-following and shelter-seeking.

To test the hypothesis that this behavior did not rely on an internal map or long-term strategy, we fit the insects' continuous turning movements to a biased persistent random walk (**Figure 9B**). The insect was modeled as having a finite group of states: "pivot" or "straight" in the center of the arena and "follow wall," "turnaround," or "depart wall" when along the wall. Each state had an associated behavior: walk in a line for "straight," turn in place for "pivot," and maintain constant wall distance for "follow wall," etc. Transitions from one state to another occurred stochastically based on state transition rates, which were extracted from the cockroach data. For example, to quantify the connection between the "follow wall" state and the "depart wall" state, we measured the wall departure rate of two departs per meter by counting the number of times animals left the wall and dividing by their total path length along the wall. Since we measured the speed of the animal along the wall to be ∼0.09 m/s, over a given time period of say, 05 s, there is a 2∗0.09∗0.05 = 0.9% chance of a wall departure. To

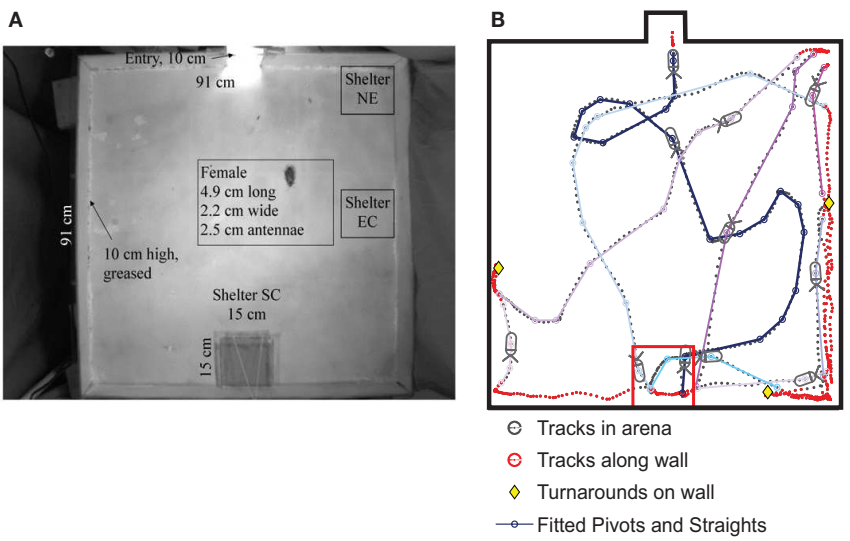

FIGURE 9 | (A) Cockroaches were released at an entry point (top) of an enclosed well-lit arena and permitted to seek darkened shelters in three locations: 33 trials with the shelter in the center of the midback wall (referred to as SC), 17 trials with the shelter in corners closest to the top (NE), 40 trials with the shelter along the side-wall (EC), and 44 trials with no shelter, as a control. **(B)**. The cockroach's path was tracked using the CTrax automated tracking system. A sample track shows the cockroach arriving at the SC shelter after a series of three turns, which we fit to pivots and straights. It then left the shelter and followed several additional (colored) tracks out to the arena and back to the shelter. Those later tracks spent more time along the walls, which is typical of cockroach behavior in the arena. From Daltorio et al. (2012).

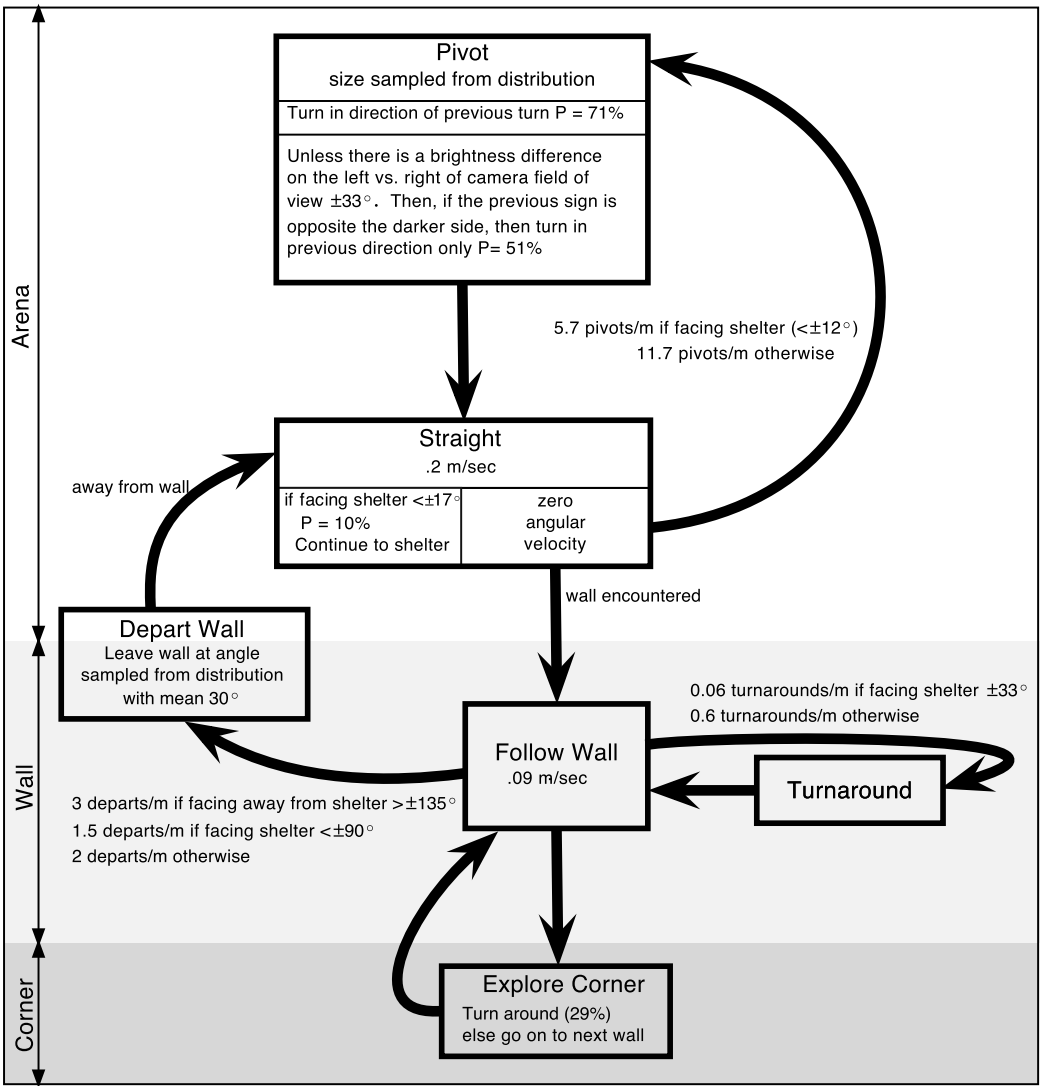

FIGURE 10 | roposed state based diagram of RAMBLER, randomi ed algorithm mimicking biased lone exploration in roaches. There are states for along the wall, in the corner, and in the arena. "Follow Wall" continues until a turnaround or departure is randomly selected at shelter-orientation dependent rates or until a corner is encountered. "Straight" continues until a wall is encountered or a pivot is randomly selected. Based upon figure in (Daltorio et al., 2012).

understand how vision affects wall-following, we parsed the data in MATLAB by the angle to the dark shelter for three different shelter locations (**Figure 9A**) and compared the results to empty arena data. We found that the animal was only more likely to leave the wall when the shelter was behind the animal (at egocentric angles of 144–180°). Similarly, the fitted insect tracks provided us with transition rates between the other states. The manner in which these parameters vary with the perception of the shelter defines our model, RAMBLER (Randomized Algorithm Mimicking Biased Lone Exploration in Roaches; **Figure 10**). Specifically, we found the following trends to be statistically significant (90% bootstrap confidence intervals not overlapping):

1. Depart wall more frequently if the shelter is behind the insect
2. Change direction on the wall less when facing a darkened shelter
3. Turn less when facing a darkened shelter (e.g., two long straight periods in initial track of **Figure 9B**)
4. Turn more to counter previous turn if the shelter is detected on the opposite side (e.g., last turn in the first track of **Figure 9B**)

When we simulated the entire model with and without a shelter, we found that these trends were sufficient to capture much of the shelter-seeking bias. **Figure 10** is a diagram that depicts the state-based algorithm that, in simulation, captured the behavior of the cockroach in the shelter-seeking task (Daltorio et al., 2012). The success of this model supports the hypothesis that insect decision making (at least in this context) is based on current perception rather than a multistep map-based plan. It is interesting to note that this model required little memory and we found no evidence that the cockroach was planning its route.

The level of complexity of the RAMBLER algorithm is an indication of the multi-sensory dependencies we expect to find in the insect's brain. In our trials, the animal neither blindly followed the wall nor perfectly tracked the goal. When wall-following, the animal was more likely to depart when the shelter was behind it. This shows that even while relying on antennal feedback to maintain the proper wall-following distance, the insect evaluated the changing visual response to decide when to leave the wall. When the antennae were not in contact with the wall, the turns the insect made may at first glance appear random. However, our analysis showed that when the shelter was in front of the cockroach, it turned away less frequently, and was more likely to correct turns away from the shelter. The presence of a visual goal seemed to modify the normal turning and wall-following behaviors to correct for undesirable changes in the animal's perception. Eventually, the subjects almost always reached the shelter, but they did not always stay there (**Figure 9B**). Indeed, they often left and returned several times. This observation in itself suggests that the cockroach was not dominated by a single-minded goal to reach the darkened shelter, but rather continuously considered several factors as it moved.

For this task, a more reflex driven algorithm that directed the cockroach toward the shelter once it saw it might actually be more efficient. However, as more competing goals (food plus shelter plus mates) are added into a more complex environment with barriers to those goals, the more directed model may not be as robust as the process that the cockroach appeared to utilize. These more complex situations would probably be closer to what the insect faces in nature.

As we begin to understand this relatively simple environment, we will add more features to try to capture the decision making that the cockroach uses as it forages in natural environments. We also must consider social interactions that occur when multiple cockroaches are present (Halloy et al., 2007; Jeanson and Deneubourg, 2007). Finally, we hope to apply the neurobiological recording, lesion, and procaine techniques to these studies. Meanwhile, we have implemented RAMBLER on a small, wheeled robot to navigate unknown environments with visual goals and tactile barriers, which it does in a remarkably insect-like fashion (Daltorio et al., 2012).

CONCLUSION

Our research on decision making in cockroach locomotion has followed a very multi-level approach. We are convinced that a thorough understanding of how insects deal with the challenges of moving through natural environments requires all of the experimental paradigms that we and others have at our disposal. We start with behavior that leads to neurobiological hypotheses that are tested with electrophysiological techniques. The results of these experiments suggest that specific regions of the central nervous system play important roles in controlling these behaviors, leading to lesion studies that examine behavioral deficits. While relatively simple behavioral choices play an important role in defining the decision processes, we feel very strongly that we must also examine more realistic situations that truly capture the parameters of foraging behavior. Other studies such as the neurogenetic observations in *Drosophila* greatly influence our thinking. We are a long way from understanding the exact role of the CC in this process, but we believe that we are on the right track.

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The dilemmas of the gourmet fly: the molecular and neuronal mechanisms of feeding and nutrient decision making in *Drosophila*

Pavel M. Itskov and Carlos Ribeiro*

Behaviour and Metabolism Laboratory, Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, Lisbon, Portugal

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Frédéric Marion-Poll, AgroParisTech, France
Greg Suh, NYU School of Medicine, USA

****Correspondence:***
Carlos Ribeiro, Behaviour and Metabolism Laboratory, Champalimaud Neuroscience Programme, Champalimaud Centre for the Unknown, Avenida Brasília s/n, 1400-038 Lisboa, Portugal.
e-mail: carlos.ribeiro@neuro.fchampalimaud.org

To survive and successfully reproduce animals need to maintain a balanced intake of nutrients and energy. The nervous system of insects has evolved multiple mechanisms to regulate feeding behavior. When animals are faced with the choice to feed, several decisions must be made: whether or not to eat, how much to eat, what to eat, and when to eat. Using *Drosophila melanogaster* substantial progress has been achieved in understanding the neuronal and molecular mechanisms controlling feeding decisions. These feeding decisions are implemented in the nervous system on multiple levels, from alterations in the sensitivity of peripheral sensory organs to the modulation of memory systems. This review discusses methodologies developed in order to study insect feeding, the effects of neuropeptides and neuromodulators on feeding behavior, behavioral evidence supporting the existence of internal energy sensors, neuronal and molecular mechanisms controlling protein intake, and finally the regulation of feeding by circadian rhythms and sleep. From the discussed data a conceptual framework starts to emerge which aims to explain the molecular and neuronal processes maintaining the stability of the internal milieu.

Keywords: behavior, sensory systems, feeding, olfaction, taste, neuromodulators, neuropeptides, internal state

INTRODUCTION

In order to survive and reproduce animals must provide themselves with an adequate supply of energy and nutrients. Under this selective pressure animals have evolved highly sophisticated and diverse repertoires of behavior to obtain food. This is especially evident in insects, which exhibit a vast variety of feeding habits some of which have been conserved through evolution between insects and mammals. Insects prefer sweet compounds (Dethier, 1976; Gordesky-Gold et al., 2008; Masek and Scott, 2010) and reject bitter substances (Dethier, 1976; Sellier et al., 2011; Weiss et al., 2011). They modulate their food preference to compensate for the lack of salt (Trumper and Simpson, 1993; Simpson, 1994, 2006) and amino acids (Simpson and Abisgold, 1985; Simpson and White, 1990; Simpson et al., 2004; Mayntz et al., 2005; Ribeiro and Dickson, 2010; Vargas et al., 2010). Furthermore, feeding habits of insects have a strong ecological, economical, and medical impact, making them highly relevant for humans. Their impact can be negative and positive: while locusts and aphids are devastating agricultural pests and blood sucking makes mosquitoes vectors of deadly diseases, agriculture would be impossible without pollinating insects.

In this review we provide an overview of the neuronal and molecular mechanisms regulating insect feeding decisions. A comprehensive description of feeding behavior in blowflies was given by Dethier (1976). We focus on the feeding behavior of the adult *Drosophila melanogaster*, since the powerful molecular genetics of this model organism has provided the scientific community with many insights into the mechanisms of insect feeding behavior.

METHODS FOR MEASURING FEEDING AND RELATED BEHAVIOR IN INSECTS

Feeding research relies on precise and robust measurements of food intake and feeding associated behaviors. In insects, especially in *D. melanogaster*, this is challenging due to the small size of the animals and the minute quantities of food they consume (Wong et al., 2008, 2009). Despite these challenges several methods have been developed to measure food intake, behavior associated with feeding or the activity of neurons involved in food consumption.

A classic approach is the two color choice assay to measure food preference (Tanimura et al., 1982; Ribeiro and Dickson, 2010; Dus et al., 2011). This assay (**Figure 1A**) is simple and allows high-throughput screening (up to 400 assays per person per week). For this test, flies are left to feed for a predetermined time from two different agarose food sources containing tastants mixed with different non-absorbable dyes. A qualitative readout can be achieved *post hoc* by visually scoring the color of the abdomen of the flies. To achieve a quantitative readout, the content of the digestive tract can be measured with the help of a spectrophotometer. Obviously, the use of one food source alone allows the quantification of food intake in a non-choice situation. A major disadvantage of this assay is that it does not allow dynamic monitoring of food intake across time as it normally relies on scoring dead flies, and does not take into account the food excreted by the flies.

An approach related to the colorimetric method is the use of a radioactively labeled food source for the acute measurement of food intake (Carvalho et al., 2006). This approach (**Figure 1B**) overcomes the background signal originating from the fly tissue in the spectrophotometric readout and is therefore more sensitive

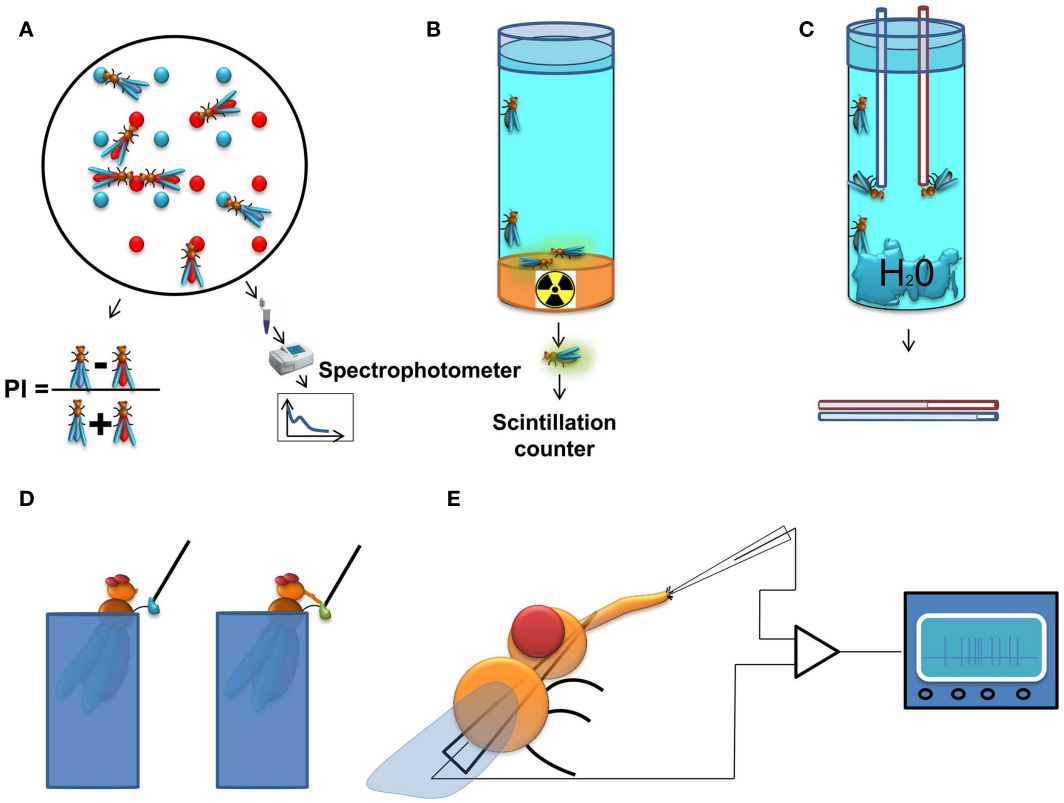

FIGURE 1 | Methods to study feeding behavior in *Drosophila.* **(A)** Two color food choice assay. Different sources of food are mixed with different dyes. The color of the abdomen of the flies is examined afterwards and a population preference index is calculated, or as an alternative approach, the dye content in the flies is determined using a spectrophotometer. **(B)** Radioactive food assay. Flies are kept on radioactive media and subsequently, the quantity of food consumed is measured with a scintillation counter. **(C)** CApillary FEeding assay. Several flies are kept in vials with a source of water and capillaries filled with food. The amount of food consumption is monitored by measuring the level of the meniscus in the capillaries. The assay can be used with either a single capillary to measure gross food intake, or with multiple capillaries with different food sources, thus providing quantitative information about the food preference of the flies. **(D)** Proboscis extension response (PER). In this assay the experimenter scores the probability of extension of the proboscis upon stimulation of the gustatory sensilla on the tarsi (depicted on the figure) or the labellum by a tastant solution. **(E)** Electrophysiological recording from gustatory sensillum. A fly is immobilized and a reference electrode is inserted through the thorax until it reaches the tip of the labellum. The recording electrode containing the tastant solution mixed with an electrolyte is positioned above the sensillum and the spiking activity of gustatory receptor neurons is registered and analyzed.

than the colorimetric assay. However, it allows only for the indirect comparison of food preferences (Vargas et al., 2010), and is therefore most useful for measurements of absolute food intake.

A method allowing dynamic measurement of food intake has recently been adapted for *Drosophila.* In the CApillary FEeding assay (CAFE; Ja et al., 2007) flies are allowed to eat from fine capillaries filled with liquid food and the consumed food is measured by assessing changes in the liquid levels within the calibrated capillary (**Figure 1C**). This assay can be used to directly measure food intake dynamically across time and has the sensitivity to discriminate individual sips of single fruit flies (Ja et al., 2007). It can also be used to measure food preference between various food sources using multiple capillaries (Lee et al., 2008; Sellier et al., 2011). Yet this method has several limitations: the flies are forced to eat in an upside-down position which could affect their feeding habits; the number of flies that are tested is rather small; and it is more laborious than the two color choice assay. The latter disadvantage could be overcome by automating the assay using a video-based imaging readout.

Among the methods for measuring behaviors associated to feeding, the Proboscis Extension Response (or reflex; PER) assay is one of the most widely used. Upon stimulation of the gustatory receptors on the labellum or the tarsae, hungry flies will extend their proboscis if the substance is palatable leading to the initiation of feeding (**Figure 1D**). Usually, the probability of the extension of proboscis is used as a quantitative measure in this assay (Dethier, 1976; Gordon and Scott, 2009; Chatterjee et al., 2010). This serves as a measure for the palatability of the tastant and the internal state of the animal, and is highly correlated with electrophysiological responses of the gustatory receptor neurons (GRNs; Dahanukar et al., 2007) as well as their calcium responses to tastants (Marella et al., 2006), but although proboscis extension always precedes a meal one can envisage that under certain circumstances it may not lead to food ingestion. Experimentally,

the PER has several attractive qualities: it is reproducible between individual animals, can be performed on immobilized animals, and flies can be conditioned to extend their proboscis to stimuli for which they are initially unresponsive (DeJianne et al., 1985; Holliday and Hirsch, 1986; Chabaud et al., 2006). This last feature has served as basis for the use of the PER in studies on learning and memory (DeJianne et al., 1985; Holliday and Hirsch, 1986; Chabaud et al., 2006).

To achieve a mechanistic molecular and neuronal understanding of the regulation of feeding it is imperative to be able to survey the activity of the neurons crucially involved in the various aspects of feeding behavior (**Figure 1E**). Measuring the spiking activity of the GRNs is a well-established method (Hodgson et al., 1955) which provides a *bona fide* signal about the taste information that is transmitted from the sensory periphery to the central nervous system. This approach has been indispensable for the characterization of GRN responses to taste stimuli, and in revealing neuronal mechanisms underlying eating habits of insects and their modulation (Abisgold and Simpson, 1988; Simpson and Simpson, 1992; Chatterjee et al., 2010; Root et al., 2011). Recently, electrophysiological recordings have been expanded by the use of genetically encoded calcium indicators, which can be expressed specifically in neurons of interest, allowing the survey of larger populations of peripheral and central neurons (Marella et al., 2006; Fischler et al., 2007; Gordon and Scott, 2009; Root et al., 2011).

Given the complexity of feeding behavior several other methods can provide useful information about the behavioral and physiological changes associated with various internal states. Some examples are automated video tracking and fly activity monitoring (Lee and Park, 2004), the four field olfactometer assay (Meiners and Hilker, 1997; Faucher et al., 2006), biochemical examination of the hemolymph content as well as survival analyses.

FEEDING DECISIONS

When animals are faced with the option to feed, several decisions must be made: whether or not to eat, how much to eat, what to eat, and when to eat. Under certain assumptions, insects can be seen as systems trying to maintain homeostasis. From this point of view, feeding behavior serves to maintain nutritional homeostasis.

TO EAT OR NOT TO EAT?

THE PHYSIOLOGY OF CHEMOSENSORY SYSTEMS IN INSECTS

Hungry animals need to locate external sources of nutrients and decide whether to ingest them in order to replenish internal resources and restore homeostasis. *Drosophila* possesses sophisticated sensory systems to detect the presence of nutrients, including the olfactory and gustatory systems, which have been extensively reviewed elsewhere (Scott, 2005; Hallem et al., 2006; Vosshall and Stocker, 2007; Benton, 2008; Vosshall, 2008; Masse et al., 2009; Montell, 2009; Tanimura et al., 2009; Touhara and Vosshall, 2009; Yarmolinsky et al., 2009; Isono and Morita, 2010). Here we will only briefly describe the key features of the gustatory and olfactory systems that are especially important to understand the regulation of feeding behavior.

The olfactory system of insects consists of olfactory sensilla, found on the antennae and maxillary palps. *Drosophila* has approximately 50 different types of olfactory receptor neuron (ORN), each of which expresses the same set of olfactory receptors or in exceptional cases receptors of the gustatory receptor gene family (Vosshall et al., 1999; Fishilevich and Vosshall, 2005). ORNs expressing the same receptor converge on the same glomeruli, dense neuropile structures in the antennal lobe (Vassar et al., 1994; Vosshall et al., 2000). Some of the ORNs express a novel gene family of glutamate Ionotropic receptors (IRs) instead of the olfactory receptors (Benton et al., 2009; Abuin et al., 2011). Unlike the olfactory receptors, several of these receptors are expressed in the same neuron (Benton et al., 2009), and at least for some of them it is clear that all of the neurons expressing the same receptor project to a single glomerulus in the antennal lobe (Benton et al., 2009). Within each glomerulus, ORNs form synapses with projection neurons (PNs) and a network of local interneurons. Approximately 180 PNs project to the mushroom bodies (MB), which are thought to be mainly involved in the formation of conditioned responses to odors (Margulies et al., 2005), and to the lateral horn (LH), which is thought to mainly mediate innate responses to odors (Masse et al., 2009).

The gustatory system of insects consists of gustatory sensilla, taste pegs, and internal taste organs. Both sensilla and taste pegs are found on the labellum, while the tarsae, and wings only harbor gustatory sensilla (Stocker, 1994). Interestingly, gustatory neurons on the *D. melanogaster* ovipositor have not yet been characterized, calling into question the existence of gustatory structures in this location. Unlike those in mammals, the gustatory sensory cells in insects are neurons (GRNs). There are four described types of GRNs in *Drosophila* (Falk et al., 1976): cells that respond to water (Fujishiro et al., 1984; Inoshita and Tanimura, 2006; Cameron et al., 2010), cells that respond to sweet substances (Fujishiro et al., 1984; Dahanukar et al., 2007), cells that respond to low concentrations of salt, and cells that respond to bitter substances (Meunier et al., 2003) and to high concentrations of salt (Fujishiro et al., 1984; Nakamura et al., 2002). The activation of the first three types of neurons promotes food consumption, while the activation of the last one triggers avoidance and suppresses feeding to prevent the animal from ingesting toxic substances (Marella et al., 2006). In addition to these taste categories, *Drosophila* is attracted to carbon dioxide in solution (carbonation) through a dedicated type of sensory neuron (Fischler et al., 2007). Recently it has been shown that some members of the IR family are expressed in the proboscis and could thus mediate some gustatory responses (Benton et al., 2009). Due to its labeled line architecture (Yarmolinsky et al., 2009), the gustatory system provides a very convenient regulatory point for feeding.

WHETHER TO EAT OR NOT AND HOW MUCH TO EAT

NEUROPEPTIDES AND NEUROMODULATORS AS CONTROLLERS OF FEEDING

Feeding starts with a motivational drive that is determined by the current demands of the organism. In *Drosophila*, as in many other animals, this demand can be mediated by neuropeptides within the nervous system (Nässel and Winther, 2010). Within the scope of this review we will focus on the following neuropeptides: Hugin, Neuropeptide F (NPF), short Neuropeptide F (sNPF), Insulin-Like Peptides, and Leucokinin.

The *hugin* gene encodes a neuropeptide homologous to mammalian Neuromedin U (Melcher et al., 2006), which is expressed in the suboesophageal ganglion of adult and larval *Drosophila* (Bader et al., 2007a,b). It was identified as a gene that is upregulated in *pumpless* and *klumpfuss*, mutants with deficits in larval feeding behavior (Melcher and Pankratz, 2005). The expression of *hugin* is suppressed in *Drosophila* larvae by both starvation and yeast deprivation. Overexpression of *hugin* suppresses feeding in the larva, while inhibition of *hugin* expressing neurons with tetanus toxin reduces the latency to initiate feeding in adult flies. Therefore *hugin* expressing neurons (and to a certain extent *hugin* itself) seem to be responsible for the control of the initiation of feeding as it suppresses immediate feeding responses and is downregulated by starvation and amino acid deprivation (Melcher et al., 2007).

Another neuropeptide, leucokinin, which is a potential homolog of mammalian Tachykinin, may signal the amount of food in the foregut and thus controls the termination of the meal (**Figure 2**). This is apparent from the behavioral phenotypes of both *leucokinin* and *leucokinin receptor* mutants: the mutant animals increase the amount of food they consume per meal and, as a compensation, increase the inter-meal interval, keeping the caloric intake constant (Al-Anzi et al., 2010). The same behavioral phenotype can be observed in animals with ablated *leucokinin* expressing neurons. Neuronal leucokinin is responsible for this phenotype since the phenotype can be rescued by the pan-neuronal expression of either the peptide or its receptor. Furthermore, the effect of leucokinin appears to be independent of hugin and npf neurons since their ablation does not affect meal size (Al-Anzi et al., 2010). In short, leucokinin appears to mediate the decision to stop feeding.

Neuropeptide F, is an ortholog of mammalian Neuropeptide Y and shares its involvement in the regulation of food intake (Tatemoto et al., 1982; Wu et al., 2005a,b; Krashes et al., 2009; Nässel and Wegener, 2011). NPF should not be confused with short Neuropeptide F, which performs different functions and which we will discuss separately (Nässel and Wegener, 2011). In *Drosophila* larvae, NPF receptor 1 (NPFR1) activation promotes feeding on noxious food as well as solid (unattractive) food, mimicking the effect of starvation (Wu et al., 2005a,b). These effects are partially mediated by the suppression of the RPS6-p70-protein kinase (S6K) and by Insulin-like receptor (InR) signaling in NPFR1 neurons. In the neurosecretory neurons that produce ILP (Insulin-like peptide), the same S6K cascade affects the intake

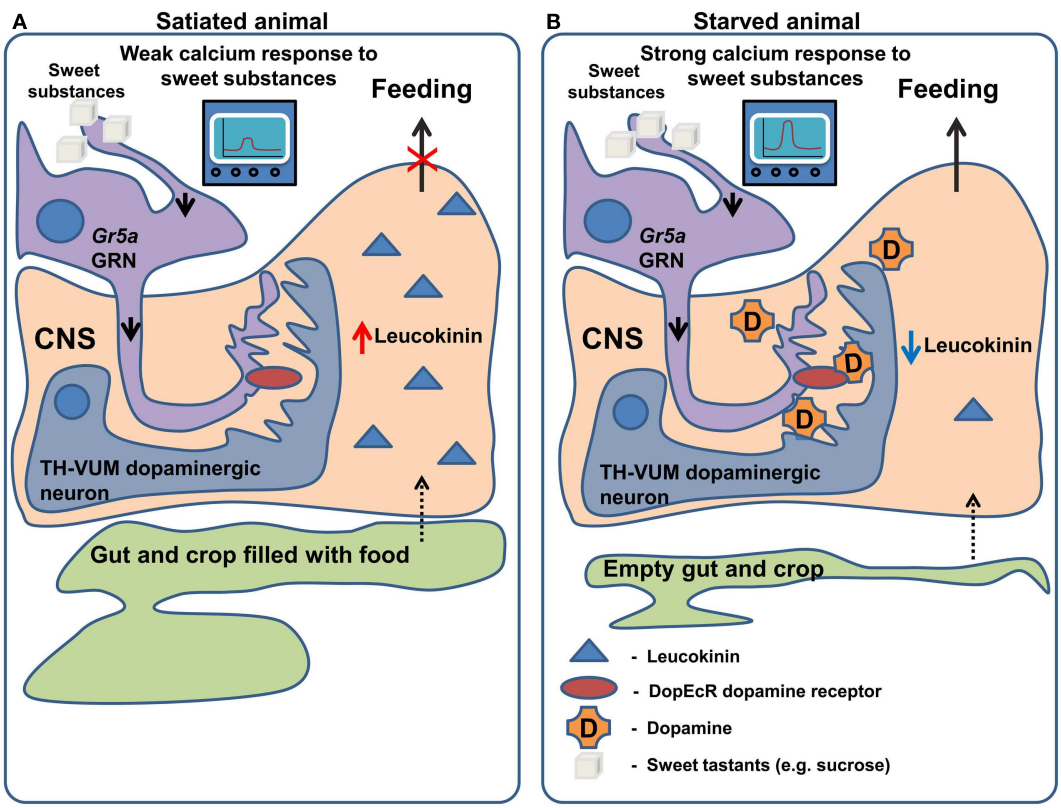

FIGURE 2 | Regulation of gustatory processing and feeding behavior by starvation. (A) In satiated animals, leucokinin is released in response to the filling of the crop and gut occurring after feeding. Leucokinin affects unknown populations of neurons in the nervous system via leucokinin receptors resulting in the termination of feeding. In the same animals TH-VUM dopaminergic neurons are less active and *Gr5a* expressing GRNs produce a weak response to "sweet" compounds. **(B)** In starved animals, the spiking activity of TH-VUM neurons is increased leading to dopamine release on the *Gr5a* expressing GRNs. In these GRNs dopamine binds to the DopEcR receptor causing increase in the calcium response to "sweet" compounds. As the crop and gut are empty leucokinin release is inhibited and feeding termination does not occur.

of both liquid and solid food and is mediated by changes in the release of ILP2 and ILP4 (Wu et al., 2005a). NPF has also been shown to be necessary for the recall of olfactory appetitive memory in adult flies through action on so called MB-MP dopaminergic neurons, which send their efferents to the mushroom body (Krashes et al., 2009). In satiated animals, the NPF neurons are silent and the output of the mushroom body is inhibited by the MB-MP neurons (**Figure 3A**). In starved animals NPF neurons are activated, leading to inhibition of the dopaminergic MB-MP neurons. The opening of the inhibitory gate from the MBs allows for the recall of the appetitive conditioned responses to odors and subsequent attraction toward presumptive appetitive food sources (**Figure 3B**).

Another peptide that conveys information about the state of internal energy resources is sNPF. Initial experiments showed that pan-neuronal overexpression of *snpf* stimulates feeding in flies, while downregulation of *snpf* using neuron-specific RNAi suppresses feeding (Lee et al., 2004). Furthermore, overexpression of *snpf* not only affected feeding behavior, but also growth, as evidenced by the induced changes in fly size, suggesting that the observed phenotypes might be at least partially due to changes in fly metabolism and growth signals (Lee et al., 2004). Recently, Root et al. (2011) uncovered an elegant mechanism by which sNPF acts to modulate neuronal circuits relevant for feeding. Signaling through the sNPF receptor, sNPFR1, in ORNs mediates an increase in olfactory sensitivity to food odors (cider vinegar) in starved flies. Using video tracking based analysis of foraging the authors demonstrated that after starvation, flies became more sensitive to the vinegar odor. This behavioral change is implemented at the neuronal level by an increased sensitivity of the ORNs carrying information about the appetitive odor. Overexpression of sNPFR1 and sNPF in Or42b expressing sensory neurons was sufficient to mimic starvation. Interestingly, the increased sensory responses to vinegar upon starvation are due to the upregulation of the expression of sNPFR1, without any changes at the level of the peptide itself. This upregulation is triggered by suppression

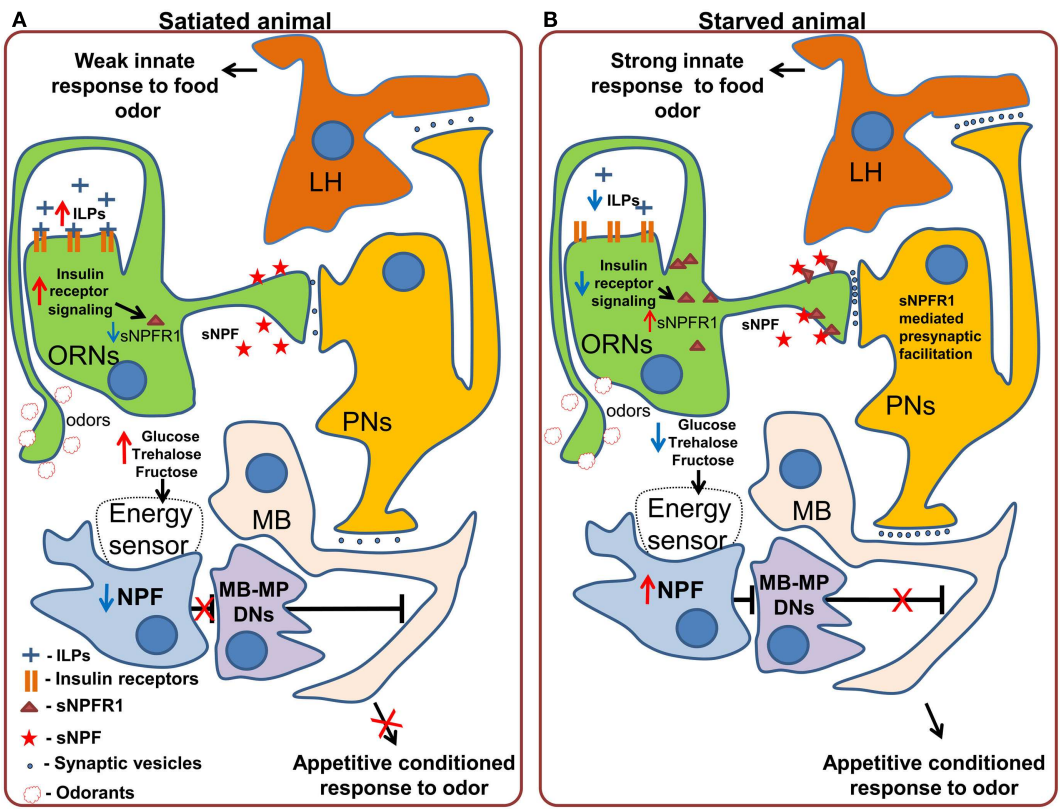

FIGURE 3 | Effects of starvation on olfactory processing in *Drosophila*. (A) In satiated animals, innate responses to food odors, which are probably mediated by the lateral horn (LH) are weak. A subpopulation of dopaminergic neurons (MB-MP DNs) projecting to the mushroom body (MB) suppresses the output of the mushroom body via tonic release of dopamine. While these neurons are active, retrieval of appetitive conditioned responses to odors does not occur. **(B)** In starved animals, the inhibition of Insulin-like receptor (InR) signaling in the olfactory receptor neurons (ORNs) stimulates the synthesis and incorporation of Short Neuropeptide F Receptor type 1 (sNPFR1) into the membrane of these neurons. sNPFR1 mediates presynaptic facilitation of the ORN response to odors, increasing the activity of projection neurons (PNs), and enhancing the innate response to attractive food odors, presumably mediated by the lateral horn neurons as well as conditioned responses to odors by the mushroom body neurons. At the same time the decrease in the hemolymph concentration of glucose and trehalose is detected by an internal energy sensor (which may or may not be directly connected to NPF neurons) which in turn activates Neuropeptide F expressing neurons. Activation of the NPF receptor 1 leads to the inhibition of MB-MP dopaminergic neurons and thus the release of the output of the mushroom body from tonic dopaminergic inhibition, allowing the retrieval of conditioned appetitive responses.

of insulin signaling in the ORNs, and is both necessary and sufficient to mimic the effect of starvation on olfactory perception. In this case, while not acting on feeding itself, but on foraging, the modulation occurs mainly at the peripheral level (**Figure 3**) and is mediated by the enhancement of olfactory attractiveness of a food odor – vinegar, a reliable cue for *Drosophila*'s favorite meal – rotten fruit.

While the examples mentioned above describe effects of neuropeptides on the olfactory system, very recently two groups (Inagaki et al., 2012; Marella et al., 2012) independently discovered that the neuromodulator dopamine mediates increased GRN sensitivity in hungry flies (**Figure 2**). The findings of Inagaki et al. (2012) rely on the use of a new method which allows the mapping of sites of action of dopamine. They demonstrate that during starvation dopamine signaling is increased in Gr5a sugar sensing GRNs, leading to an increased probability of proboscis extension, a key step in food intake. The effects of dopamine on sugar sensing neurons are in turn mediated specifically by the DopEcR dopamine receptor, a receptor whose physiological function had previously remained elusive. A second group (Marella et al., 2012) identified a dopaminergic neuron named TH-VUM, which projects to the suboesophageal ganglion (the site of the brain to which GRNs project). This neuron is activated by starvation increasing the probability of the extension of the proboscis to sucrose. In fact, activation of the TH-VUM in isolation using TRPA1 induces proboscis extension, while silencing of this neuron inhibits it (Marella et al., 2012). These studies demonstrate that dopamine is a key player in enhancing gustatory sensitivity toward sugars upon starvation, shedding light on the longstanding question of how hunger facilitates the extension of the proboscis.

In the picture which emerges, starvation causes changes in levels of specific neuropeptides and neuromodulators affecting feeding decisions via central and peripheral mechanisms. Acting on the periphery, they enhance innate responses to attractive odors and sugars (InR > sNPFR1 > presynaptic facilitation in the ORNs and TH-VUM > DopEcR > increased Calcium responses of the GRNs) while their action in the brain (NPF > MB-MP neurons > MB; **Figure 3**), alters the acquired attractiveness to odors in a metabolic state dependent way (**Figures 2** and **3**).

WHETHER TO EAT AND WHAT TO EAT

INTERNAL STATE SENSORS

To ensure feeding homeostasis, internal sensor mechanisms must be present that signal the lack or excess of internal nutritional resources. Internal sensors are necessary not only to initiate feeding *per se*, but also to assist in the selection of the optimal food source to compensate for the lack of specific nutrients. In insects, there is behavioral evidence for the existence of internal energy sensors (Burke and Waddell, 2011; Dus et al., 2011; Fujita and Tanimura, 2011) as well as protein/essential amino acid sensors (Simpson and Abisgold, 1985; Lee et al., 2008; Ribeiro and Dickson, 2010; Vargas et al., 2010). This extends earlier behavioral findings in rats demonstrating the existence of internal energy sensors (Sclafani and Nissenbaum, 1988). The molecular and neuronal substrates of the internal sensors are currently under intense investigation and the first details of their functioning are the main focus of this review. In general these sensors could directly enable the neurons to sense levels of nutrients, or could act on neurons via surrogate signals (hormones) secreted by nutrient sensing cells outside the nervous system.

EXPERIMENTAL EVIDENCE FOR THE EXISTENCE OF ENERGY SENSORS

While neuropeptides mediate changes in behavior by modifying information processing in the nervous system, the question remains as to how the neuropeptide releasing neurons detect the internal nutritional state of the animal. Recently, three groups have independently produced behavioral evidence for an internal energy sensor in *Drosophila*. Similar mechanisms are thought to exist in mammals (Sclafani and Nissenbaum, 1988; de Araujo et al., 2008; Oliveira-Maia et al., 2011) and have been recently reviewed (De Araujo, 2011). An early line of evidence for the existence of an internal energy sensor comes from the work of Sclafani and Nissenbaum (1988). Their work demonstrated that pairing of flavored water with intra-gastric infusions of hydrolyzed starch in rats led to strong and robust flavor preference in favor of the starch-paired flavor. This work demonstrated that the caloric value *per se* can be rewarding, and is capable of modifying behavior. Recently, It has been demonstrated that flies too can be conditioned by the caloric value of the food (Burke and Waddell, 2011; Dus et al., 2011; Fujita and Tanimura, 2011). This is shown by the fact that flies can be conditioned using sorbitol, an alcohol that can be used by flies as an energy source, but to which they do not show any gustatory responses (Fujita and Tanimura, 2011). In contrast, if flies are conditioned with sweet but non-caloric food (arabinose) the memory trace is weak and unstable. This memory trace can, however, be stabilized if attractive but non-metabolizable sugar (arabinose) is mixed with tasteless but energy-rich substance (sorbitol; Burke and Waddell, 2011). These experiments demonstrate the existence of an internal energy sensor, working in parallel with gustatory perception that is crucial for memory formation and stabilization.

These observations are supported by a second set of experiments showing that starved "taste-blind" fruit flies prefer sucrose over a non-caloric alternative. Several genes have been shown to be crucial for the gustatory detection of sucrose. Among these are the trehalose receptor Gr5a (Dahanukar et al., 2001) and Gr64a, a receptor for maltose, sucrose, and glucose (Jiao et al., 2007). Both of these receptors are expressed in sugar sensitive GRNs (Dahanukar et al., 2007). The other gene in which mutations lead to a severe taste deficit is *Pox neuro*, which encodes a transcription factor (Awasaki and Kimura, 1997). In *Pox neuro* mutant flies all chemosensory sensilla are transformed into mechanosensory organs, leading to a loss of gustatory perception. Dus et al. (2011) tested several "sugar – blind" flies, either *Gr5a* and *Gr64a* double mutants or *Pox neuro* mutants, and found that neither of the taste-blind flies showed a significant PER when presented with 100 mM sucrose before or after starvation. However, in the two color choice assay they ate significantly more of the sucrose-containing agar gel than the agar gel alone. Furthermore, when given the choice between two sugars that are both perceived as sweet by the wild type flies, but differ in their nutritional content (non-metabolizable sucralose or L-glucose and metabolizable sucrose or D-glucose), starved *Gr5a* and *Gr64a* double mutant flies ate significantly more of the metabolizable sugars (Dus et al., 2011). This preference for calorie-rich food was correlated with the

depletion of glycogen reserves and decreased hemolymph levels of glucose and trehalose. These findings suggest that flies are capable of postingestive identification of calorie-rich food through a putative internal energy sensor independent from the characterized gustatory "sweet" receptors (*Gr64a* and *Gr5a*) or other chemoreceptors on *poxn* positive taste sensilla.

These studies provide converging evidence for the existence of a behaviorally relevant energy sensing mechanism in *Drosophila*, but multiple questions still remain. Are energy levels sensed by the nervous system directly? Which molecular machinery is used to sense energy in the nervous system? Which cells act as energy sensors?

Current research is starting to answer these questions. Following up previous observations (Thorne and Amrein, 2008; Park and Kwon, 2011), a gustatory receptor (Gr43a) has been identified as being expressed not only in the gustatory organs but also in the digestive tract, uterus, and most importantly in the central brain of *Drosophila* where it acts as an internal energy sensor (Miyamoto et al., 2012). In a series of elegant experiments the authors demonstrated that Gr43a is necessary for fructose sensing and that, unexpectedly, fructose levels in the hemolymph constitute a reliable postingestive signal to estimate the energy content of a meal. Only three to four neurons in the dorsolateral protocerebrum express Gr43a receptors and show robust Calcium responses to fructose within the physiological range of concentrations. The activity of these fructose sensing neurons is likely to play an important role in mediating the metabolic effect of carbohydrate ingestion on feeding behaviour and short term memory (Miyamoto et al., 2012).

These findings do not contradict the possibility that in *Drosophila* either the NPF neurons themselves (as described above) can act as energy sensors, or that a different subset of neurons or tissues act as energy sensors and indirectly exert their function via NPF release.

EXPERIMENTAL EVIDENCE FOR THE EXISTENCE OF INTERNAL PROTEIN/AMINO ACID SENSORS

Research on the neuronal basis of food intake and energy expenditure has largely concentrated on energy-rich bulk food intake and energy homeostasis, largely ignoring other types of nutrients such as proteins. This stands in contrast to the substantial body of evidence that has accumulated over the last 30 years suggesting that different species of animals, both vertebrates and invertebrates, are capable of selecting food sources that optimize not only the gross energy intake, but also the intake of macronutrients such as amino acids, salts, and sterols (Trumper and Simpson, 1993; Behmer and Joern, 1994; Simpson, 1994, 2006; Behmer et al., 1999; Behmer, 2009). A comprehensive review of the different behavioral adaptations to imbalanced diets in insects has recently been discussed in detail elsewhere (Behmer, 2009). We would like to focus on the emerging neuronal and molecular mechanisms underlying the regulation of protein intake.

Locusts are herbivores that change their food intake upon exposure to a low protein diet. They increase total food consumption by decreasing inter-meal interval without changing the size of individual meals (Simpson and Abisgold, 1985). This seminal discovery spurred a large body of work which has made important contributions to our current understanding of the physiological and neuronal mechanisms underlying protein homeostasis. In locusts kept on a low protein diet, hemolymph osmolality and amino acid concentrations decrease, followed by an increase in food intake (Abisgold and Simpson, 1987). Accordingly, injecting of amino acids directly into the hemolymph or raising hemolymph osmolality partially reverses the increase in food consumption. Furthermore, simultaneous increase of hemolymph osmolality and amino acid concentrations resulted in even larger inter-meal intervals, suggesting that both physiological parameters independently influence the increase in food intake. The same authors ruled out feedback from stretch receptors as being involved in regulating this behavior (Abisgold and Simpson, 1987). These results suggested the existence of an internal amino acid sensor controlling feeding behavior in locusts.

Interestingly, in locusts the sensitivity of maxillary palp GRNs (**Figures 4A,B**) is correlated with the increase in food intake seen in response to the low protein diet: the sensitivity of the GRNs to leucine and a mixture of 10 amino acids increased, with no apparent change in the sensitivity to sucrose (Abisgold and Simpson, 1988). Importantly, injection of amino acids into the hemolymph reversed the change in receptor sensitivity to pre-deprivation levels (Abisgold and Simpson, 1988). The effects on the sensitivity of GRNs were not mediated by a top-down effect from the central nervous system, since transection of the maxillary nerve did not affect the changes in sensitivity, which could be reversed by injection of amino acids directly in to the isolated maxillary palp (Simpson and Simpson, 1992). In locusts the current hypothesis is that the amino acid sensor is likely to be located in the GRNs themselves, and that the increased consumption of proteins is largely determined by elevated sensitivity of GRNs to amino acids. This stands in contrast to vertebrates, where protein homeostasis is thought to rely on amino acid sensing in the brain (Hao et al., 2005; Maurin et al., 2005; Gietzen et al., 2007). Following these discoveries, further research suggested that protein intake is tightly regulated on a behavioral level in many different species (Raubenheimer and Simpson, 1997), leading to the development of a unifying methodological and theoretical framework which was termed "nutritional geometry" (Raubenheimer and Simpson, 1993; Lee, 2006).

Despite these important contributions the locust as a model organism is not very well suited for the dissection of molecular and neuronal mechanisms. Recently *D. melanogaster* has been shown to be able to tightly regulate protein intake (Ribeiro and Dickson, 2010; Vargas et al., 2010) to achieve maximal reproductive success (Lee et al., 2008), opening up the possibility to use its sophisticated neurogenetic toolkit to study this important nutritional process (**Figures 4C,D**).

When given the choice between protein rich food (yeast) and carbohydrate-rich food (sucrose) using the two color choice assay, fruit fly males and females differ dramatically in their response to protein deprivation (Ribeiro and Dickson, 2010). Flies of both sexes normally prefer sucrose solutions over yeast. Males switch their preference to yeast after 10 days of protein deprivation, and while virgin females behave much like males, females switch their preference to protein rich food much faster after mating, i.e., after 3 days of yeast deprivation. This behavioral change is at least partially mediated by the Sex peptide, a short peptide contained in

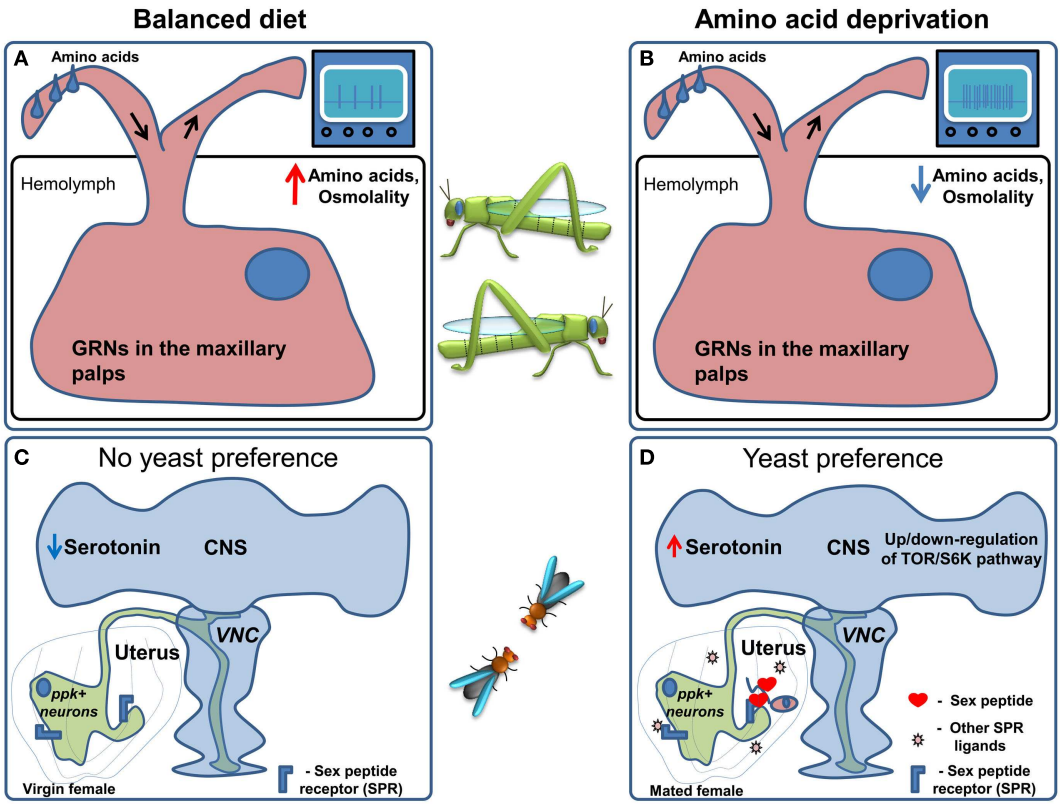

FIGURE 4 | Regulation of amino acid preference. (A) When locusts are kept on a balanced diet the sensitivity of amino acid responsive GRNs in the maxillary palps is moderate. **(B)** Upon amino acid deprivation hemolymph amino acid concentration and osmolality are decreased. This leads to an increase in the sensitivity of amino acid responsive GRNs followed by an increase in food consumption. **(C)** In *Drosophila* kept on a balanced diet ppk^+ neurons are active, causing flies to prefer sucrose. **(D)** When flies are deprived of amino acids, it is conceivable that, TOR/S6K signaling in neurons is altered presumably indicating an internal amino acid deficiency and serotonin levels in the brain are thought to increase. Furthermore, upon mating Sex Peptide, as well as other ligands of the Sex Peptide receptor, are transferred to the uterus causing the inhibition of the ppk^+ neurons which project to the Ventral Nerve Cord (VNC) and the brain. These changes lead to an increase in yeast (amino acid rich food) preference.

the seminal fluid of males that is injected into the female during copulation and is thought to act on the Sex peptide receptor (Fox et al., 1959; Kubli, 2003; Yapici et al., 2008). Furthermore, Sex peptide receptor acts in neurons in the female genital tract called *pickpocket*$^+$ *(ppk)*$^+$ neurons (Häsemeyer et al., 2009; Yang et al., 2009) sending projections to multiple brain areas (Häsemeyer et al., 2009; Rezával et al., 2012) to modulate food preference (Ribeiro and Dickson, 2010). In addition to ppk^+ neurons, Target of Rapamycin/S6K (TOR/S6K) signaling in the nervous system and serotonin are likely regulators of this nutritional decision. In fact, modulation of the TOR pathway or the activity of one of its downstream targets – S6K – in the nervous system of males or virgin flies causes a clear preference for yeast over sucrose (Ribeiro and Dickson, 2010; Vargas et al., 2010). Given the fact that the TOR/S6K pathway is best known as a cellular nutrient sensing pathway reporting the lack of amino acids (Wullschleger et al., 2006; Liao et al., 2008), and has been shown to regulate feeding behavior in vertebrates (Cota et al., 2006), it is very attractive to speculate that this pathway could act as a neuronal nutrient sensor, underlying changes in feeding decisions upon ingestion of imbalanced diets.

Despite these encouraging first insights into possible molecular mechanisms regulating feeding decisions upon protein deprivation, many questions still remain open. It will be important to determine whether TOR/S6K signaling indeed acts as a nutrient sensor in the nervous system and, if so, in which neurons it acts. A further important question is how changes in nutrient sensing, be it mediated by TOR/S6K activity and serotonin or by another mechanism, are translated into neuronal activity and ultimately changes in feeding decisions. To answer these questions, the identification of further molecular players mediating this homeostatic nutritional behavior will be essential. Regarding the modes of action, multiple hypotheses of how internal nutrient sensing leads to changes in nutrient choice can be envisaged. It is possible that, similar to what has been proposed in locusts, nutrient sensing in *Drosophila* acts at the level of peripheral chemosensory neurons. An obstacle to gaining further insight into this aspect of protein homeostasis is that, in contrast to locusts and many other insects, *Drosophila* has not been shown to have functional GRNs sensitive to amino acids. The recent demonstration, however, that fruit flies can taste amino acids (Toshima and Tanimura, 2012) is an important step toward the identification of *Drosophila* amino

acid receptor neurons. Due to the specialization for yeast feeding, *Drosophila* is furthermore likely to have evolved to use yeast metabolic products, such as carbon dioxide (Fischler et al., 2007) or glycerol (Koseki et al., 2005; Wisotsky et al., 2011), as proxies signaling the availability of amino acids. Further insights into nutrient choice in this genetically tractable organism will therefore require a better understanding of the chemosensory basis for detection of amino acid rich food. A different hypothesis is that a postingestive mechanism to detect the lack of amino acids in the diet affects nutrient decisions through the modulation of higher brain centers, as has been described in vertebrates (Gietzen et al., 2007). Ultimately, a combination of peripheral and central modulation, as is the case for energy homeostasis, is most likely to occur. In any case, further understanding of the molecular basis for nutrient choice in *Drosophila* will rely on the identification of more molecular and neuronal players and a better electrophysiological, cellular, nutritional, and behavioral understanding of how they act within the nervous system to modify feeding decisions.

WHEN TO EAT

THE INFLUENCE OF CIRCADIAN RHYTHMS AND SLEEP ON FEEDING DECISIONS

To achieve an optimal expenditure of organismal resources and to maximize fitness (Xu et al., 2011), physiological processes are orchestrated by the circadian clock machinery (Sehgal, 1995). It is therefore not surprising that the same holds true for feeding behavior (Krishnan et al., 2008; Tanoue et al., 2008; Xu et al., 2008; Chatterjee and Hardin, 2010).

In *Drosophila*, the sensitivity of GRNs to tastants does not remain constant throughout the day (Chatterjee et al., 2010) with a maximum sensitivity in the morning (Chatterjee and Hardin, 2010). This phenomenon is mediated by the G-Protein coupled receptor regulatory kinase 2 (GPRK2), which, in turn, is regulated by the circadian molecular clock machinery in the GRNs themselves (**Figure 5**). The food intake of flies follows the sensitivity of the receptor neurons, with peak food intake in the morning. Diurnal variations of sensitivity in *Drosophila* ORNs have also been described. They are also mediated by GPRK2 (Tanoue et al., 2008), but display the opposite regulation to that observed in the gustatory system, such that the peak of olfactory sensitivity is at night, when gustatory sensitivity is minimal. Surprisingly, the changes in sensitivity were proposed to be mediated not only by the alteration of the firing rate in response to stimuli, but also by changes in the amplitude and duration of the action potential generated in the GRNs and ORNs (Krishnan et al., 2008; Chatterjee et al., 2010). Confirmation of these observations and the investigation of the exact nature of this phenomenon remain to be uncovered by future studies and will require either calcium imaging or ideally patch clamp recordings as opposed to the extracellular tip recordings used in these studies. Furthermore, circadian clock components in peripheral tissues also regulate feeding. Interfering with components of the circadian clock in the fat body, for example, disrupts the circadian pattern of feeding while increasing food consumption and decreasing the levels of glycogen and resistance to starvation (Xu et al., 2008).

Another mechanism that has been proposed to mediate changes in food intake across the diurnal circle is mediated by the protein Takeout. *takeout* has strong sequence homology to the Juvenile Hormone (JH) binding protein genes, whose products are involved in the transport of the lipophilic JH to its tissue targets. Takeout was isolated as a protein whose expression is strongly regulated by the circadian rhythm and was shown to affect feeding: *takeout* mutant flies overeat when food is available *ad libitum* (Sarov-Blat et al., 2000; Meunier et al., 2007). In addition, *takeout* mutants do not show a decrease in GRN sensitivity to glucose after feeding, suggesting that the mechanism of overeating is caused by a lack in modulation of the sensitivity of the peripheral GRNs (Meunier et al., 2007). These results provide further evidence for the importance of peripheral chemosensory modulation in the regulation of feeding.

As feeding and sleeping are mutually exclusive behaviors, any description of the regulation of food intake would be incomplete without a discussion of its coordination with sleep. In vertebrates, long term sleep deprivation stimulates appetite (Rechtschaffen and Bergmann, 2002) while starvation leads to a decrease in sleep (MacFadyen et al., 1973). Mechanistically these behaviors are coordinated by the orexin/hypocretin system, which controls both food intake and wakefulness (De Lecea et al., 1998; Sakurai et al., 1998) and which is regulated by blood amino acid and glucose levels (Burdakov et al., 2005, 2006; Frederick-Duus et al., 2007; Karnani et al., 2011). Fruit flies also decrease the time they sleep when they are starved (Lee and Park, 2004; Keene et al., 2010), and yeast feeding can shorten or terminate the sleep of flies (Catterson et al., 2010). As the effect of starvation on sleep is mediated by nutrient deprivation it is likely to involve internal energy sensing. Accordingly feeding sucralose, a non-nutritious sweet compound, to starved flies does not lead to an increase in sleep (Keene et al., 2010). Furthermore, this starvation-induced sleep alteration is mediated by *clock* and *cycle* in the dorsally located population of the *clock* expressing neurons (Keene et al., 2010), opening a window to a better understanding of how these essential, but mutually exclusive behaviors are coordinated.

CONCLUDING REMARKS, OPEN QUESTIONS, AND FUTURE DIRECTIONS

Despite the recent increase in knowledge of the molecular and neuronal components as well as the mechanisms controlling feeding decisions in *Drosophila*, important questions still remain to be answered.

One of the main open questions is the exact nature of the nutrient sensing mechanisms. We are just starting to identify the molecular machinery allowing the nervous system to detect the lack of energy available to the fly, the neurons in which this machinery acts, and to which extent nutrient sensing happens within the nervous system. It will be interesting to differentiate between two possibilities. One in which nutrient sensing happens centrally in a small set of neurons or in a peripheral organ that systemically regulates the activity of all neurons involved in feeding decisions. The other possibility would be that all or a majority of neurons are able to sense the lack of specific nutrients, and use this information to specifically modify their mode of action to modulate feeding.

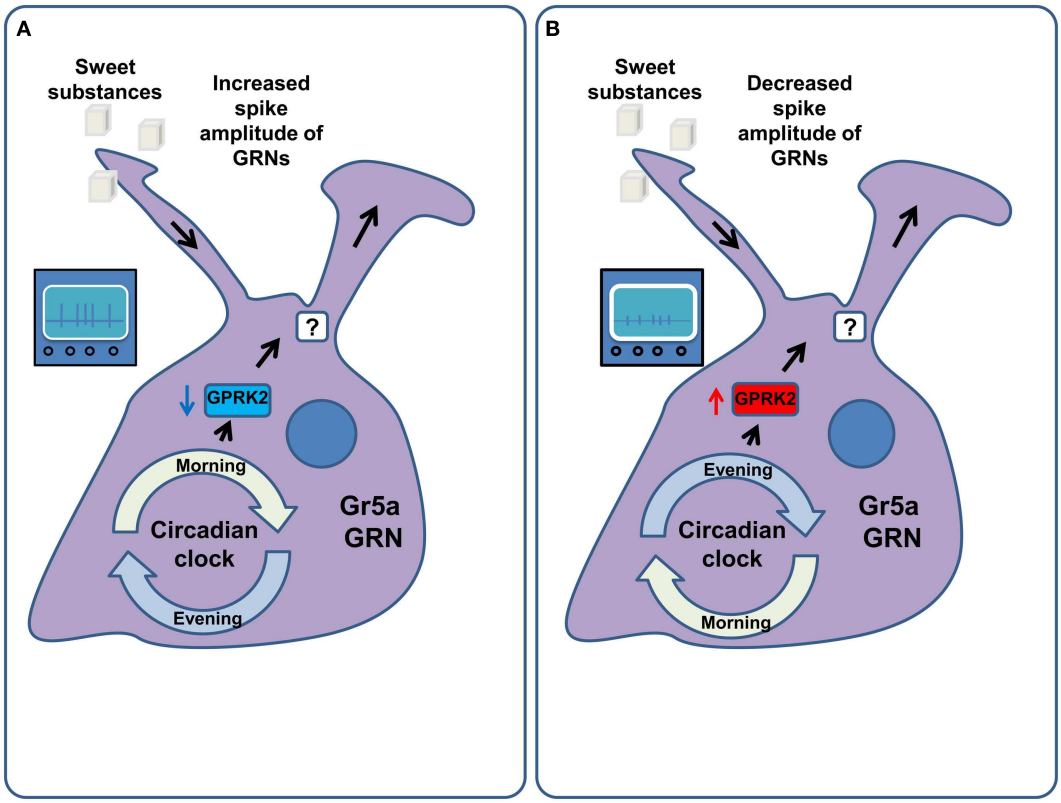

FIGURE 5 | Circadian regulation of GRN sensitivity. (A) In the morning the molecular machinery of the circadian clock down-regulates the activity of the G-Protein coupled receptor regulatory kinase 2 (GPRK2) in the Gr5a gustatory receptor neurons. This amplifies the responsiveness of these GRNs to sucrose via an increase in their firing rate as well as the width and amplitude of their action potentials. As a result flies consume more food in the morning. **(B)** In the evening and at night the activity of GPRK2 is increased, leading to a decrease in the sensitivity of Gr5a GRNs to sucrose. This is mediated by decreasing the spike width and amplitude of these GRNs. As a result flies consume less food in the evening.

The emerging picture of how feeding decisions are modulated in *Drosophila* is that upon changes in nutrient state, both peripheral chemosensory and central neurons change their firing properties to elicit a change in feeding behavior (**Figures 2** and **3**). Interestingly, gustatory receptors are also expressed outside of the taste organs, for example in the midgut and in the uterus (Miyamoto et al., 2012). The function of these "ectopic" receptors is not defined; however it is interesting to speculate that they may also be involved in the evaluation of the internal state of the animal.

As the molecular and neuronal mechanisms underlying nutrient sensing, and the ways in which they elicit changes in feeding behavior, are better understood, the challenge will be to integrate this knowledge into a systems-level framework of how changes in neuronal output are translated into a whole-animal response to ensure homeostasis. This will also require an understanding of how the different systems ensuring the homeostasis of energy, protein, and other nutrients interact at the behavioral, neuronal, and molecular level to maximize survival chances and reproduction.

This systems-level understanding will rely on expanding the repertoire of behavioral assays used to study feeding (**Figure 1**) in order to be able to capture, quantitatively and dynamically, the full complexity of feeding and associated fly behaviors. Video tracking and automatic analysis of behavior, which arose from the intersection between machine vision and ethology (Branson et al., 2009; Fontaine et al., 2009; Straw et al., 2011), might fulfill these requirements, particularly if they are expanded by methods for online monitoring of food consumption. Complementary monitoring of neuronal activity during behavior will be important to understand how neuronal computations lead to feeding decisions to ensure homeostasis.

Ultimately, the combination of the identification of molecular and neuronal mechanisms and fine behavioral data in genetically and nutritionally manipulated animals, together with associated changes in neuronal dynamics, will allow us to build an understanding of how the animals make feeding decisions allowing them to maintain the stability of the internal milieu.

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The decision to fight or flee – insights into underlying mechanism in crickets

Paul A. Stevenson*[1] *and Jan Rillich*[2]

[1] *Institute for Biology, University of Leipzig, Leipzig, Germany*
[2] *Institute for Neurobiology, Free University of Berlin, Berlin, Germany*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Björn Brembs, Freie Universität Berlin, Germany
Jens Herberholz, University of Maryland, USA

***Correspondence:**
Paul A. Stevenson, Institute for Biology, University of Leipzig, Talstr. 33, D-04103 Leipzig, Germany.
e-mail: stevenson@rz.uni-leipzig.de

Ritualized fighting between conspecifics is an inherently dangerous behavioral strategy, optimized to secure limited resources at minimal cost and risk. To be adaptive, potential rewards, and costs of aggression must be assessed to decide when it would be more opportune to fight or flee. We summarize insights into the proximate mechanisms underlying this decision-making process in field crickets. As in other animals, cricket aggression is enhanced dramatically by motor activity, winning, and the possession of resources. Pharmacological manipulations provide evidence that these cases of experience dependent enhancement of aggression are each mediated by octopamine, the invertebrate counterpart to adrenaline/noradrenaline. The data suggest that both physical exertion and rewarding aspects of experiences can activate the octopaminergic system, which increases the propensity to fight. Octopamine thus represents the motivational component of aggression in insects. For the decision to flee, animals are thought to assess information from agonistic signals exchanged during fighting. Cricket fights conform to the cumulative assessment model, in that they persist in fighting until the sum of their opponent's actions accumulates to some threshold at which they withdraw. We discuss evidence that serotonin, nitric oxide, and some neuropeptides may promote an insect's tendency to flee. We propose that the decision to fight or flee in crickets is controlled simply by relative behavioral thresholds. Rewarding experiences increase the propensity to fight to a level determined by the modulatory action of octopamine. The animal will then flee only when the accumulated sum of the opponent's actions surpasses this level; serotonin and nitric oxide may be involved in this process. This concept is in line with the roles proposed for noradrenaline, serotonin, and nitric oxide in mammals and suggests that basic mechanisms of aggressive modulation may be conserved in phylogeny.

Keywords: aggression, biogenic amines, octopamine insects, assessment, motivation, experience dependent plasticity, decision making

AGGRESSION AND THE DECISION TO FIGHT OR FLEE

There are many forms of aggression but no uniform definition (see, e.g., Nelson, 2006). In this paper we review insights into how sexually mature insects decide whether to fight or flee when contacting a conspecific of the sex and age under laboratory conditions. Notably, the "struggle for life" is most severe between individuals of the same species, after all, they rival for the same foods, shelter, territory, and sexual partners (Darwin, 1859). This intra-specific aggression is a widespread behavioral strategy in the animal kingdom, which is generally thought of as serving to optimize an animal's chances of securing limited resources at minimal risk of injury or cost. For aggression to be adaptive, animals must be able to weight up potential benefits and costs in order to "decide" when it would be more opportune to fight or to flee. A variety of hypotheses address how this could be done (c.f. Hurd, 2006). Game theory predicts that aggressive behavior between conspecifics is optimized in "evolutionarily stable strategies" (Maynard Smith and Price, 1973). These are typically stereotyped contests involving the ritualized exchange of agonistic signals, which are thought to convey increasingly more accurate information for assessing the contenders' "resource holding potential" (RHP), or put simply – win chances (Parker, 1974). The latter will not only depend on physical factors, such as size, strength, and weaponry, but also on metabolic factors (see, e.g., Briffa and Elwood, 2005) and a wide variety of experiences including winning, defeat as well as on the presence, and subjective value of resources at stake such as shelter, territory, food, and mates, that will all determine an animal's willingness to invest energy in fighting – i.e., its "aggressive motivation" (see **Figure 1**). These, largely theoretical considerations, provide a neat framework to explain most behavioral observations, such as all else being equal the stronger wins, but that the weaker can prevail when fighting in defense of its offspring. But what are the proximate mechanisms controlling aggression? How do experiences such as resource possession determine "aggressive motivation" and how is this encoded in the nervous system? How do animals "assess" agonistic signals and by which means do they influence the expression of aggressive behavior? Just how exactly do animals make the "decision to fight or flee"?

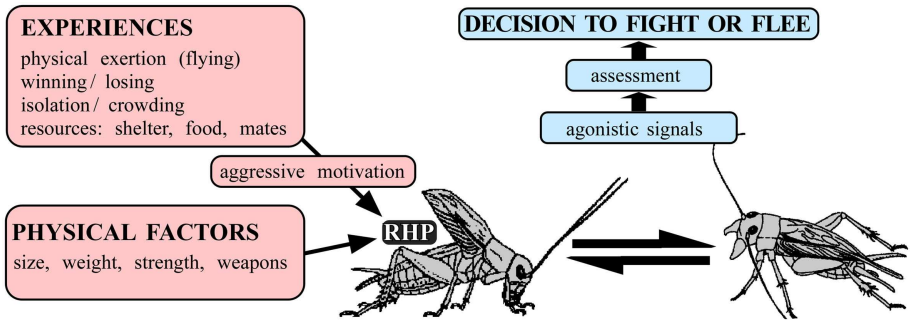

FIGURE 1 | Factors influencing the decision to fight or flee in intra specific aggression. An individual's prowess at fighting (Resource Holding Potential, RHP) and hence win chances, depends on physical factors (e.g., size) as well as on numerous experiences (e.g., presence and value of resources) that influence aggressive motivation. On confronting a competitor, agonistic signals exchanged during escalating ritualized fighting convey increasingly more accurate information on the individual's RHP in order to assess whether it would be more opportune to persist in fighting or to flee.

CRICKETS AS MODEL ANIMALS FOR THE STUDY OF AGGRESSION

In this review we summarize insights into these questions gained from studies on insects, primarily field crickets (*Gryllus bimaculatus* de Geer). Crickets possess a conveniently sized and comparatively simple, segmentally organized nervous system, and above all have a rich and robust behavioral repertoire (Huber et al., 1989). Their fighting behavior is highly stereotyped and involves a series of easily quantifiable agonistic acts (**Figure 2**) that can escalate into impressive wrestling contests lasting over a minute and resulting in serious injury (Hofmann and Stevenson, 2000). Fighting establishes clear winners and losers, whereby winners sing a rivalry song, and the losers avoid other males for hours. This is just one example of many illustrating that aggression in crickets is experience dependent. Crickets thus offer the opportunity to investigate nervous mechanisms of context dependent plasticity of social interactions.

THE DECISION TO FIGHT – THE ROLE OF THE ANTENNAE

When two crickets meet they first contact each other with their large moveable antennae and this guides the decision to court, fight, or flee. Species and sex is signaled by the pheromonal signature (Iwasaki and Katagiri, 2008), which induce males to court conspecific females. Females seldom interact, but can fight vigorously in the presence of a male or his courtship song (Rillich et al., 2009). In Drosophila the two sexes adopt different fighting strategies (Nilsen et al., 2004), controlled by the expression of the fruitless gene in specific neurons (Vrontou et al., 2006; Chan and Kravitz, 2007). When male crickets meet they fence vigorously with their antennae and this is both sufficient and necessary to evoke aggressive behavior (Hofmann and Schildberger, 2001). Agonistic responses, such as mandible spreading, can be evoked by simply lashing the antennae with a bristle (Alexander, 1961), or alone by highly volatile male odors (Iwasaki and Katagiri, 2008), which have been identified in fruit flies (Wang and Anderson, 2010). It is thus not surprising that when the antennae are ablated, male crickets frequently court each other but no longer engage each other in fighting (Hofmann and Schildberger, 2001; see also Fernandez et al., 2010 on Drosophila). Striking the antennae directly activates a set of fast conducting descending interneurons (Schöneich et al., 2011), that trigger directed turning responses in some insects (Baba et al., 2010), but their role in cricket aggression remains speculative. Higher brain centers are almost certainly involved in triggering aggression, as indicated by the original finding of Huber (1960) that local electrical stimulation in the vicinity of the mushroom bodies can evoke reproduction of the male rivalry song (Huber, 1960; english summary: Huber et al., 1989).

THE DECISION TO FIGHT – THE ROLE OF OCTOPAMINE

In mammals, the adrenergic/noradrenergic systems are generally accredited with preparing the animal to fight or flee. Insects and other protostomes lack the catecholamine adrenaline and noradrenaline and convert instead the substrate amino acid tyrosine first to tyramine and then to octopamine (c.f. Pflüger and Stevenson, 2005). Recent studies in crickets and fruit flies provide evidence that noradrenaline's analog octopamine promotes the expression of aggressive behavior in insects.

Fighting behavior in crickets leads to elevated levels of octopamine in the hemolymph (Adamo et al., 1995). Treatment with agents that deplete octopamine and dopamine from the nervous system markedly reduces their aggressiveness and general excitability, which can both be at least partially restored by treatment with the octopamine agonist chlordimeform (CDM), indicating that the defect is most likely due to octopamine depletion (**Figure 3A**; Stevenson et al., 2000, 2005). Depleting central nervous stores of serotonin, an amine with many functionally antagonistic actions to octopamine (Erber et al., 1993), induces hyperactivity and enhances startle responses, but without affecting aggression. This infers that octopamine's effect on aggression is selective, and not simply due to increasing general excitability (Stevenson et al., 2005). Similarly in Drosophila, tyramine-β-hydroxylase mutants, which cannot synthesize octopamine and have 10-fold elevated tyramine levels in their brains, have either deficits in aggressive behavior (Baier et al., 2002), or tend to court rather than fight each other (Certel et al., 2007). Hoyer et al. (2008) confirmed that mutant flies lacking octopamine, or octopamine and tyramine, display almost no aggression, and that the defect could be rescued partially by octopamine treatment, or substituting gene function. Furthermore, Zhou et al. (2008) identified a subset of octopaminergic neurons important for aggression in

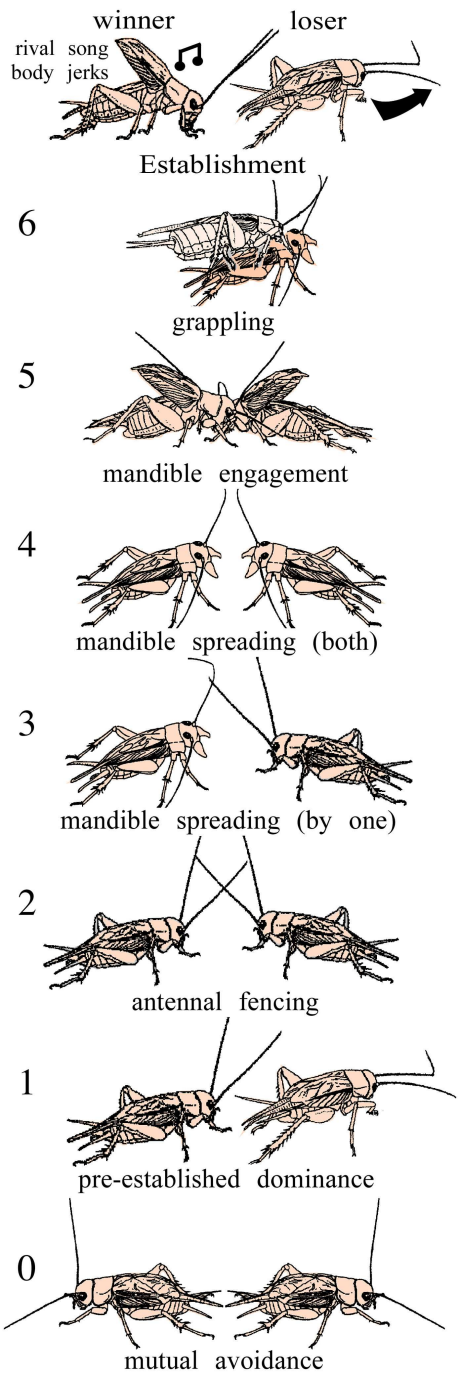

FIGURE 2 | tereotyped levels of escalating aggression shown by male adult crickets. *Level 0* mutual avoidance: no aggressive interaction. *Level 1* pre-established dominance: one cricket attacks, the other retreats. *Level 2* antennal fencing. *Level 3* mandible spreading (by one): one cricket displays spread mandibles. *Level 4* mandible spreading (both): both crickets display spread mandibles. *Level 5* mandible engagement: the mandibles interlock and the animals push against each other. *Level 6* grappling: an all out fight, the animals may disengage, and reengage to bite other body parts. Establishment: the fight can be concluded at any level by one opponent, the loser, retreating, the established winner typically produces the rival song and body jerking movements (modified from Stevenson et al., 2000; Stevenson et al., 2005).

Drosophila and showed that enhancing octopaminergic signaling enhanced aggressiveness.

It is important to stress that octopamine is not essential for actually initiating aggression. For example, crickets lacking octopamine can still exhibit practically all the basic element of aggressive behavior, though usually only when coaxed by repeated antennal stimulation (Stevenson et al., 2000). Taken together, the data suggest that octopamine acts as a modulator that promotes the tendency of insects to fight (Stevenson et al., 2005) and perform agonist acts such as lunging (Hoyer et al., 2008; Zhou et al., 2008) and mandible spreading (Rillich and Stevenson, 2011). This basically corresponds to the modulatory role of octopamine in promoting cholinergic initiating of motor behaviors such as flying (Buhl et al., 2008). As outlined below, experiments on crickets revealed that octopamine mediates the promoting influences of diverse experiences on aggression.

EXPERIENCE DEPENDENT PROMOTION OF AGGRESSION

As in mammals and other vertebrates, aggressive behavior in crickets is promoted by a variety of experiences including physical exertion (flying), winning, and the possession of key resources such as food, mates, and shelter (e.g., Alexander, 1961; Dixon and Cade, 1986; Simmons, 1986; Hofmann and Stevenson, 2000; Stevenson et al., 2000, 2005; Nosil, 2002; Killian and Allen, 2008; Rillich and Stevenson, 2011; Rillich et al., 2011). Below we first highlight three illustrative examples, and then summarize evidence showing that octopamine plays a key role in each case (data summarized in **Table 1**).

THE EFFECT OF FLYING

Cricket fighting has been a popular pastime in China for centuries (Hofmann, 1996). Surprisingly, "punishing" submissive crickets by shaking and launching them in the air, as recommended by knowledgeable aficionados, significantly increases their aggressiveness, but it is more effective to make the animals fly tethered in a wind stream for a minute or two (Hofmann and Stevenson, 2000). Flown crickets are exceptionally aggressive (**Figure 3B**), and fight two to three times longer than usual (Stevenson et al., 2005). Moreover, while losers usually avoid other males for hours, flown losers regain their aggressiveness within only 15 min. These effects highlight the impact that motor activity can have on the operation of seemingly unrelated behaviors.

THE WINNER EFFECT

Winning a conflict makes an individual more aggressive and more likely to win a subsequent encounter in numerous species (reviews: Hsu et al., 2006, 2009; Rutte et al., 2006) including crickets (Khazraie and Campan, 1999; Iwasaki et al., 2006), but little is known of the proximate causes. Recent work implicates androgens as physiological mediators in vertebrates (Oliveira et al., 2009), while in crickets octopamine is involved (Rillich and Stevenson, 2011).

The effect of experiencing successive wins on aggression in crickets has been quantified by staging knockout tournament (Rillich and Stevenson, 2011). With each round, fights between winners of preceding contests become progressively more severe and longer (**Figure 4**). This winner effect is transient and lasts

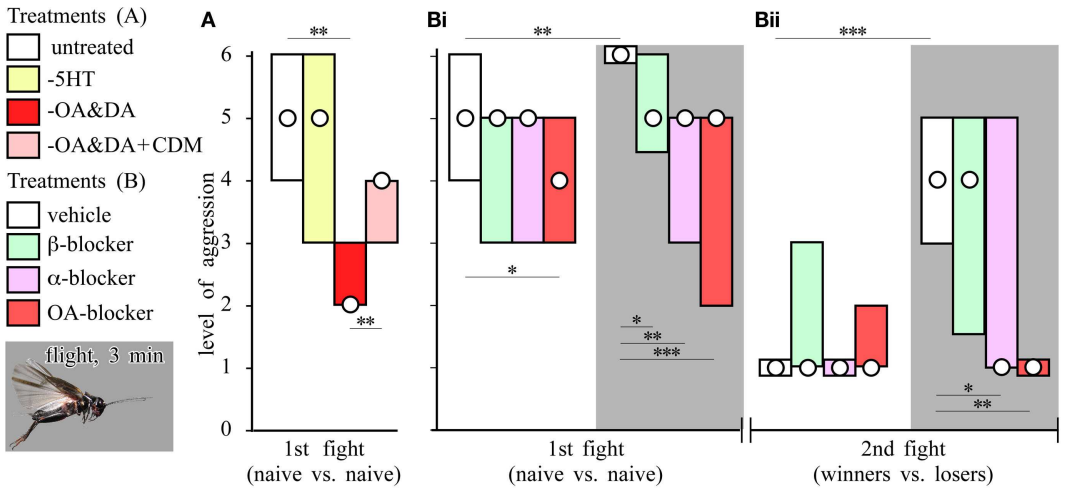

FIGURE 3 | Aminergic drugs and the effect of flying. (A) Effect of amine depletion on aggression. Bars giving the level of aggression (median, interquartile range) for fights between pairs of socially inexperienced crickets (initial fight) that were either untreated (white bar, n = 24), serotonin depleted (yellow bar, −5HT, n = 27), octopamine/dopamine depleted (red bar, −OA/DA, n = 45) or octopamine/dopamine depleted and treated with the octopamine agonist CDM (pink bar, −OA/DA + CDM, n = 10). **(B)** Effect of aminergic blockers on aggression and the effect of flying (**Bi** initial fight; **Bii** winners vs. losers 15 min later). Before the initial fight the crickets were injected with vehicle (white bars, n = 20), the β-adrenergic blocker propranolol (green bars, n = 19), α-adrenergic blocker phentolamine (violet bars n = 14), or the specific octopamine (OA) blocker epinastine (red bars, n = 20). Seperate groups receiving the same treatments were flown for 3 min just before the initial fight (gray background: vehicle n = 24; propranolol n = 19; phentolamine n = 23; epinastine n = 24). Asterisks denote significant differences between columns indicated (Mann–Whitney *U*-test $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; simplified from Stevenson et al., 2005).

able 1 | Effect of various behavioral experiences on the fighting behavior of adult male crickets (control), and how these effects are influenced by selected pharmacological treatments.

Behavioral experience	harmacological treatment and its influence on the effect of behavioral experience					
	Control (vehicle)	A agonist (CDM)	A blocker (epinastine)	DA or A blockers	A depletion (AM)	5 depletion (AM)
Control	–	None	Reduced	None	Reduced	None
Losing	Reduced	Restored	None	None	None	None
Flying	Enhanced	–	Blocked	None	Blocked	None
Winning	Enhanced	–	Blocked	None	Blocked	None
Residency	Restored	–	Blocked	None	Blocked	None

*The table summarizes the key results depicted in **Figures 3–5** (based on original data from Stevenson et al., 2005; Rillich and Stevenson, 2011; Rillich et al., 2011). OA, octopamine; DA, dopamine; TA, tyramine; 5HT, 5-hydroxytryptamine (serotonin); CDM, chlordimeform; AMT, alpha methyltyrosine; AMTP, alpha methyltryptophan.*

less than 20 min, which is far shorter than in rodents, and is thus not necessarily associated with learning and memory, as suggested for fruit flies (Yurkovic et al., 2006). But what exactly constitutes a win? When fights between two crickets are interrupted before either experiences an actual win, both contestants become hyper-aggressive in subsequent encounters. However, a winner effect also developed in crickets that experienced an opponent's retreat prior to any physical interaction. Hence, a winner effect can result both from the physical exertion of fighting, as well as from some non-physical aspect of the actual winning experience.

RESOURCES AND THE RESIDENCY EFFECT

Animals in possession of a key resource, an essentially non-physical experience, are more likely to win disputes against contenders, but it is hotly debated how this is controlled (reviews: Kemp and Wiklund, 2004; Hsu et al., 2006, 2009). For male field crickets, burrows are valuable assets offering shelter from predators and an aid in attracting females, which mate preferentially with burrow owners, and these zealously fight off any intruding male (Alexander, 1961; Simmons, 1986; Rodriguez-Munoz et al., 2011).

Under laboratory conditions, initially submissive crickets become highly aggressive after occupying an artificial shelter and frequently win against an aggressive intruder (Rillich et al., 2011; **Figure 5**). This residency effect is transient and has a similar time course as the winner effect. It first becomes significant after 2 min of residency, maximal after a 15-min, and declines 15 min after removing the shelter. Hence, the effect does not depend on the initial sensory experience of shelter occupation *per se*. There also

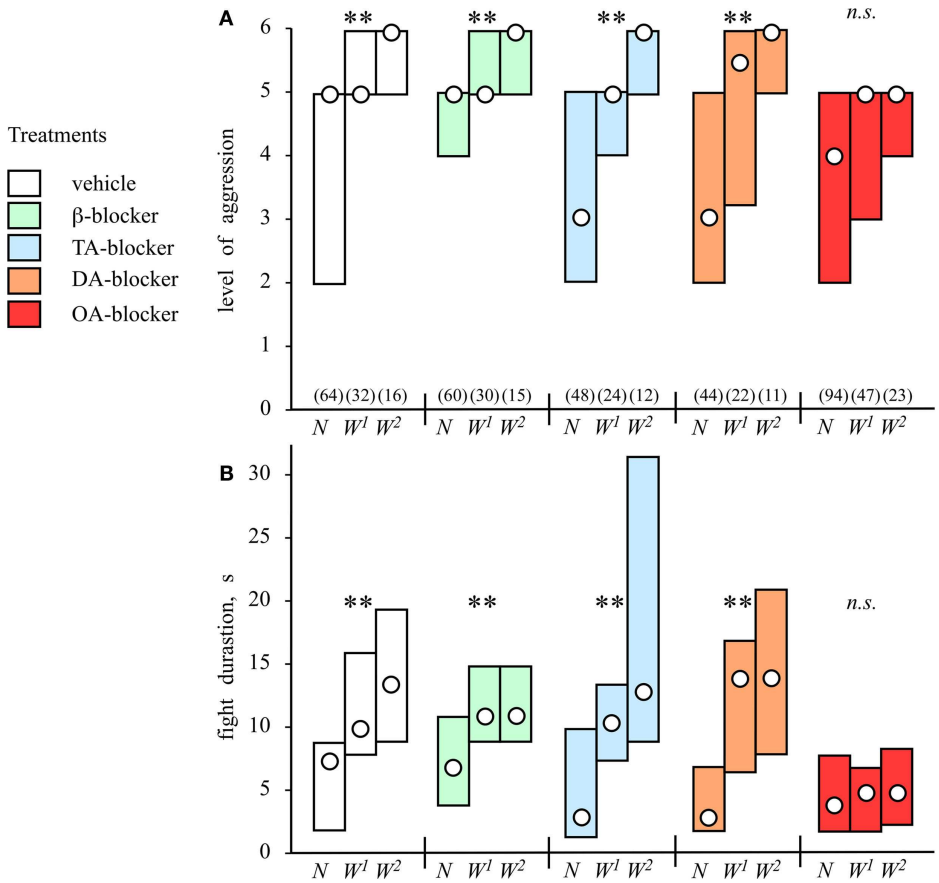

FIGURE 4 | he winner effect and influences of amine receptor blockers. Bar graphs giving **(A)** the level and **(B)** duration of aggression (median, interquartile range) for fights between pairs of weight matched male crickets that were socially inexperiences (naive, N), winners of one previous encounter (W^1), or winners of two encounters (W^2) for an inter-fight-interval of 5 min. *Pretreatments:* before the initial fight the animals were injected, with: vehicle (white bars), β-adrenergic blocker propranolol (green bars), tyramine (TA) blocker yohimbine (blue bars), dopamine (DA) blocker fluphenazine (brown bars), or octopamine (OA) blocker epinastine (red bars). Numbers in parenthesis in (A) give the pairs for each round, significant differences between tournament rounds are indicated (Kruskal–Wallis one way variance test, $^{**}p < 0.01$, n.s. not significant; adopted from Rillich and Stevenson, 2011).

seems to be no single feature of the shelter causing the effect. For example, wire shelters, or shelters with a transparent roof are less effective, although darkness alone has no effect. Increased aggressiveness with prolonged residency or territoriality is known in many animal species (Cromarty et al., 1999) and is thought to reflect the increase in value of the resource with time as the animal gathers more information on it or invests increasingly more in it (Bradbury and Vehrencamp, 1998).

OCTOPAMINE DEPENDENCY

Pharmacological manipulations to evaluate the impact of different biogenic amines on aggression have shown that the effects of flying, winning, and resource possession (residency) are mediated in each case by octopamine. First, the effect of flying can be mimicked by activating the octopaminergic system with CDM, but it is no longer evident in octopamine/dopamine depleted crickets, and it is also selectively blocked by octopamine receptor antagonists (Stevenson et al., 2005; **Figure 3B**). Similarly, the winner effect is blocked by the selective octopamine receptor antagonist epinastine, but not by β-adrenergic-, tyramine-, or dopamine-receptor antagonists (Rillich and Stevenson, 2011; **Figure 4**). Finally, the residency effect is prohibited in octopamine/dopamine depleted crickets, while being unaffected by serotonin depletion, and it is selectively blocked by treatment with octopamine antagonists (Rillich et al., 2011; **Figure 5**).

OCTOPAMINE, REWARD, AND AGGRESSIVE MOTIVATION

The paradoxical question now posed is how experiences as diverse as flying, winning, and resource possession, which encompass extremes of the locomotory and energy expenditure spectrum, can all lead to activation of the octopaminergic system promoting aggression? Activation of the insect octopaminergic system is generally thought to occur under stressful conditions and prepare the animal for a period of prolonged activity, or assist in recovering from increased energy demand (Verlinden et al., 2010). Flying and fighting both lead to a considerable increase in the hemolymph titer of octopamine (Adamo et al., 1995), although the concentration is too low to pass the "blood-brain" barrier and

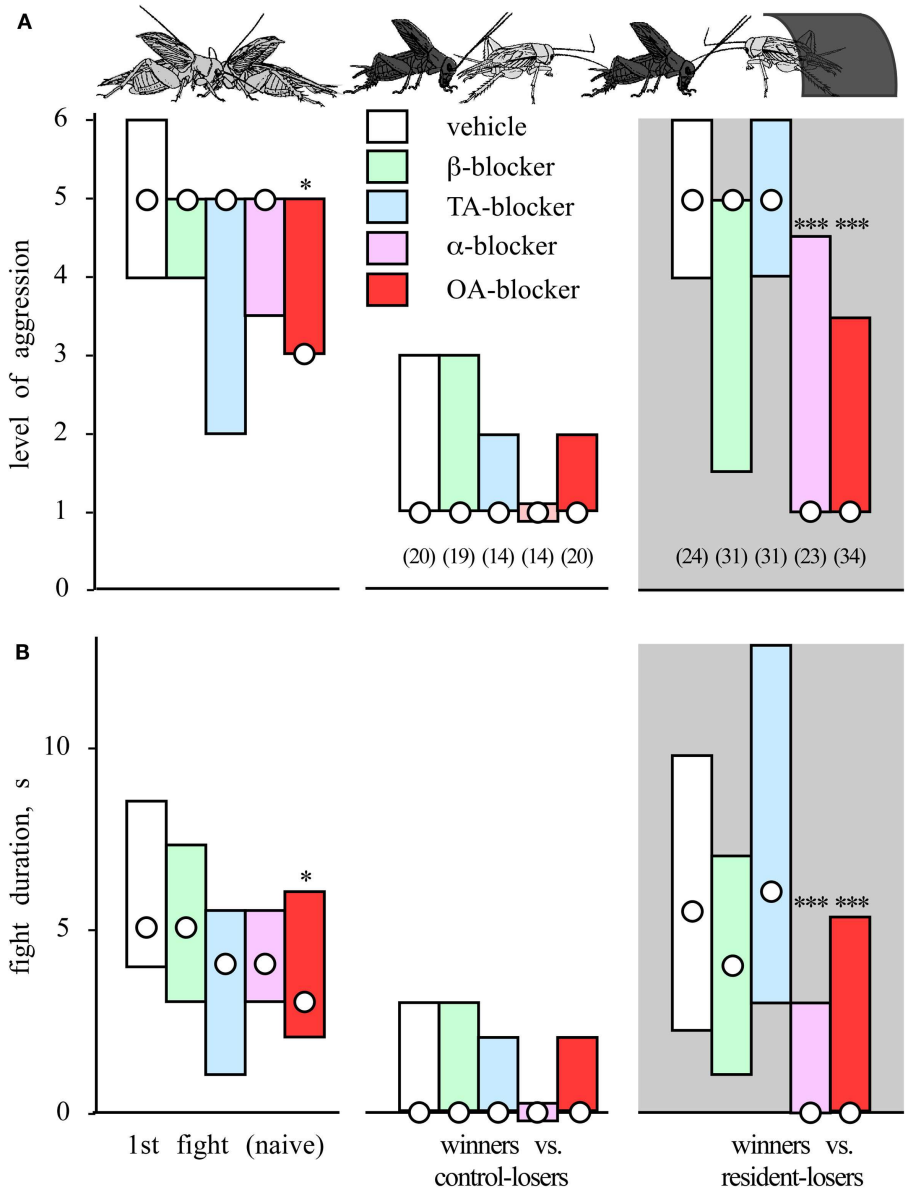

FIGURE 5 | he residency effect and influences of amine receptor blockers. Bar charts giving in **(A)** the level of aggression and **(B)** fight duration after residency (median and interquartile range). The crickets were treated prior to the initial fight with either vehicle (white bars), a β-adrenergic blocker (green bars), a tyramine (TA) blocker (blue bars), an α-adrenergic blocker (violet bars), or octopamine (OA) blocker (red bars). The aggressiveness of treated animals was evaluated in an initial fight (naïve) and in a second contact 15 min later before which the losers remained in the arena without a shelter (winners vs. control-losers) or occupied a shelter in the arena (winners vs. resident-losers, gray background). The number of contests evaluated n is given in parenthesis beneath each column, excepting initial fight, which is pooled. Asterisks denote statistically significant differences (Mann–Whitney *U*-test *, **, ***: $p < 0.05$, 0.01, 0.001 respectively; adopted from Rillich et al., 2011).

influence aggression (c.f. Stevenson et al., 2005). The increase must result partly from "spill over" from efferent octopaminergic neurons, such as the dorsal and ventral unpaired median neurons (DUM/VUM cells, reviews: Stevenson and Sporhase-Eichmann, 1995; Bräunig and Pflüger, 2001), which innervate skeletal muscles and are excited during flying (Duch et al., 1999), walking (Baudoux et al., 1998), and by a variety of mechanosensory signals (Morris et al., 1999). Although the activation of octopaminergic neurons due to the physical exertion of flying and fighting could explain the effects of these activities on aggression, the argument is less compelling for the influences of winning without fighting and residency. The latter, essentially non-physical experiences, are also unlikely to represent stressful conditions.

As an alternative hypothesis, we propose that all experiences that enhance aggressiveness in crickets do so because they in someway represent a positive, reinforcing, or rewarding experience (Rillich and Stevenson, 2011; Rillich et al., 2011). Physical exercise in mammals, including humans, seems to be equated with reward

(Raichlen et al., 2011), and can act as a mood elevator that alleviates symptoms of depression by invoking changes in a variety of neurotransmitter systems including dopamine (Craft and Perna, 2004). Aggression itself also leads to increased activity in dopaminergic pathways and androgen receptor expression in regions of the mammalian brain that mediate motivation and reward (Barron et al., 2010; O'Connell and Hofmann, 2011). Even watching a previous victory can evoke similar effects in humans (Carre and Putnam, 2010). In insects, evidence suggests that reward is signaled by octopamine, rather than dopamine (review: Barron et al., 2010). In honeybees, the nutritional value of food sources may be encoded by octopamine modulating associative reward pathways (Barron et al., 2010). Octopamine conveys reward signals in appetitive learning in honeybees (Hammer and Menzel, 1995), fruit flies (Schwärzel et al., 2003), and crickets (Mizunami et al., 2009). In the honeybee, the activity of even a single identified octopaminergic DUM/VUM-type neuron can substitute for the sucrose reward in an associative learning paradigm (Hammer, 1993). This neuron is one of a group of less than 20 octopaminergic DUM/VUM-neurons occurring in the subesophageal ganglion of honeybees (Schröter et al., 2007) and other insects including crickets (Stevenson and Sporhase-Eichmann, 1995). In Drosophila, a distinct subset of these octopaminergic neurons was shown to be functionally important for expressing aggression (Zhou et al., 2008). Another subset expresses the sex determining factor fruitless, and is involved in mediating the choice between courtship and aggression (Certel et al., 2007, 2010). The function of these neuron types in crickets is unknown.

THE DECISION TO FLEE – THE CUMULATIVE ASSESSMENT MODEL

For the decision to flee, animals are thought to assess information from ritualized agonistic signals exchanged during fighting. This decision could be based on average differences in signals (Sequential Assessment Model), the total sum of own actions (Energetic War of Attrition Model), or the total sum of opponent actions (Cumulative Assessment Model; c.f. Payne, 1998). Work on crickets revealed that agonistic signals act to reduce the aggressiveness of the receiver, but not the sender (Rillich et al., 2007). For example, pairs of crickets with lamed mouthparts not only fight, they escalate higher and fight longer than sham treated crickets (**Figure 6A**). Fights became progressively longer the more the animals were handicapped, lasting minutes rather than seconds for example between opponents with lamed mandibles, blackened eyes, and clipped foreleg claws to limit body flipping. Furthermore, whereas "blinded" crickets, or crickets with lamed mouthparts fought non-handicapped crickets with almost unaltered win chances, the blinded crickets practically always (98%) defeated crickets with lamed mouthparts (Rillich et al., 2007; **Figure 6B**). These findings are fully conform with predictions of the cumulative assessment model postulated by Payne (1998). We suggest, in accord with this model, that Mediterranean field crickets persist in fighting until the sum of the perceived adversary's actions accumulated during fighting surpasses some threshold to flee. Hence, the blinded cricket persists because it receives no visual and limited physical input from an opponent with lamed mandibles, whereas the latter accumulates the full brunt of his adversaries actions and becomes the first to flee. This model also accommodates the effects

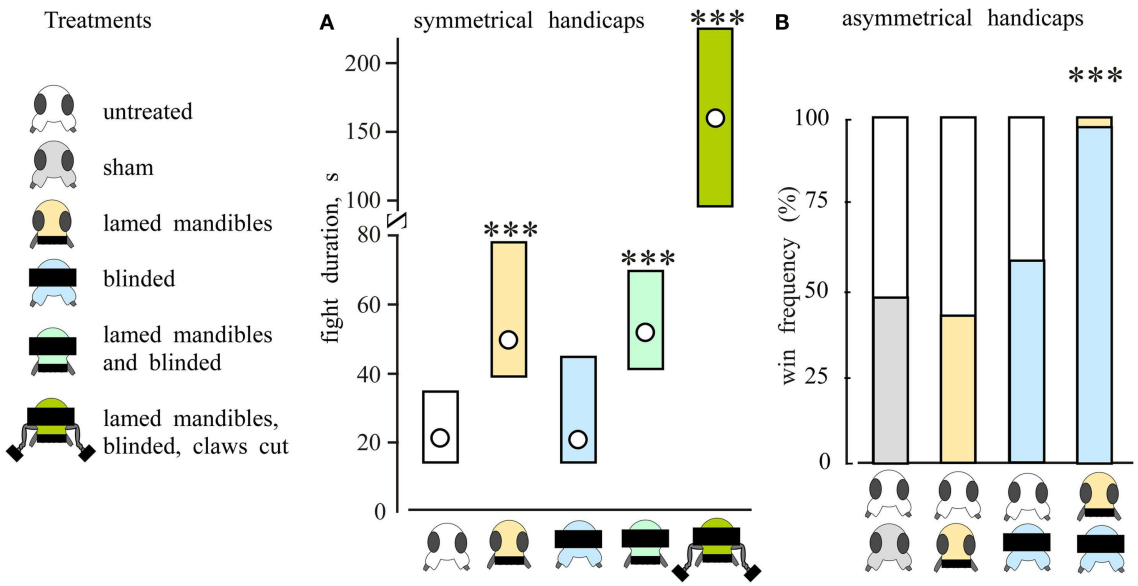

FIGURE 6 | andicaps reveals assessment strategy in crickets. (A) Symmetrical handicaps. Bars giving the level of aggression (median, interquartile range) for fights between pairs of crickets that were both (from left to right) untreated (n = 38), had lamed mandibles (n = 26), blinded (n = 19), had lamed mandibles and blinded (n = 26) or lamed mandibles, blinded, and clipped foreleg claws (n = 23). **(B)** Asymmetrical handicaps. Bars giving the win frequencies (%) for handicapped crickets, from left to right: sham vs. untreated (n = 33); lamed mandibles vs. untreated, (n = 45) blinded vs. untreated (*n* = 50), blinded vs. lamed mandibles (n = 35). Statistically significant differences between data sets are indicated [**(A)**, *U*-test; **(B)**, Chi-square; *, **, ***: $p < 0.05$, 0.01, 0.001 respectively; adopted from Rillich et al., 2007].

of physical disparities such as size strength and weaponry on fight outcome (see, e.g., Judge and Bonanno, 2008; Hall et al., 2010), since an animal with any physical advantage will have a greater sensory impact on its opponent, which in turn increments opponent agonist signals more rapidly, and is thus more likely to flee first. It also fits our personal observation that crickets often fight on even after serious injury, such as losing part of a leg or antenna, only to retreat seconds later for no obvious reason.

THE DECISION TO FLEE – CANDIDATES FOR ITS CONTROL

It is not known how the sensory information of various modalities conveyed by an opponent's agonistic signals is summated in the nervous system and how this triggers a cricket to retreat. Whatever its cause, defeat results in longer-term submissiveness in many animal species (reviews: Rutte et al., 2006; Hsu et al., 2009). This loser effect lasts over 3 h in crickets, during which time they avoid contact to other males, even if unfamiliar (Hofmann and Stevenson, 2000). Nonetheless, losers are still potentially aggressive, since they will fight for example when their eyes are blackened, a manipulation that effectively eliminates the visual sensory impact of the approaching opponent (Rillich et al., 2007). Accordingly, the reduced tendency of losers to fight seems to be due to an increased tendency to flee, rather than reduced aggressiveness *per se*. Supporting this idea, retreat, and the loser effect appears not to result from depressed octopaminergic signaling. For one, octopamine levels are similar in winners and losers (Adamo et al., 1995). Furthermore, the octopamine agonist chlordimeform (CDM), which binds almost irreversibly to octopamine receptors (c.f. Evans, 1985), can restore aggression in losers, but cannot protect them from actually losing, and subsequently behaving submissively for a short time, after which their aggression is once again restored under the continued influence of CDM (Stevenson et al., 2005). There must, therefore, be some opposing control mechanism, which could involve the following neuromodulators.

SEROTONIN

The actions of octopamine in arthropods are often functionally antagonized by serotonin (Erber et al., 1993). Serotonin's role in insect aggression is, however, unclear. In crickets, serotonin depletion induces hyperactivity and enhances startle responses, but without affecting aggression (Stevenson et al., 2000, 2005). Supporting this, Baier et al. (2002) found that aggression in Drosophila is unaffected when serotonin synthesis is either disrupted, or its level elevated by treatment with serotonin's precursor (5HTP). In contrast, Dierick and Greenspan (2007) observed that 5HTP promotes aggression in fruit flies, and Alekseyenko et al. (2010) using genetic manipulation report that activating serotonergic neurons resulted in flies that escalated faster and fought fiercer, while disrupting serotonergic transmission yielded flies with reduced fighting ability (see also Dyakonova et al., 1999 on crickets). These conflicting findings may be due to differences in behavioral protocols together with difficulties in dissecting out differential effects of serotonin operating via different receptor subtypes. Johnson et al. (2009) for example, found that pharmacological activation of 5HT2-type receptors reduced total aggression in Drosophila, and conversely that activating 5HT1A-type receptors increased it.

In mammals, different serotonin receptor subtypes also seem to influence different aspects of the total aggressive behavioral repertoire (de Boer and Koolhaas, 2005). This and other findings now challenge the dogmatic view of serotonin acting simply to suppress aggression. Currently, serotonin in mammals is thought to limit impulsivity (review: Nelson and Trainor, 2007) or promote the drive to withdraw (Tops et al., 2009), rather than suppress aggression *per se*. We envisage an analogous scenario in insects, since it fits our observations in crickets that losers have an increased tendency to flee, rather than suppressed tendency to fight. The current evidence is however limited. While serotonin seems to depress escape responses in aggressive crickets (Dyakonova et al., 1999), losers are claimed to exhibit enhanced escape behavior due to lower serotonin levels (Murakami and Itoh, 2001). Similarly in crayfish, the effects of serotonin on escape and body posture change with social status due to a shift in the relative expression of different serotonin receptor subtypes to a pattern more appropriate for the new status (Edwards and Spitzer, 2006; Cattaert et al., 2010). Finally in locusts, visual, and tactile inputs from conspecifics induce the release of serotonin, which promotes social tolerance (Anstey et al., 2009). It is thus conceivable, that serotonergic pathways are activated in crickets by the perceived agonistic signals of an opponent during fighting.

PEPTIDES

The expression of aggression in insects is also influenced by the action of neuropeptides. In crickets, treatment with the opiate antagonist naloxone elevates aggressiveness in losers, without affecting winners, or socially naive animals (Dyakonova et al., 2002) and in Drosophila aggression is increased following genetic silencing of circuitry employing neuropeptide-F, the invertebrate homolog of neuropeptide-Y (Dierick and Greenspan, 2007).

NITRIC OXIDE

Aggression in mammals is suppressed by the action of the gaseous modulator nitric oxide (NO), at least partly by influencing serotonergic signaling (Nelson and Trainor, 2007), but its role in insect aggression needs clarification. Dyakonova and Krushinskii (2006) report that treatment with an NO-synthesis inhibitor prohibits the aggression promoting effects of flying in crickets, indicating that NO enhances aggressiveness. Iwasaki et al. (2007) in contrast, report that inhibiting NO-synthesis relieves the loser effect, but has no effect on socially naive crickets. On going work indicates that disrupting the NO/cGMP pathway causes socially naive crickets to persist longer at fighting (Stevenson, 2011; Stevenson, in preparation), suggesting that accumulating NO may be involved in triggering the decision to flee.

A RELATIVE THRESHOLD MODEL FOR THE FIGHT OR FLEE RESPONSE

We propose that the decision to fight or flee could be accounted for in crickets by simply modulating the initiation thresholds for these two opposing behaviors relative to each other (**Figure 7**). As argued above, experiences evaluated as being in someway rewarding (winning, resource possession) promote the tendency to fight to a level determined by the modulatory action of octopamine. Accordingly, octopamine can be considered as representing the motivational component of aggression. Opposing this, and in

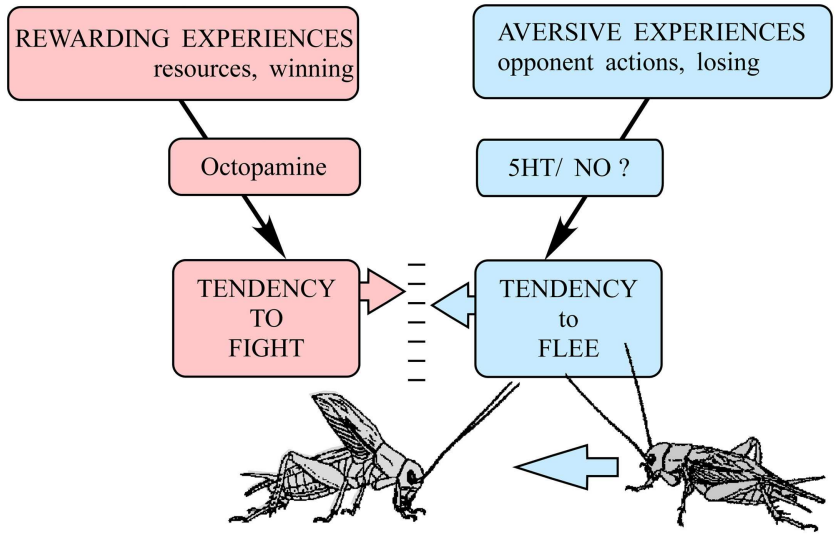

FIGURE 7 | A relative threshold model for the decision to fight or flee in crickets. Rewarding experiences (winning, resource possession) promote the tendency to fight via the action of octopamine. In this respect, octopamine can be regarded as representing the motivational component of aggression. Aversive experiences accumulated during fighting (opponent's agonistic signals, losing) promote the tendency to flee via actions of other neuromodulators, which probably include serotonin (5HT), nitric oxide (NO), and selected peptides. In accord with the cumulative assessment hypothesis (Payne, 1998), a cricket will fight and persist as long as the perceived sum of rewarding experiences (motivation) exceeds the sum of accumulated aversive fighting experiences, but flee the moment the latter is greater. (adopted from Simpson and Stevenson, 2012).

accordance with the cumulative assessment hypothesis (Payne, 1998), aversive experiences, i.e., the opponent's agonistic signals, trigger the tendency to flee when the accumulated sum surpasses a set level. It appears likely that serotonin, nitric oxide, and selected peptides are involved in integrating agonistic signals for the decision to flee. This model is in line with the roles proposed for noradrenaline, serotonin, and nitric oxide in mammals (Tops et al., 2009), suggesting that basic mechanisms of aggressive modulation may be conserved in phylogeny. However the principle actions of serotonin and octopamine on aggression are apparently reversed in crustaceans (Kravitz and Huber, 2003), so they do not fit into this schema. Regardless of the actual modulators involved, the relative threshold model would allow the animal to optimally adapt its aggressive behavior toward an opponent by taking into account both physical disparities as well as experience dependent disparities in aggressive motivation.

CONCLUSION AND FUTURE DIRECTIONS

The brains of insects may be comparatively simple in terms of neuron number, they nonetheless have the integrative power to sculpture social interactions of a complexity approaching that of our own (Simpson and Stevenson, 2012). Insects are thus ideal models for investigating how animals make appropriate adaptive behavioral choices. As such, they should provide insights for the currently en-vogue discipline of neuroeconomics (Glimcher et al., 2009), which seeks to determine how costs, and benefits are represented in the brain for optimizing decision-making. The studies on crickets outlined here illustrate that insects have the capacity to compute potential rewards and costs of aggression for making the adaptive behavioral decision to fight or flee on confronting a competitor. They achieve this it seems quite simply, by exploiting the powers of neuromodulation, primary using biogenic amines, which act at the interface between the animal's social environment and central brain circuits (Simpson and Stevenson, 2012).

It has been shown that the tendency to fight in insects is promoted by the amine octopamine, the analog of noradrenaline. Rather than acting as a releaser of aggression, or simple "arousal" agent, octopamine appears rather to function as a selective neuromodulator that mediates the aggression promoting effect of experiences, including physical exertion, winning, and the possession of resources. In this respect octopamine represents the motivational component of aggression that drives the tendency of a cricket to fight. In correspondence with the envisaged role of dopamine on aggression in mammals (reviews: Barron et al., 2010; O'Connell and Hofmann, 2011), we propose that experiences that promote aggression in crickets are evaluated as being in someway rewarding. What we now need to know is whether octopamine encodes the actual value of a resource for the decision to fight. This seems feasible considering that foraging honey bees appear to exploit the octopaminergic system to report the quality of discovered food sources (Barron et al., 2009).

The decision to flee, on the other hand, appears to be controlled in line with predictions of the cumulative assessment hypothesis (Payne, 1998) in that crickets persist in fighting until the sum of the perceived adversary's actions surpasses some threshold to flee. Defeated crickets have a reduced tendency to fight, but are still potentially aggressive, indicating that losing increases the tendency to flee, rather than reduce aggressiveness *per se*. Future studies must now be directed toward discovering how information from an opponent's agonistic signals are summated in the nervous system and how this could promote the drive to withdraw. The first step must be to evaluate the extent to which serotonin, nitric oxide, and possibly peptides are involved in this process. At present we also do not know how the decision to flee is influenced by the energetic

costs of physical fighting, which is high in crickets (Hack, 1997). In hermit crabs it appears that the depletion of energy reserves and accumulation of harmful by-products are cues for the decision to give up (Briffa and Elwood, 2005). In crickets we predict (in accord with the cumulative assessment hypothesis), that accruing metabolic costs may correlate with abating physical fitness, and hence lowered sensory impact on the opponent, which will hence tend to persist longer and be less likely to flee first.

In conclusion, we propose that the decision to fight or flee in crickets is controlled by the action of separate neuromodulator system that set the relative behavioral thresholds for these opposing behaviors. A simple threshold mechanism also has the power to control sophisticated collective decision-making in eusocial insects (Robinson et al., 2011). The model we propose is in line with the roles envisaged for noradrenaline, serotonin, and nitric oxide in mammals (Tops et al., 2009), but differs to that in crustaceans in which the principle actions of serotonin and octopamine are apparently reversed (Kravitz and Huber, 2003).

While some progress has been made in elucidating the nervous centers and neuroanatomical pathways underlying aggression in rodents and non-human primates (review: Nelson and Trainor, 2007), we are far from knowing this in crickets, despite their reputedly more accessible and comparatively simpler nervous system. Individual octopaminergic cells involved in the neuronal representation of rewarding qualities have been identified in the honeybee as individuals of the group of ascending DUM/VUM cells in the subesophageal ganglion (Hammer, 1993). Neurons of this class may also be involved in the expression of aggression in fruit flies (Zhou et al., 2008). They also occur in crickets and other orthopterans (Stevenson and Sporhase-Eichmann, 1995), but their functions are largely unknown. In the insects investigated, individual DUM/VUM neurons were found to invade all major brain neuropils, including the mushroom bodies, a region where focal electrical stimulation was shown to elicit discrete elements of aggressive behavior in crickets more than 50 years ago (Huber, 1960). We now need to discover the synaptic connectivity of the ascending octopaminergic DUM/VUM cells in crickets, and in particularly the locality and types of receptors they activate. These and other aminergic neurons are often equipped with a host of co-transmitters, including nitric oxide, amino acids, and peptides (e.g., Bullerjahn et al., 2006), but it is not known under which behavioral circumstances co-transmitters are released, nor how they affect modulation at their targets. Finally, on a topic we have brushed past, aggression can have longer-term changes on the operation of the nervous system than those discussed here. Agonistic behavior can trigger neurogenesis (Ghosal et al., 2009) and FOS-like protein expression in the male cricket brain (Ghosal et al., 2010), but it is not know whether this leads to changes in behavior. A hint of the complexities involved is given by the finding that aggressive behavior in Drosophila is affected by over 50 novel genes with widespread pleiotropic effects (Edwards et al., 2009).

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Re-visiting of plentiful food sources and food search strategies in desert ants

Harald Wolf *, Matthias Wittlinger and Siegfried Bolek

Institute of Neurobiology, University of Ulm, Ulm, Germany

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Wael F. Asaad, Harvard Medical School, USA
Ken Cheng, Macquarie University, Australia
Kathrin Steck, Max Planck Institute for Chemical Ecology, Germany

****Correspondence:***
Harald Wolf, Institute of Neurobiology, University of Ulm, D-89069 Ulm, Germany.
e-mail: harald.wolf@uni-ulm.de

North African desert ants, *Cataglyphis fortis*, are established model organisms in animal navigation research. *Cataglyphis* re-visit plentiful feeding sites, but their decision to return to a feeder and the organization of food searches has been little studied. Here we provide a review of recent advances regarding this topic. At least two parameters determine the ants' assessment of site quality, namely, amount of food available and reliability of food encounter on subsequent visits. The amount of food appears to be judged by the concentration of items at the food uptake site. Initially the amount of food in a feeder dominates the foragers' decision to return, whereas learning about reliability takes precedence in the course of a few visits. The location of a worthwhile site is determined by the animals' path integration system. In particular, the distance of the feeding site is memorized as the arithmetic average of the distances covered during the previous outbound and homebound journeys. Feeding sites that are small and inconspicuous cannot be approached directly with sufficient certainty, due to inevitable inaccuracies of the path integrator. Instead, desert ants steer downwind of the goal to encounter the odor plume emanating from the food and they follow this plume to the feeder. The angle steered downwind reflects the animals' maximal navigation error and is adjusted according to experience. In summary, food searches of desert ants provide an unexpected wealth of features that may advance our understanding of search, navigation, and decision strategies. There are several aspects that warrant further scrutiny.

Keywords: desert ant *Cataglyphis*, navigation, feeding site assessment, path integration, error compensation

INTRODUCTION

The life of animals, including more "simple" invertebrates, abounds with decisions, most of which have a bearing on reproductive fitness or even survival. And while the individual decision may not be too important, a balanced strategy for arriving at viable decisions in the long term is certainly essential. Food acquisition is a good example here since it has direct consequences for survival and reproduction. When a forager encounters a plentiful feeding site it cannot fully exploit, is it useful to return later? Or are there better chances of finding food elsewhere, due to high food abundance or because other foragers will have removed the bounty next time around?

Desert ants are good study objects in this context because, firstly, food availability is easily manipulated in the barren and open desert habitat and, secondly, appreciation of food sources by the ants can be measured quantitatively as the focusing of their food search behavior. Moreover, the North African species *Cataglyphis fortis* is a well-studied model system in navigation research (Wehner, 2003), with good associated knowledge of behavioral aspects. For instance, the ants may be trained to re-visit plentiful feeders, a property regularly employed in navigation research. Feeder location is determined by a path integrator that keeps track of a forager's position with respect to its nest throughout foraging excursions (Wehner and Wehner, 1986; Müller and Wehner, 1988; Wehner and Srinivasan, 2003).

By contrast, comparatively little is known about either the parameters used by the ants in evaluating whether a food source is valuable enough to re-visit or about other features associated with food searches. We thus provide an overview of recent results with a focus on the following questions.

1. What prompts the ants to re-visit a feeding site in the first place? Is it the amount of food or the reliability of food encounter on sequential visits?
2. Is it the previous outbound or the last inbound journey that is used to establish the memory of the feeding site location?
3. In the case of small and inconspicuous food sources, is the accuracy of the path integrator sufficient to find the food source again? And if not, what strategies are used for a reliable encounter?

We use these recent data to identify important points for further study in this area.

WHAT PROMPTS DESERT ANTS TO RETURN TO A FEEDING SITE?

FOOD AMOUNT AND RELIABILITY OF FOOD ENCOUNTER

We examined whether it is the amount of food available on the previous visit or the reliability of food encounters on sequential visits that influences the return to a feeding site by *Cataglyphis*

ants (Bolek et al., 2012b). Only novice foragers were used in these experiments to avoid any influence from previous experience.

Experimental situations

Experiments in artificial channels make the recording of quantitative data much easier compared to the open desert terrain. It was thus first necessary to establish whether or not food searches performed in channels do indeed reflect normal search behavior as performed in the open field (**Figure 2**). Food searches were therefore initially recorded in the open desert terrain by placing a feeder 10 m from the nest (**Figure 1A**) and recording the ants' foraging trips by means of a 2-m by 2-m grid painted on the desert floor around the feeder. When an ant had encountered a full feeder on its first trip to the feeding site, its next food search was clearly centered on the previous position of the now absent feeder (**Figure 2A**). This demonstrates that, in this situation at least, the ants memorize the vector to the food site quite exactly. When projecting the two-dimensional search trajectory onto the nest-feeder axis (details in legend **Figure 2**, see also Bolek et al., 2012a), the resulting search distribution (**Figure 2B**) was similar to the search pattern recorded in a channel under otherwise identical conditions (**Figure 2C**; same data set as **Figure 3A**, bottom box). This observation attests to the validity of the channel experiments carried out in the following experiments.

The setup for the channel experiments in this and the subsequent experiments (including those in section Is it the Previous Outbound or the Last Inbound Journey that Establishes the Memory of the Feeding Site Location?) consisted of two parallel channels that were both connected to the nest via a Y-shaped junction (**Figures 1B,C**). In the training channel, a feeder was established at 10 m distance from the ants' nest. The channel arrangement increased the number of ants foraging at the feeder by restricting their foraging excursions to the channel, and it also facilitated the recording of search behavior. For testing, the ants were led into the test channel that extended for more than 20 m beyond the feeder in parallel to the training channel. A switch door in the channel near the nest allowed selection of the ants to be tested (**Figure 1C**). The ants' search behavior was recorded by noting their U-turns in the test channel. For each ant individual, search medians (**Figures 2B,C**, **3**, and **4B**) and spreads (**Figures 3D–F**) were calculated from the initial six turns. Spreads were calculated as variances of the individuals' searches. For the individuals' values, means, and percentiles were determined for the experimental groups.

To test what prompts an ant to search for a food site, the ants were left to find the feeder by chance (similar to the situation in **Figure 1A**, though in the training channel). In the different test situations, the feeder was equipped with either one, five, 25, or

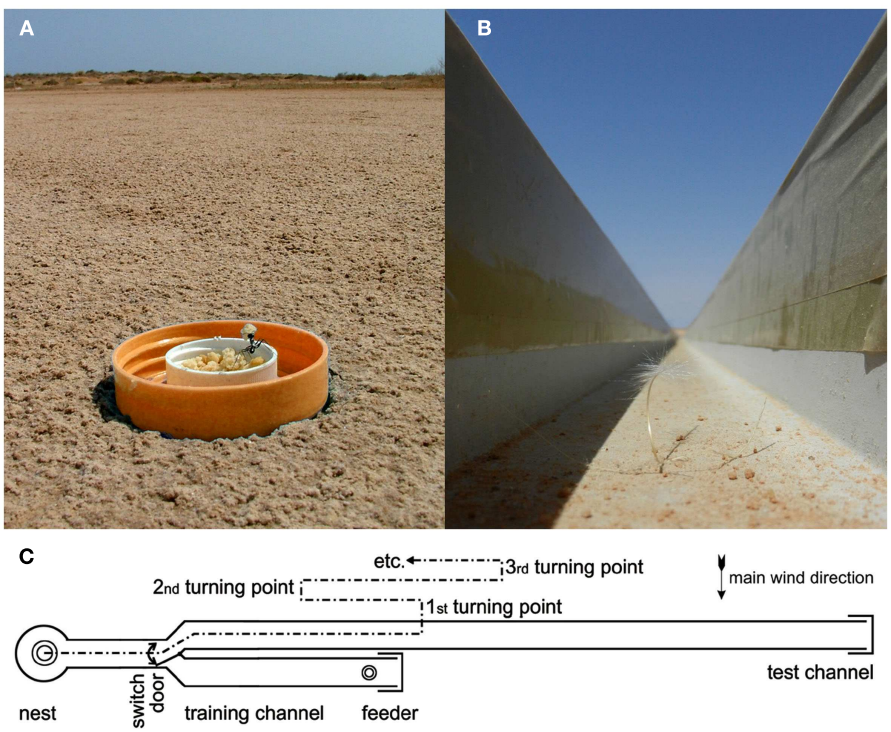

FIGURE 1 | Experimental feeding station (A) and channel arrangement (B,C). (A) A feeding station on the desert floor is visited by a *Cataglyphis fortis* forager (Forel 1902; Wehner, 1983). The lid of a marmalade jar is usually pressed into the desert floor (rather than left lying on top as shown here for clarity) to avoid any visual cues extending above desert floor level. The lid captures any food crumbs that are blown out of the small central food container or that are removed by the foragers but dropped during sampling of different items. **(B)** View along a training channel (width 7 cm, walls 7 cm high) from the nest entrance. Tape covers on the walls provide a slippery surface that dissuades most ants from escaping from the channel, thus increasing the number of animals that find the feeder. **(C)** Arrangement of training (bottom) and test (top) channels and their connection to the nest via a Y-junction with a switch door.

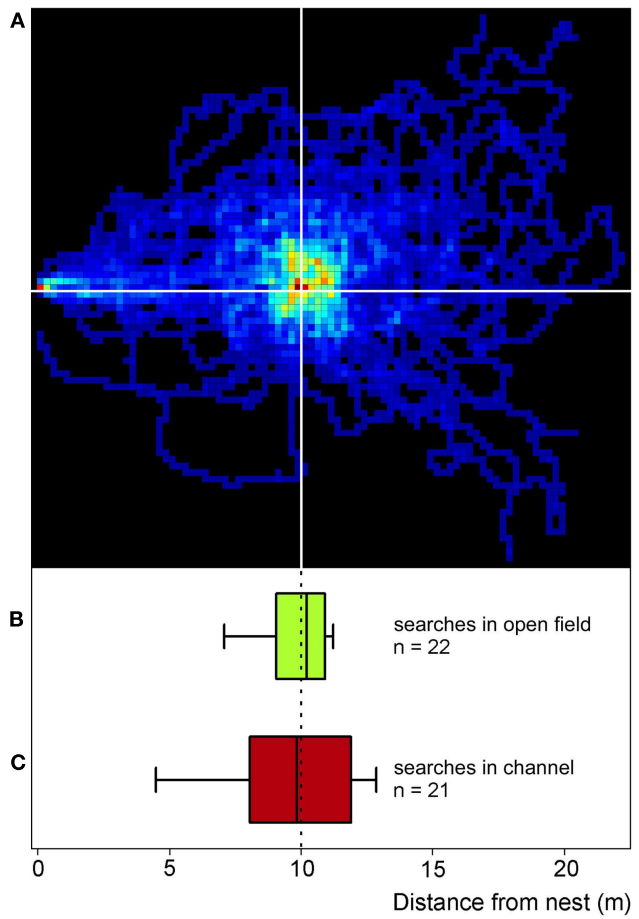

FIGURE 2 | Desert ants' search behavior in the open field (compare Figure 1A) and in channels (compare Figures 1B,C). For the two-dimensional search density plot in **(A)**, the number of ants' visits to each 25 cm × 25 cm pixel of the feeder surrounds was recorded, summed, and normalized to the maximum number of visits per pixel in the plot. The darkest red represents the highest density (100%), the darkest blue just a single visit, and black areas were not visited at all (0%). Recordings lasted for 2.5 min after an animal had left the nest (note red pixel on the left hand margin); nest-feeder distance was 10 m. The ants ($n = 31$) had visited the full feeder (>800 biscuit crumbs) once before the recordings were made. To construct the box plot in **(B)**, the data in **(A)** were projected onto the nest-feeder axis, i.e., any movements along the axis perpendicular to the nest-feeder direction were disregarded. Like in the channel experiments [see **(C)**], the initial six turning points on the nest – feeder axis were used to calculate medians and percentiles (below; $n = 22$, since not all 31 ants performed six turns in the projected path as required for the analysis). The box plot in **(C)** presents searches recorded in the test channel used in all the other experiments described in this report (**Figure 1C**). The ants had visited a full feeder once in the training channel (as in the open field) before the recordings were made. Note the similarity of the plots in **(B,C)**, attesting to comparable search behavior in the channel and in the open field. Box plots show medians, box margins (+75th, −25th percentiles) and whiskers (+90th, −10th percentiles) in this and all following figures.

many (>800) food morsels. Once an ant had visited the feeder and returned to the nest with a food morsel, the next foraging trip was recorded in the test channel (**Figures 3A,D**). This experimental series thus examined the effect of different food amounts in the feeder on search behavior. Alternatively, a minimum number of five visits were allowed before recording the search (**Figures 3B,E**). This experimental series examined the effect of the foragers' experience with the food site on search behavior. The recordings of all ants in a given experimental situation were used to calculate search medians (box-and-whisker plots, **Figures 2B,C** and **3**) and spreads (**Figures 3D–F**).

Experimental results

The experiments demonstrated that both parameters, the amount of food in the feeder, and experience regarding reliability of food encounter, influence the desert ants' search for the feeding site. Ants that had encountered the feeder just once (**Figures 3A,D**) exhibited rather different search patterns upon their next visit, depending on the amount of food presented in the feeder. Different from the initial chance encounter, this second visit appeared goal-oriented, as indicated by the more or less narrow search distributions in **Figure 3D**. Searches for a feeder with just a few food items or only a single food item had search centers noticeably beyond the original feeder position and larger spreads (**Figures 3A,D**, top three boxes). This is not at all surprising, since the respective ant had removed much or all food from the feeder on its previous visit and should not necessarily expect further morsels in that particular location, reflecting the typical situation for *C. fortis* foragers that usually scavenge on scattered insect carcasses (Wehner et al., 1983). These searches appear to reflect sector fidelity as reported previously (Wehner et al., 1983; Schmid-Hempel, 1984), with the search extending roughly into the previously successful direction but without a clear search for the previous food location. When the feeder was equipped with many (>800) standardized biscuit crumbs (ca. 1.5 by 1.5 mm in size; **Figures 3A,D**, bottom box), the searches were much more focused and the search center coincided almost exactly with the previous feeder location.

A larger number of successful visits had similar effects on search density as had food abundance, i.e., repeated successful visits overrode the effect of abundance just described. If the ants were allowed to visit the feeder 5 or more times before being tested, all searches were well-focused just beyond the previous feeder position (**Figures 3B,E**), even if the feeder had yielded just a single item on each previous visit (**Figures 3B,E**, top box; quantitative data on search densities in Bolek et al., 2012b).

In summary, the ants assess both food abundance and the reliability of food encounter. Increases in both parameters lead to more focused searches for the food source, with learning about reliability overriding food abundance after several visits. It is an important additional result that desert ants *C. fortis* exhibit a well-defined food vector (**Figure 2**; see also below, **Figure 4**), in addition to the sector fidelity reported previously (Wehner et al., 1983; Schmid-Hempel, 1984). Sector fidelity appears to be applied for single food items that are removed upon being met by the forager. If food is left back at the food site or food is encountered reliably over several visits, the ants show well-focused searches for a familiar food source, thus memorizing a food vector. The observation of such point fidelity in a quantitative manner is an important novel finding in *Cataglyphis*. Although point fidelity is routinely employed when training ants to visit a feeding site, this aspect had as yet received almost no attention,

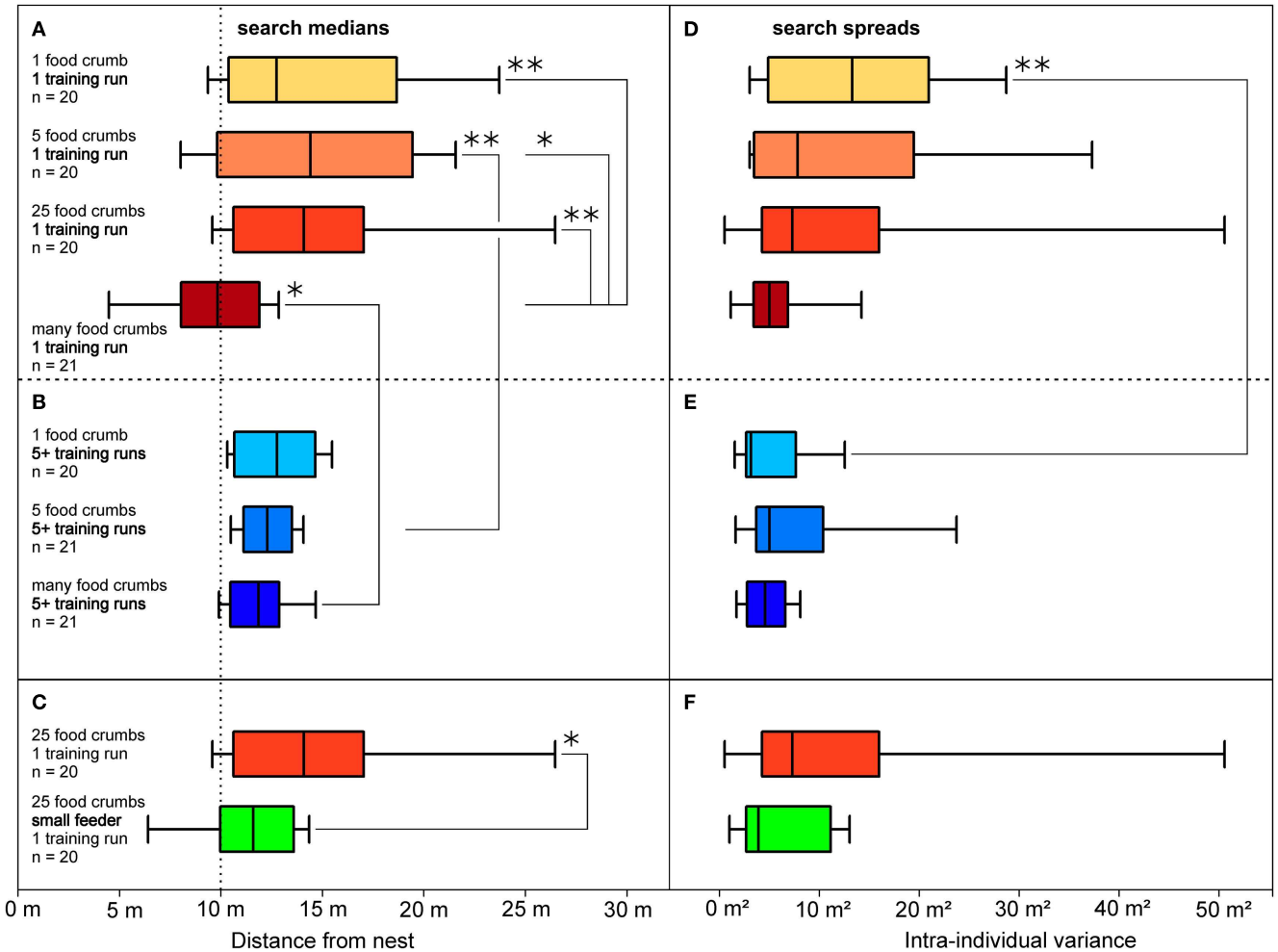

FIGURE 3 | Distributions of food searches (A,D), according to their dependency on the amount of food presented, and (B,E) according to rewarded experience with the feeding site. (A,D) Data from ants that had performed a single (training) visit to a feeder located in a channel, 10 m from the nest. The experimental groups differed in the amount of food available in the feeder, as noted on the left and indicated by the box color (darker colors represent more food items). Also noted are numbers of experimental animal. Boxes and whiskers as in **Figure 2**. Significant differences are indicated by brackets and asterisks; one asterisk, $p < 0.05$; two asterisks, $p < 0.01$; absence of significant difference is not indicated. **(B,E)** Data from ants that had performed five or more (training) visits; the experimental groups differed in the amount of food available in the feeder; other labels as in **(A)**. **(C,F)** Data from ants that had visited a feeder equipped with 25 food items once before being tested. The feeder was either of standard size (32 mm diameter; same situation as in **(A)**, bright red box) or small (8 mm, green box); food density was thus 16-fold higher in the small feeder. Other labels as in **(A)**. Search medians are plotted on the left **(AC)** , search spreads on the right **(DF)** as variances of the individuals' searches.

in contrast to the well-known sector fidelity (Wehner et al., 1983; Schmid-Hempel, 1984; see also Buchkremer and Reinhold, 2008). In summary, point fidelity appears to be used for feeders that are worthwhile re-visiting due to large food supply or high reliability, while sector fidelity would appear to represent the normal mode of foraging for isolated prey items such as scattered arthropod carcasses.

The emergence of a food vector after sufficient reinforcement further demonstrates that experience shapes the ants' food search behavior. This is interesting when one considers that the same navigational toolkit is employed as when determining the home vector, but the home vector does not improve or otherwise change with increasing experience (Merkle et al., 2006).

ASSESSMENT OF FOOD SOURCES, IN *CATAGLYPHIS* AND OTHER SPECIES

A preliminary experiment indicates that the ants may judge the food amount not by counting items – which would not be expected anyway (Franks et al., 2006) – but by assessing the density of food items at the location of food uptake (Bolek et al., 2012b). **Figures 3C,F** illustrate that *Cataglyphis*' food search becomes more focused if 25 food items are offered in a small feeder of 8 mm diameter, rather than in the standard feeder of 32 mm diameter that is used in all other experiments. This increases the density of food items 16-fold, which is the only change that should be noticeable to the ants under the experimental conditions (Bolek et al., 2012b).

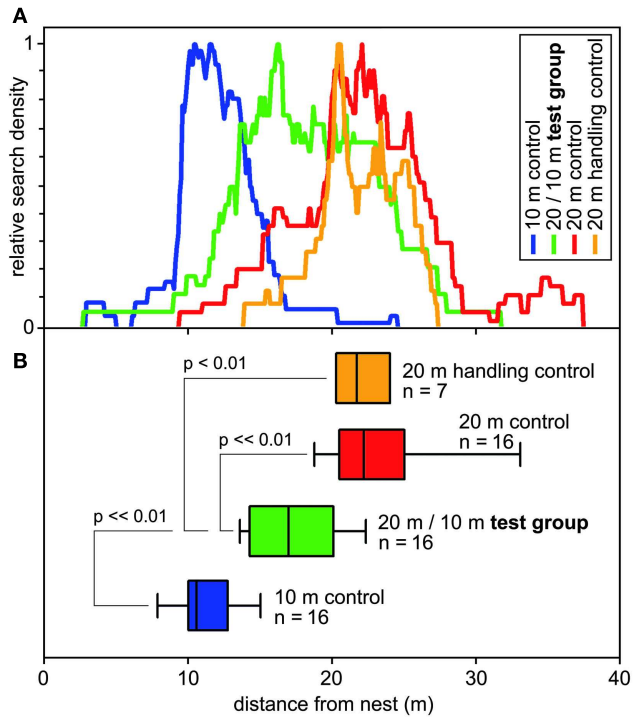

FIGURE 4 | earch behavior of desert ants on their outbound journey, from the nest to the site of a feeder (that was removed for testing). (A) Normalized search densities (no. of visits per 10 cm bin of test channel). Color code noted in top right inset. **(B)** Corresponding box-and-whisker plots (boxes and whiskers as in **Figure 2**); abscissa, distance from the nest. Color code corresponds to **(A)**; indicated are numbers of animals and significant differences including significance levels. Medians of the turning points of the food searches of the ant individuals were used to calculate ANOVAs, with pair-wise comparisons according to Holm–Sidak *post hoc* test.

It has yet to be established how the animals assess density. Mechanosensory input from legs and mouthparts is an obvious possibility, as is food odor, because a higher concentration of odorants would be expected to emanate from a higher density of food items, even at some distance. It is further conceivable that the visit to a plentiful feeder initiates associative learning in desert ants, similar to the situation in honeybees (Pelz et al., 1997). This is a viable option since associative learning in response to odor stimuli has recently been reported in *Camponotus* ants (Guerrieri and d'Ettorre, 2010) and appears also to be present in *Cataglyphis* according to preliminary experiments (Klein, 2011; Wohlfarth, 2011). Pelz et al. (1997) have shown that odorant concentration has an influence on associative learning in honeybees. When extrapolating the findings in honeybees to *Cataglyphis*, food odorants represent a conditioning stimulus that is associated with the food reward. The conditioned response may be the food vector that takes the ant back to the previously visited food source in this case. And differences in odor concentration may make a plentiful food source a more intense and more salient olfactory stimulus than a poor food source. By the same line of argument, repeated successful visits to the food source may represent repeated conditioning trials in an associative learning process, sharpening the conditioned response, i.e., focusing the food search. Such an interpretation sees decisions in the light of reward-dependent learning, a topic considered in detail in other contributions to this issue.

The Australian desert ant, *Melophorus bagoti*, occupies an ecological niche very similar to that of the Saharan *Cataglyphis*. Comparison of two species that have evolved their desert life independently is thus tempting, although there are few studies as yet on food site vectors in either species, *Melophorus* or *Cataglyphis*. A recent study by Schultheiss and Cheng (in review) demonstrated that *Melophorus* adjust their search behavior differently for protein and carbohydrate foods, with carbohydrate food eliciting more concentrated searches (compare data on *Formica schaufussi* (Traniello et al., 1992; Fourcassié and Traniello, 1993, below). This corresponds to the natural distribution of these food supplies, with carbohydrates offered mainly in plentiful patches by fruiting plants and proteins occurring primarily as scattered arthropod carcasses. Apart from this adaptive search layout, *Melophorus*' food search patterns are centered on the familiar food site, resembling the well-known searches for the nest.

Assessment of feeding sites in *Cataglyphis* appears comparable to the food assessment strategies in other ants. A major difference concerns the fact that most ant species use their social organization to exploit food sources through recruitment of nest mates. Such group foraging allows adjustment of deployed forager forces to feeding site yield, for instance (e.g., in *Monomorium pharaonis*; Sumpter and Beekman, 2003). Recruitment is absent in the desert ant, *C. fortis*, which only forages individually.

The amount of food available is judged in ants by parameters such as satiation (e.g., in *Lasius niger*; Mailleux et al., 2000) or portability of larger items (e.g., in *Pheidole pallidula*; Detrain and Deneubourg, 1997). If a large amount of food is encountered, nest mates are usually recruited by laying pheromone trails on the return journey to the nest. Other recruiting mechanisms are also observed, however, including the leading of novice foragers to promising feeding sites in tandem runs (e.g., in *Temnothorax albipennis*; Franks and Richardson, 2006). Indeed, recruitment is often used to measure the assessment of feeding sites by ant species in experimental paradigms. And while pheromone trails eliminate the need for establishing food vectors, the tandem-running *Temnothorax*, and scouts of other species will need to memorize the location of a food supply for future return visits. As is the case with *Cataglyphis*, this aspect has, as yet, received little attention.

Other species, such as *F. schaufussi*, do not exhibit noticeable assessment of food amount (Robson and Traniello, 1998) but appear to focus primarily on food quality (Traniello et al., 1992; Fourcassié and Traniello, 1993), for example, regarding protein, carbohydrate, and fat contents (see also Schultheiss and Cheng, in review for *Melophorus*, above). Learning of the reliability of a food supply or assessment of food amount was not observed in this species, and the evaluation of food quality also requires further scrutiny. What the ants consider high-quality food may vary, depending on the time of the year, the food naturally available at a given time, requirements of the brood, and other factors, even though no such variations were observed in *Formica schafussi* (Traniello et al., 1992). These complications may as yet have prevented a detailed study of this aspect.

IS IT THE PREVIOUS OUTBOUND OR THE LAST INBOUND JOURNEY THAT ESTABLISHES THE MEMORY OF THE FEEDING SITE LOCATION?

Cataglyphis desert ants primarily use path integration to navigate in their desert habitat while foraging (Wehner and Srinivasan, 2003), although landmarks (e.g., Wehner et al., 1996), ground structures (Seidl and Wehner, 2006), and odor marks (Steck et al., 2009, 2010) are also used if available. The ants use a skylight compass (Wehner, 1997; Wehner and Müller, 2006) and a stride integrator (Wittlinger et al., 2006, 2007) to monitor their meandering search paths and constantly update distance and direction back to the nest. The ants also use their path integrator to return to plentiful or reliable feeding sites (Wolf and Wehner, 2000). It has remained unclear, however, what is used to memorize the feeder position: is it the straight homebound path from the feeder or the state of the path integrator when finding the food, i.e., on the outbound journey, or are these two measures combined, and if so, in what manner?

These questions were addressed (Bolek et al., 2012a) by connecting an ants' nest to a U-shaped metal channel, as described above (**Figures 1B,C**). A plentiful feeder was placed in this training channel at 20 m (or 10 m, below) distance from the nest. A much longer test channel was arranged just next to and in parallel to the training channel. This setup allowed selective assessment of the distance component, or odometer, of the ants' path integrator. Once an ant had visited the feeder and returned to the nest with a food morsel, its next foraging trip was monitored by leading it into the (empty) test channel *via* a switch door. These (control) ants searched for food quite reliably close to the previous nest-feeder distance of 20 m (**Figure 4**). This was to be expected, not least on the basis of the preceding experiments on feeder assessment (**Figure 3A**, bottom box; see also **Figure 2**). In the following set of experiments, the distance of the experimental animals' homebound journey was altered by gently catching them at the feeder once they had taken up a food item, and releasing the ants closer to the nest, at half the outbound distance, i.e., 10 m. One would expect these animals to concentrate their searches for food at around 10 m if they took their homebound journey for memorizing the feeder position, or at around 20 m if they took their outbound journey.

The data in **Figure 4** demonstrate that the ants follow neither of these expectations but rather average the out- and inbound path lengths. Furthermore, they consider the linear average, instead of the harmonic average or other averaging options, at least in the present experimental situation. It will be interesting to see if, with more typical outbound search trajectories, i.e., that are meandering and much longer than the straight inbound path, the weighting of the two legs of the foraging trip changes.

The present result corresponds well to the few previous reports on food site vectors, particularly Cheng and Wehner (2002). Similar observations were made in honeybees (Otto, 1959), although more recent contradictory results also exist for bees (Srinivasan et al., 1997). In the latter report, estimation of the distance to a feeder was examined by a forager honeybee's return journey to that feeder under controlled artificial laboratory conditions (rather than observing the bee's dance back in the hive). Experimentally interfering with the bees' optic flow odometer demonstrated that outbound travel distance apparently determines the bee's memory of nest-feeder distance. Similar to the situation in desert ants, however, more natural foraging situations may possibly reveal additional mechanisms. This may be particularly true when considering that odometer information appears to be determined in different ways for a forager bee's own return travel to a feeder and for its dance communication to fellow foragers back in the hive (Dacke and Srinivasan, 2008).

IS THE ACCURACY OF THE PATH INTEGRATOR SUFFICIENT TO FIND THE FOOD SOURCE AGAIN?

Any navigation system has a limited accuracy, although present technical systems may be very precise. In the case of desert ant navigation, the accuracy is surprisingly good, considering the meandering foraging paths, large foraging distances, and inherent errors in the path integrator as a dead reckoning system (Müller and Wehner, 1988). This accuracy is not sufficient, however, to steer precisely toward a goal smaller than a few degrees in azimuth without landmarks or other structures supporting orientation (Wolf and Wehner, 2000, 2005; Wolf, 2008). This is acceptable for *Cataglyphis* ants for two reasons. Firstly, the immediate nest surroundings will be familiar to a forager both after its initial few trips and also from refuse deposition and short exploratory outings by the ant before assuming its foraging task (Wehner et al., 2004). This ensures finding of the nest on return journeys even in the nest location is not met spot-on. Secondly, the ants possess a number of backup strategies, including olfactory orientation (Wolf and Wehner, 2000) and an efficient search strategy (Wehner and Srinivasan, 1981; Merkle and Wehner, 2010). Nonetheless, navigation inaccuracy may present a problem for returning to a plentiful feeding site, at least initially.

DOWNWIND APPROACH STRATEGY

Cataglyphis ants minimize these problems by using other cues to localize a food source, if such cues happen to be available. Landmarks are an obvious possibility (e.g., Wehner et al., 1996), and odors are another important cue (Steck et al., 2009, 2010). Desert ant food – typically arthropod carcasses, or occasionally biscuit crumbs from experimenting biologists – will normally exude some odor. This odor not only alerts the ants to novel food items over a distance (Wehner and Duelli, 1971), it also allows guided approaches to a familiar feeding site. The ants usually steer downwind of a known food source to encounter the odor plume emanating from the food (similar to the situation depicted in **Figure 5A**). Once the ants have encountered the plume, it will safely guide them to the food source. Such a strategy affords a detour and thus a longer path than a direct approach. In return, it avoids missing the goal or small food locations in particular. With a direct approach, the ants would inadvertently walk into an area upwind of the food in about 50% of cases due to navigation inaccuracies. The resultant searches are usually much longer than the detour required by the downwind strategy (**Figure 5B**; Wolf, 2008). In the sample traces shown in **Figure 5B**, the average detour required by the downwind approach extends the walking path by about 28% of the nest-feeder distance, i.e., from 5.7 m nest-feeder distance to 7.3 m walking trajectory. The search trajectory initiated after passing the food on the upwind side is an

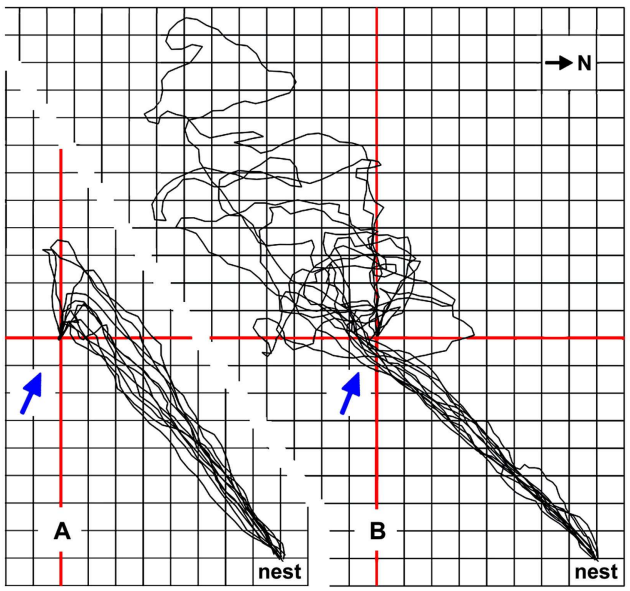

FIGURE 5 | he downwind approach strategy exhibited by *Cataglyphis* ants reduces the average foraging path length. Typical downwind approaches of four foragers are shown in **(A)**, the final upwind segments guided by the odor traces emanating from the small and inconspicuous feeder (see **Figure 1A**). Feeder position is marked by red cross lines; grid line distance is 0.5 m. Ambient wind direction is indicated by blue arrows. The downwind approach afforded an additional 1.6 m walking distance compared to a beeline approach, on average. **(B)** The same ants occasionally choose a slightly different path, probably due to lack of experience and slight shifts in wind direction (Wolf, 2008). Approaches of the same four animals, performed just before or after the sample runs shown in **(A)** and leading into an area upwind of the food source, are superimposed in **(B)**. They demonstrate that the ants missed the feeder by passing upwind of the odor traces and illustrate the ensuing searches of an average 9.7 m walking distance, in addition to the direct approach distance of about 5.7 m. Compass north is indicated in the top right corner; nest-feeder distance is 5.7 m.

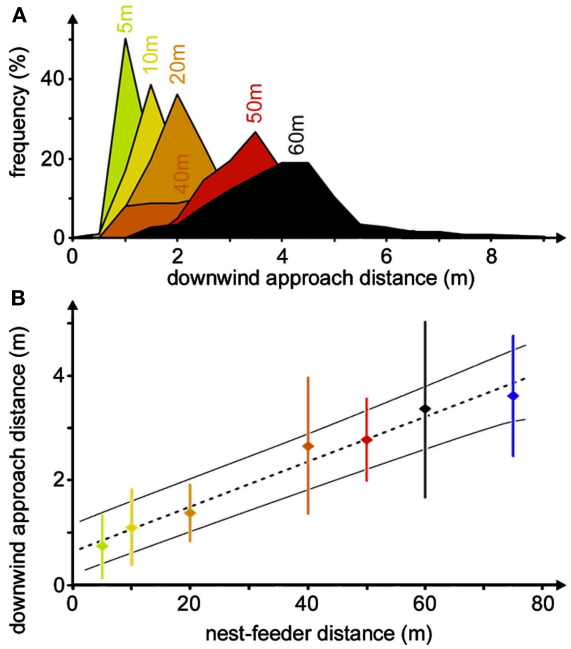

FIGURE 6 | Compensation of navigation inaccuracy by downwind approach. (A) Distributions of downwind approach distances for different nest-feeder distances. Histograms were recorded at the nest-feeder distances noted above the peak bins (bin widths 0.5 m). Different histograms are distinguished by different colors. For the different histograms, the numbers of ant individuals were between 8 and 29, yielding between 42 and 747 recordings, except for 75 m nest-feeder distance with only three ants and six recordings (see Wolf and Wehner, 2005). **(B)** The same data set is shown as a plot of downwind approach distance against nest-feeder distance. Dotted line indicates the best-fit regression, thin lines mark 95% confidence intervals. Measurements for each individual were pooled before calculating means, SD, and regression line. Color code as in **(A)**.

average of twice (198%) the nest-feeder distance, extending the approach from 5.7 m nest-feeder distance to an average 17.0 m walking trajectory. Despite some effort, the downwind approach strategy thus appears clearly advantageous.

ERROR COMPENSATION

The downwind approach strategy is used by desert ants quite reliably, although adjustment of the downwind distance occurs during the initial four to six visits to a food source (Wolf, 2008). It represents a so-called error compensation strategy (Biegler, 2000), i.e., inevitable navigation errors are compensated for, or rather accounted for, by tailoring the approach strategy to minimize search effort. In the case of the desert ants' downwind approach, this means that the animals consider their angular steering error by keeping downwind of the assumed goal direction by their expected maximum error angle, which will safely lead them into the downwind area and allow encountering of the food odor. It also means that the distance steered downwind of a food source should increase linearly with nest-feeder distance. And this is indeed borne out when establishing feeding stations at different distances from an ants' nest, ranging from 5 to 60 m (**Figure 6A**), or even 75 m (**Figure 6B**), although ants are difficult to train to such distant feeding sites. The angle steered downwind of the food site is in the range of 4°–8°, which should correspond to the maximum navigation error according to the error compensation strategy (Wolf and Wehner, 2005).

In other words, *Cataglyphis* desert ants are able to judge their own navigation accuracy. Although this knowledge appears to be inexact initially and is adjusted during the first three to five visits to a familiar site (Wolf, 2008), this result is remarkable for an insect navigator. It is particularly unexpected in view of the fact that the typical prey items of *Cataglyphis* are scattered arthropod carcasses that do not warrant a return to the previously visited site.

CONCLUSION AND OUTLOOK

Food searches in desert ants, *C. fortis*, provide an unexpected wealth of features that may advance our understanding of search, navigation, and learning and decision strategies. More detailed studies would appear promising, particularly for the following aspects: (i) The assessment of food site quality, beyond food abundance and reliability of food encounter; this concerns the

chemical quality, the density, or the size of food items and possible learning mechanisms; (ii) the mode of memorizing food site vectors in more typical food searches, with meandering outbound search paths and straight homebound paths; (iii) the downwind approach strategy of desert ants with regard to the adjustment of the downwind distance under different circumstances, such as wind conditions, desert floor structure, presence of landmarks.

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Visually guided decision making in foraging honeybees

***Shaowu Zhang*[1]*, *Aung Si*[1,2] *and Mario Pahl*[3]**

[1] *Centre of Excellence in Vision Science, Research School of Biology, The Australian National University, Canberra, ACT, Australia*
[2] *Department of Linguistics, School of Culture, History and Language, College of Asia and the Pacific, Australian National University, Canberra, ACT, Australia*
[3] *BEEgroup, Biocentre, Würzburg University, Würzburg, Germany*

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Benjamin Hayden, University of Rochester, USA
Christoph Grüter, University of Sussex, UK

***Correspondence:**
Shaowu Zhang, Centre of Excellence in Vision Science, Research School of Biology, The Australian National University, Biology Place, Canberra, ACT 0200, Australia.
e-mail: shaowu.zhang@anu.edu.au

Honeybees can easily be trained to perform different types of discrimination tasks under controlled laboratory conditions. This review describes a range of experiments carried out with free-flying forager honeybees under such conditions. The research done over the past 30 or so years suggests that cognitive abilities (learning and perception) in insects are more intricate and flexible than was originally imagined. It has become apparent that honeybees are capable of a variety of visually guided tasks, involving decision making under challenging situations: this includes simultaneously making use of different sensory modalities, such as vision and olfaction, and learning to use abstract concepts such as "sameness" and "difference." Many studies have shown that decision making in foraging honeybees is highly flexible. The trained animals learn how to solve a task, and do so with a high accuracy, but when they are presented with a new variation of the task, they apply the learnt rules from the earlier setup to the new situation, and solve the new task as well. Honeybees therefore not only feature a rich behavioral repertoire to choose from, but also make decisions most apt to the current situation. The experiments in this review give an insight into the environmental cues and cognitive resources that are probably highly significant for a forager bee that must continually make decisions regarding patches of resources to be exploited.

Keywords: honeybee, top down, pattern vision, ma e learning, learning concept, delayed matching to samples, working memory, long term memory

INTRODUCTION

Honeybees are social insects with a rich and easily observable behavioral repertoire, and an excellent capability for learning and memory. For an adult worker bee, successful foraging is the primary task necessary for the survival and maintenance of the whole colony. In order for foraging strategies (i.e., strategies that take into account the time, frequency, and geographic location of foraging, as well as the flowers to be targeted) to be successful, honeybees need to have evolved the sensory and cognitive mechanisms necessary to implement those strategies. Indeed, an individual foraging bee is able to ascertain whether or not it is on the correct path either to a food source or back to the hive, and make any necessary corrections by comparing the currently viewed scene with the appropriate stored image (Collett and Kelber, 1988; Wehner et al., 1990, 1996; Collett et al., 1993; Collett, 1996; Judd and Collett, 1998; Zhang et al., 1999; Pahl et al., 2011). The foragers that find a rewarding food source return to the hive, and dance to inform recruits about the location of the food source. Individual bees following the dance then have to decide whether or not to forage at the food source being advertised (von Frisch, 1967; Esch et al., 2001; Dyer, 2002; Grüter et al., 2008; Menzel et al., 2011). Even while foraging at the advertised location, they have to decide which patches of flowers to visit; such decisions are presumably made after taking into consideration a range of factors, such as shape, color, and time of day, all of which may be influenced by the memories and experiences of past foraging trips. Finally, foraging bees might have to find their way back to the hive from previously unexplored locations. Decision making is undoubtedly required in determining which path to take, and much research has been carried out on the topic of search strategies (Wolf and Hainsworth, 1990; Greggers and Menzel, 1993; Riley et al., 2005). Thus, in every moment of its foraging life, a bee has to continually make numerous decisions that not only ensure that the tasks vital to the colony's well-being are completed, but also that the bee is able to safely return home thereafter.

Honeybees provide a classic example of a symbolic communication system among non-human animals (von Frisch, 1967, 1971). They are able to communicate information by performing dances about potential nesting sites and food sources after scout or forager bees find such locations. In the context of swarming behavior, Seeley and his colleagues have examined the group decision-making process in detail, and shown that in the early stages of swarming, the scout bees locate potential nest sites in all direction and at distances of up to several kilometers. They communicate to each other through dancing, allowing the comparison of different potential sites. Finally, there is a crescendo of dancing just before liftoff. They proposed that a swarm's overall strategy of decision making was a "weighted additive strategy" (Seeley et al., 1991; Seeley and Buhrman, 1999). The evolution and precise workings of such phenomena – which have been observed in a number of invertebrate taxa – are discussed in further detail in the contributions by Jeanson et al. (2012) and Stroeymeyt (2012).

In the present article, we review research, from the last two decades, that has explored the cognitive processes involved in

decision making in honeybees. This review focuses on individual, free-flying honeybees trained to perform complex, artificial tasks in a laboratory setting. These experiments therefore attempt to explain the factors that govern the behavior of foraging honeybees, as they navigate to a precise location (which may be known from a previous trip, or unknown), and make decisions regarding which patches of flowers (or even which individual flowers) should be preferentially targeted for nectar or pollen. Visually based tasks dominate our experimental protocols, although some olfactory cues are infrequently used to test for the transfer of learnt rules across sensory modalities (The contribution by Ritzmann et al. (2012) provides an account of interactions between tactile and visual sensory input in cockroach decision making). The experiments described in this review illustrate how honeybees use not only bottom-up sensory information (i.e., information from their immediate physical environment), but also memorized top-down information (i.e., stored conceptual information) in decision making (Zhang and Srinivasan, 1994). They are able to use abstract visual features of objects to make a decision in discrimination tasks, make a series of decisions while negotiating a complex maze, and learn abstract concepts or rules that guide them toward making correct decisions. In Delayed-Matching-to-Sample (DMTS) tasks or Symbolic Delayed-Matching-to Sample (SDMTS) tasks, they have to use a combination of working memory and long-term-memory to make a correct decision.

MEMORIZED INFORMATION IS ACTIVELY INVOLVED IN DECISION MAKING

Like big animals, bees can learn to distinguish camouflaged patterns if they are first trained on a related, but simpler task. This demonstrates that bees apply acquired prior "knowledge" in decision making, and use it to choose the correct camouflaged pattern (Zhang and Srinivasan, 1994).

It is well-known that prior knowledge or experience aids us tremendously in uncovering objects that are poorly visible, partially hidden, or camouflaged. Many of us who view the scene in **Figure 1** for the first time would not see a familiar object, especially if we are unaware of the picture's content. Once the camouflaged Dalmatian has been discovered, however, it is detected and recognized instantly every time the picture is re-encountered. Evidently, prior experience or knowledge aids the visual system significantly in the task of uncovering objects (Lindsay and Norman, 1977; Goldstein, 1989; Cavanagh, 1991).

"Top-down" processing of this kind can speed up the analysis of the retinal image when a familiar scene or object is encountered, and help fill-in, or complete, details that are missing in the optic array (Cavanagh, 1991). Is the ability to enhance processing in this way restricted to highly developed visual systems, such as those of humans and higher mammals? Or does it extend to relatively simple visual systems, such as those of invertebrates?

Zhang and Srinivasan (1994) approached this question by investigating whether bees are able to use prior experience to facilitate the detection of objects and discrimination of their shapes. They first attempted to train bees to distinguish between two shapes – a ring and a disk – when each shape was presented in a camouflaged fashion as a textured figure, positioned 6 cm in front of a similarly textured background in a Y-maze (**Figures 2A,B**). It turned out that bees were unable to learn to make this discrimination, despite lengthy training incorporating over 100 rewards per bee. Next, Zhang and Srinivasan examined whether bees could learn to distinguish the camouflaged patterns if they were first trained on a related, but simpler task: that of distinguishing between a black ring and a black disk, each presented 6 cm in front of a white background. The ring and the disk were of the same size and shape as their textured counterparts, and their spatial configuration in relation to the background was identical to that in the previous experiment. The bees were able to learn this new task (**Figure 2C**). When these pre-trained bees were tested on the task of **Figure 2B**, they could distinguish between the patterns almost immediately (**Figure 2D**). Although the figures in **Figure 2D** are camouflaged, they can be detected by virtue of the relative motion between the images of the figure and the more distant background, as the bee approaches the figure. Evidently, the bees were able to learn to use this motion parallax as a cue to break the camouflage – but only after they had been pre-trained on uncamouflaged versions of the same shapes.

FIGURE 1 | A familiar, but camouflaged object (readers experiencing difficulty in recogni ing the Dalmatian dog may wish to view the picture upside down). Photo courtesy R. C. James. Reprinted from Lindsay and Norman (1977), with permission of authors and publishers.

BEES ARE ABLE TO USE ABSTRACT, GENERAL PROPERTIES OF VISUAL PATTERNS IN DISCRIMINATION TASKS CHOOSING THE CORRECT PATTERN

What kind information can be stored in a honeybee's memory? Honeybees are able to use concrete features of objects, such as color, shape, scent, and so on (Menzel and Bitterman, 1983; Gould and Gould, 1988; Menzel, 1990; Chittka et al., 1993; Lehrer et al., 1995). Important insights into visual perception can be gleaned by examining whether honeybees are capable of perceiving and abstracting the general properties of objects. There can be little doubt that bees use some kind of neural "snapshot" to remember and recognize patterns and landmarks (Collett and Cartwright, 1983; Judd and Collett, 1998). However, it is hard to imagine that this is all there is to pattern recognition. In their daily lives, bees are required to remember a number of different patterns and

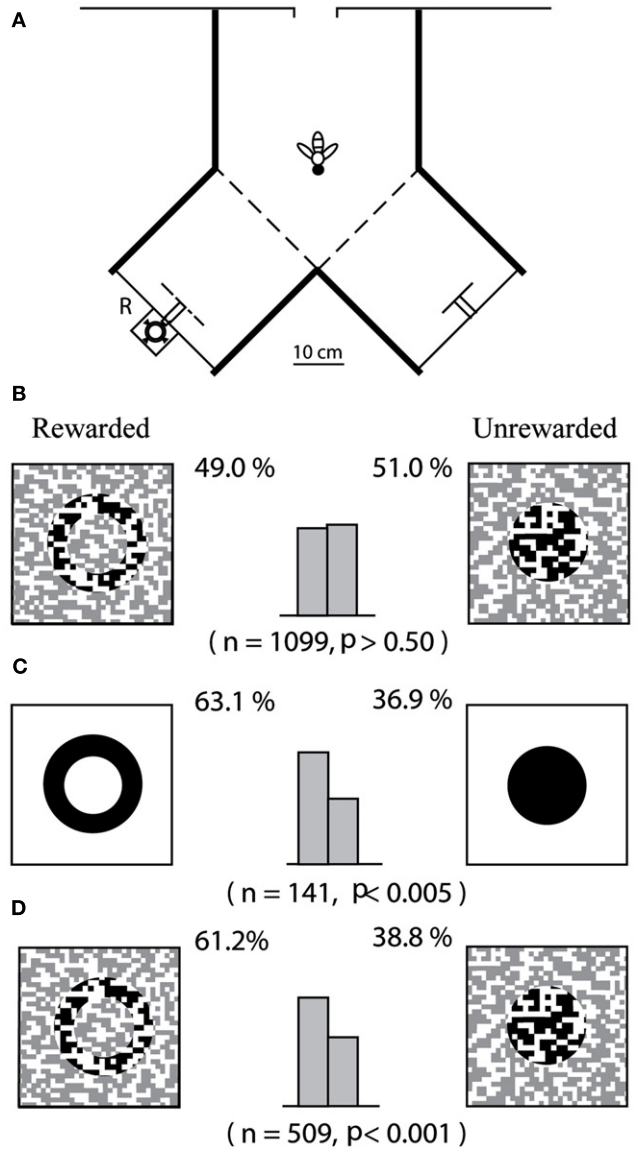

FIGURE 2 | Investigation of top down processing in honeybees. Adapted from hang and rinivasan (1994). (A) Experimental setup; **(B)** bees are trained and tested directly on camouflaged patterns; **(C)** bees are first trained on simple patterns; and **(D)** tested on camouflaged patterns.

their properties. Some examples would be the shape of the nest or hive, shapes representing nectar-bearing flowers, and shapes of important landmarks on the way to the food source and back. If snapshots were the only mechanisms for remembering shapes, bees would require a large memory to store all of these images (Horridge et al., 1992). Given that the brains of bees contain far fewer neurons than do ours, it seems very unlikely that they can afford the luxury of a large memory. One would imagine, therefore, that bees also possess other, more economical means of representing patterns. Can bees extract general properties of form? We tackled this question in the early 1990s with a series of Y-maze experiments.

Learning to abstract pattern orientation

It is of interest to ask whether honeybees can learn to abstract a particular attribute of a pattern, such as its orientation, without having to memorize the pattern precisely. An early paper by Wehner (1971) hinted that bees could indeed abstract pattern orientation in this way. This question was pursued further by Van Hateren et al. (1990), who used a Y-maze apparatus and stimuli consisting of black-and-white gratings of random amplitude.

Van Hateren et al. (1990) found that bees could be trained to distinguish between the vertical and horizontal orientations, as well as between two oblique directions. Furthermore, bees trained to distinguish between two mutually perpendicular orientations were able to discriminate the overall orientations of other patterns which they had never encountered previously. Thus, bees are able to extract orientation information from patterns on which they are trained, and to use this information to evaluate novel patterns in decision making.

Similarly, honeybees are capable of discriminating patterns with radial symmetry from circular symmetry (Horridge and Zhang, 1995), as well as with vertical symmetry from horizontal symmetry (Horridge, 1996). Giurfa et al. (1996) showed that bees can learn to discriminate bilaterally symmetrical patterns from non-symmetrical ones; bees can also learn other abstract properties of objects, such as their color and size, without having to memorize the objects' images exactly (Horridge et al., 1992; Ronacher, 1992).

It is important to emphasize that, in all of the above experiments the ability to "generalize" has been demonstrated by training bees to not one, but a number of stimuli that differ individually in detail but share the property that is to be generalized. For example, the rewarded patterns could all possess the same orientation or the same kind of symmetry (say, left-right symmetry). These stimuli are shuffled randomly during the training. Such a training procedure ensures that the bees learn the critical cue that is associated with the reward (Horridge, 1999).

HONEYBEES ARE ABLE TO MAKE A SERIES OF DECISIONS IN NEGOTIATING COMPLEX MAZES

The discovery of "top-down processing" by bees inspired us to pursue further investigation of their learning and memory. We subsequently initiated a series of experiments, using mazes, to examine whether honeybees can learn "rules" in making a series of decisions to deal with complex tasks and then to apply them to novel situations.

The ability to learn mazes has been investigated extensively in a number of higher vertebrates, notably rats, mice, and pigeons (Pick and Yanai, 1983; Dale, 1988). Relatively few studies, however, have explored the capacity of invertebrates to learn mazes. Can bees learn complex labyrinths, requiring several correct decisions to be made to reach the goal? Zhang et al. (1996) explored this question by attempting to train bees to fly through a variety of complex mazes to find a reward of sugar solution, in the presence, or absence of specific visual cues. Each maze consisted of a 4×5 matrix of identical cubic boxes. Each wall of a box carried a hole in its center. The path through the maze was created by leaving open some of the holes between boxes, and blocking others. Bees had to fly through a sequence of boxes to reach the goal, which

was a feeder containing sugar solution. The experimental maze was placed on a movable table and its position and orientation were varied frequently to prevent the bees from using landmarks external to the maze as navigational cues.

Honeybees are able to negotiate a maze by following a mark

One series of experiments investigated the ability of bees to find their way through the maze by *learning to following a color mark* that signaled the correct exit in each box. The mark was a 4 cm × 4 cm green square affixed immediately below the appropriate hole in each box to indicate the correct path (**Figure 3A**). Bees were trained to enter the maze and take the correct path through it. This was accomplished by moving a feeder step-by-step along the correct path, until it reached the third box in the path. During this period, the bees had the opportunity to learn that the mark in each box signaled the correct exit. After the bees had reached this stage, the feeder was moved directly to the final box on the path, left there briefly, and then moved to its final destination, namely, the feeder compartment behind the final box.

The bees' performance was tested immediately thereafter. During the test, only one bee at a time was allowed into the maze.

The results show clearly that bees, trained initially to follow color marks through only a small, initial part of the maze, are immediately able to "blaze a trail" by using the same cue to find their way through the rest of the maze (Test 1 in **Figure 3B**). Performance continues to be good when the bees are tested on a new path, created by rearranging the boxes and marks (Test 2 in **Figure 3B**). Evidently, the trained bees had learnt to follow the

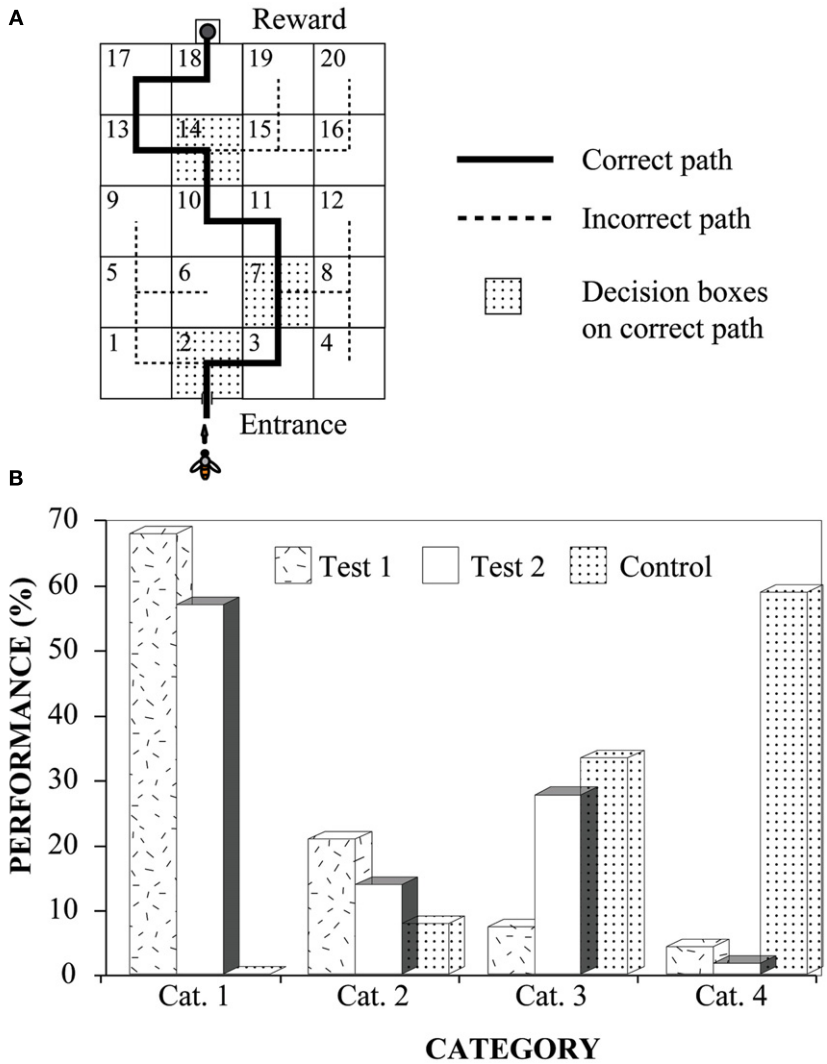

FIGURE 3 | Learning to negotiate ma es by following marks. Modified from hang et al. (1996). (A) The experimental setup, indicating the correct path through the maze; **(B)** Experimental results. Performance was scored by assigning each flight into one of four categories. Cat. 1: a bee flew through the entire path and arrived at the goal without making any mistakes; Cat. 2: flights in which the bee turned back and retraced her path (once or many times) but remained on the correct path; Cat. 3: flights in which a bee made one or more wrong turns at the decision boxes, but still arrived at the goal within 5 min; Cat. 4: unsuccessful searches, defined as flights in which the bee did not reach the goal within 5 min of entering the maze (regardless of whether she was on the correct path or not).

marks to the goal and were immediately able to use this rule to trace a novel path through the maze.

Honeybees are able to negotiate a maze by using a symbolic cue

Another series of experiments examined whether bees could *learn to negotiate mazes by using a symbolic cue* (Zhang et al., 1996). Left and right turns were signaled according to a color placed on the back wall of each box where a turn had to be made (**Figure 4A**, left panel). Bees were trained and tested for learning performance in a specific path (**Figure 4A**, left-hand panel, Path 3). The training and testing procedures were similar to those described above. The results showed that bees learned this task very well too (Test 3, **Figure 4B**). In fact, their performance in this maze was just as impressive as in the mark-following maze. Here again, bees trained to use the symbolic cue on a particular route were immediately able to use the cue to trace novel paths (**Figure 4A**, middle and right-hand panels) though the maze (Test 4 and 5, **Figure 4B**). The performance in all tests (Test 3, Test 4, and Test 5) was significantly better than in the control (**Figure 4B**).

Honeybees negotiate unmarked mazes

Zhang et al. (1996) have also explored the ability of bees *to learn to negotiate unmarked mazes*. Here bees were trained step-by-step through the entire path, from the entrance to the reward box. After training for 5 days, tests carried out on the same path revealed that the bees had indeed learnt to find their way through the maze, although performance was significantly poorer than when they followed a color mark. Nevertheless, performance was significantly better than the control. Presumably, this is accomplished by memorizing the sequence of turns that have to be made at specific distances (or box counts) along the route. There is evidence that bees use visual odometry to estimate distance flown (Srinivasan

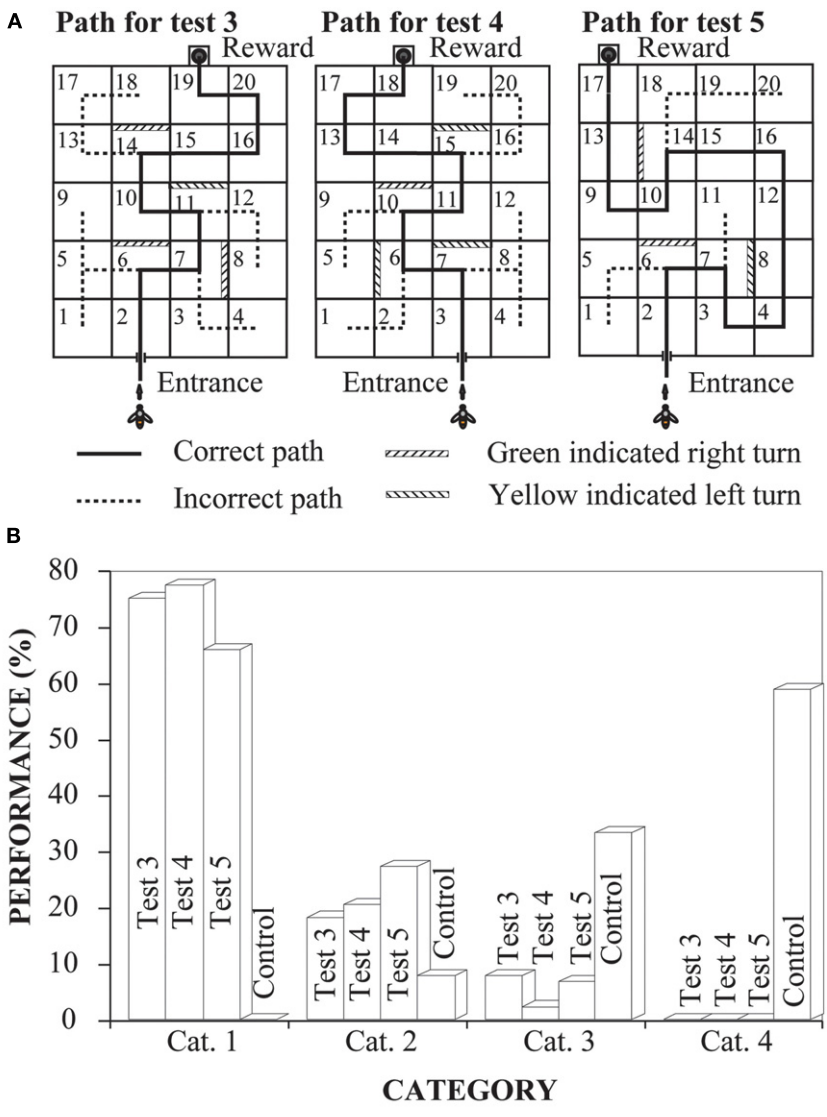

FIGURE 4 | Learning to negotiate ma es by following marks. (A) The experimental setups, indicating the correct path through the maze. **(B)** Experimental results. Performance was scored by assigning each flight into one of four categories as in **Figure 3**. Modified from Zhang et al. (1996). Details in text.

et al., 1997, 2000) and that they are even able to "count" landmarks en route to a goal (Chittka and Geiger, 1995).

Interestingly, when bees that have learned to negotiate a maze with the aid of marks or symbolic cues (as in the experiments of **Figures 3** and **4**) are tested on the same routes with the marks or cues removed, their performance is significantly poorer than when bees are trained on unmarked routes in the first place (Zhang et al., 1996). Evidently when bees are given marks or symbolic cues, they rely almost exclusively on these signals for navigation: they hardly pay any "attention" to the route that they take through the maze, unlike the bees that are forced to learn an unmarked route.

Honeybees negotiating mazes by using path regularity

We have seen above that the bees' performance in the unmarked maze was not as good as that in the mazes with color marks, where there was information on the appropriate turn to be made at each stage in the maze. This is because the only way that a bee can navigate an unmarked maze, in general, is to memorize the path through it – that is, memorize the entire sequence of turns that are necessary to go through the maze successfully. It is conceivable, however, that some unmarked mazes are easier to learn than others. For example, mazes that require a regular pattern of turning might be learned more readily than those that do not, if bees possess the ability to recognize such patterns.

Zhang et al. (2000) explored this question by investigating the ability of bees to learn unmarked mazes of various configurations, some of them with path regularity and some of them without it. Four different configurations were used, each in a different experimental series: (a) constant-turn mazes, in which the appropriate turn is always in the same direction in each decision chamber; (b) zig-zag mazes, in which the appropriate turn is alternately left and right in successive decision chambers; (c) irregular mazes, in which there is no readily apparent pattern to the turns; and (d) variable irregular mazes, in which the bees were trained to learn four irregular mazes simultaneously (**Figure 5**).

A bee flying a correct path through the maze entered a cylinder through one hole and could leave through one of two exit holes, positioned 45° to the left and right of the "straight ahead" direction. One of these holes represented the correct path continuing through the maze, while the other one led to a cylinder representing a "dead-end." The final cylinder on the correct path contained a feeder that provided a solution of sugar water, which the bees could drink *ad libitum*. After they had fed, bees were released from this cylinder by raising the transparent cover of the cylinder temporarily. The bees' performance under the various experimental conditions was evaluated by using the same categories as described in **Figure 3**, as well as flight time through the maze.

Learning to negotiate a right-turn maze

One series of experiments (noted as Series 1 in the Tables) investigated the ability of bees to negotiate a maze in which every turn is to be made in the same direction – a *constant-turn maze*. A right-turn maze is shown in **Figure 5A**. The performance of bees, trained on this maze for 1 day, and then tested in an identical maze is summarized in **Table 1**, as evaluated by the four categories, and in **Table 2**, as evaluated by the five time categories. The performance shows that most flights have a relatively short duration (T1: flight duration <30 s) and most of the test flights belong to the category Cat. 1 (no errors). Thus, the trained bees are able to fly through the maze quickly and accurately.

Bees trained in the right-turn maze (**Figure 5A**) were tested in an extended right-turn maze with an additional decision chamber added at the end, also requiring a right turn. These bees showed a clear tendency to make correct choices (right turns) even in the extension, indicating that they applied the rule that they had learned during the training to the extended part of the maze in this test. Bees trained in the right-turn maze were tested in an irregular maze, which they had never experienced (**Figure 6A**). Interestingly, these bees succeeded in arriving at the feeder, as shown by the sample trajectory in **Figure 6A**. They achieved this by simply using the "always turn right" rule. This rule always made them reach the goal eventually, even if they entered some dead-end chambers en route. The relative frequencies of right and left turns made by the trained bees, when tested in a number of irregular mazes are shown in **Figure 6B**. Bees trained in a right-turn maze show a strong and significant preference for making right-hand turns, no matter what maze they encounter. Bees trained in a right-turn maze can also negotiate left-turn and zig-zag mazes, because the right-turn rule (or left-turn rule, for that matter) can, in principle, be applied to all of these mazes to eventually get to the reward, even though this entails entering a number of dead-end cylinders en route (Zhang et al., 2000).

Learning to negotiate a zig-zag maze

The second series of experiments (noted as Series 2 in the Tables) examined whether bees could learn to negotiate a *zig-zag maze*, where the correct turns were alternately to the right and to the left, as shown in **Figure 5B**. It was shown that, bees learn a zig-zag maze nearly as well as a constant-turn maze (Zhang et al., 2000). Can bees extrapolate the zig-zag rule that they have learned, and apply it to extended or altered mazes? We investigated this question by testing the bees that were trained in the zig-zag maze of **Figure 5B**, in a set of altered mazes – one of these experiments is discussed below.

Bees were tested in a maze similar to that of **Figure 5B**, but in which a special chamber (chamber 5) was added in the middle, as shown in **Figure 7A**. However, this new chamber had only one exit, diametrically opposite to the entrance, so that the bees could not choose "left" or "right" while passing through it. The question here was: how would the bees behave in the *next* chamber (chamber 7), given that they had made a left turn in the previous chamber (chamber 5). The tests (**Figure 7B**) revealed that the bees showed a clear tendency to turn *left* in chamber 7. This implies that they had treated chamber 5 as though they had made a right turn in it, even though it was a "dummy" chamber that offered no turning choice. Evidently, in applying the zig-zag rule, even dummy chambers are treated as valid ones.

The above experiments show that honeybees can negotiate mazes by recognizing and learning regularities in the paths through them, if such regularities exist. The performance in the mazes with path regularities is better than in the mazes without path regularities (for details of statistical tests see Zhang et al., 2000). Honeybees can negotiate novel mazes in transfer tests by using the "rules" that they acquire during training.

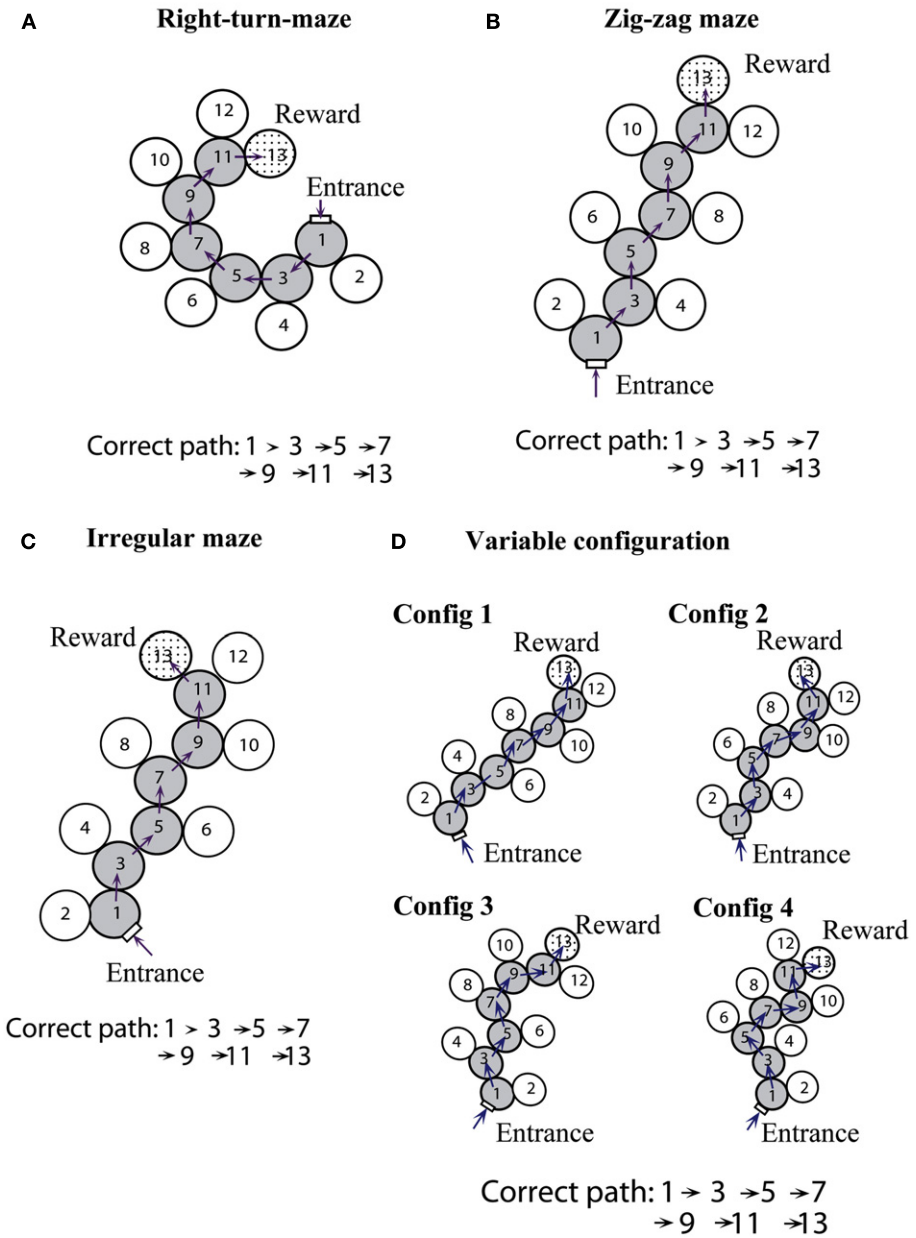

FIGURE 5 | Four types of ma e configurations. (A) Constant-turn maze: same turn direction in each decision chamber. **(B)** Zig-zag maze: alternating turns in successive decision chambers. **(C)** Irregular maze: random sequence of turns. **(D)** Variable irregular mazes: four configurations have to be learned simultaneously. Modified from Zhang et al. (2000). Details in text.

HONEYBEES USE WORKING MEMORY AND LONG-TERM-MEMORY IN DELAYED-MATCH-TO-SAMPLE TASKS OR SYMBOLIC-DELAYED-MATCH-TO-SAMPLE TASKS

One of the more complex tasks that has been used to investigate principles of learning and memory is the so-called "DMTS." This task has been investigated in a number of vertebrate species such as the monkey (e.g., D'Amato et al., 1985), dolphin (e.g., Herman and Gordon, 1974), and pigeon (e.g., Roberts, 1972). Honeybees need to use two memory systems to successfully complete this task: working memory for remembering a sample pattern, and long-term-memory for remembering what criterion or rules are to be used in making decisions.

Most DMTS tasks follow the same general procedure. Each trial begins with the presentation of a sample stimulus. The sample is followed by a delay or retention interval and then by the presentation of two or more test stimuli, one of which is identical to the sample stimulus. If the animal chooses the test stimulus that corresponds to the sample, it then obtains a reward (hence, the name "delayed match-to-sample"). Most experiments use two or three sample stimuli, which are varied randomly from trial to trial.

able 1 | ummary of ma e performance as evaluated by categories.

	1	2	3	4	5	otal
ERIE 1						
Number of flights	138	78	30	13	7	266
Percentage	51.8	29.3	11.3	4.9	2.6	
ERIE 2						
Number of flights	64	45	11	3	0	123
Percentage	52.0	36.6	8.9	2.4	0	
ERIE 3						
Number of flights	39	49	27	10	0	125
Percentage	31.2	39.8	21.6	8.0	0	
ERIE 4						
Number of flights	7	23	11	3	12	56
Percentage	12.5	41.1	19.6	5.4	21.4	
C N R L						
Number of flights	3	13	10	3	13	42
Percentage	7.1	31.0	23.8	7.1	31.0	

*For each series of experiments, performance is indicated by number and percentage of flights in each category: Cat. 1 to Cat. 4 (see **Figure 6** caption for details).*

able 2 | ummary of ma e performance as evaluated by flight time.

	Cat. 1	Cat. 2	Cat. 3	Cat. 4	otal
ERIE 1					
Number of flights	87	18	161	0	266
Percentage	32.7	6.8	60.6	0	
ERIE 2					
Number of flights	33	4	86	0	123
Percentage	26.8	3.3	69.9	0	
ERIE 3					
Number of flights	21	5	99	0	125
Percentage	16.8	4.0	79.2	0	
ERIE 4					
Number of flights	0	1	55	0	56
Percentage	0	1.8	98.2	0	
C N R L					
Number of flights	1	0	34	7	42
Percentage	2.4	0	80.9	16.7	

For each series of experiments performance is indicated by number and percentage of flights in each time category (the time taken to successfully navigate the test maze) – T1: 1–30 s; T2: 31–60 s; T3: 61–90 s; T4: 91–120 s; and T5: 121–300 s (5 min). T1 therefore represents the best performance and T5 the worst.

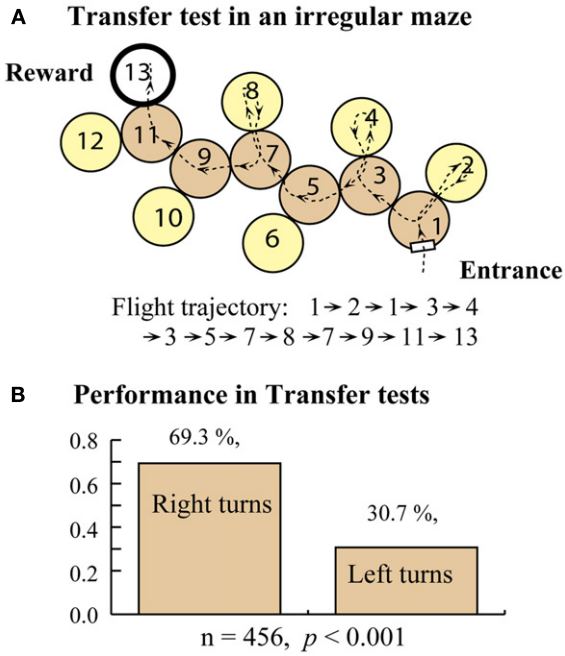

FIGURE 6 | raining in a right turn ma e and transfer test in an irregular ma e. (A) Transfer test setup. **(B)** Test results. Modified from Zhang et al. (2000). Details in text.

A more complex variant of the above task is called a "SDMTS" task. In this task, none of the test stimuli physically match the sample: the experimenter arbitrarily designates the correct choice. Here, the animal has to learn to associate the correct test stimulus corresponding to each sample stimulus.

Can bees learn such tasks? Their foraging behavior may give us some hints. Honeybees have evolved a number of navigational skills that enable successful foraging. Collett and Wehner suggested that foraging insects traveling repeatedly to a food source and back to their homes navigate by using a series of visual images, or "snapshots," of the environment acquired en route (Collett and Kelber, 1988; Wehner et al., 1990, 1996; Collett et al., 1993; Collett, 1996; Judd and Collett, 1998). By comparing the currently viewed scene with the appropriate stored image, the insect is able to ascertain whether or not it is on the correct path, and make any necessary corrections. Successful foraging may require the bee to be able to solve tasks analogous to SDMTS tasks. Thus, it is of interest to explore whether bees can learn DMTS and SDMTS tasks.

Learning Symbolic-Delayed-Matching-To-Sample task in the visual domain

One series of experiments examined the bees' ability to learn an SDMTS task in the visual domain (Zhang et al., 1999). Honeybees were trained to fly through a compound Y-maze consisting of a series of interconnected cylinders (**Figure 8A**). The first cylinder carried the sample stimulus.

The second and third cylinders each had two exits. Each exit carried a visual stimulus, between which the bees had to choose. If a bee made a correct choice in the second as well as in the third cylinder, she arrived in a fourth cylinder where she found a feeder with sugar solution. Thus, the second and the third cylinder acted as decision stages: at each of these cylinders the bee had to choose between two stimuli. It was the single sample stimulus in the first cylinder that determined the choices that the bees had to make in the subsequent decision stages.

During training, the sample stimulus was a black-and-white grating oriented either horizontally (Stimulus A) or vertically (Stimulus A′), respectively. The second cylinder (first decision

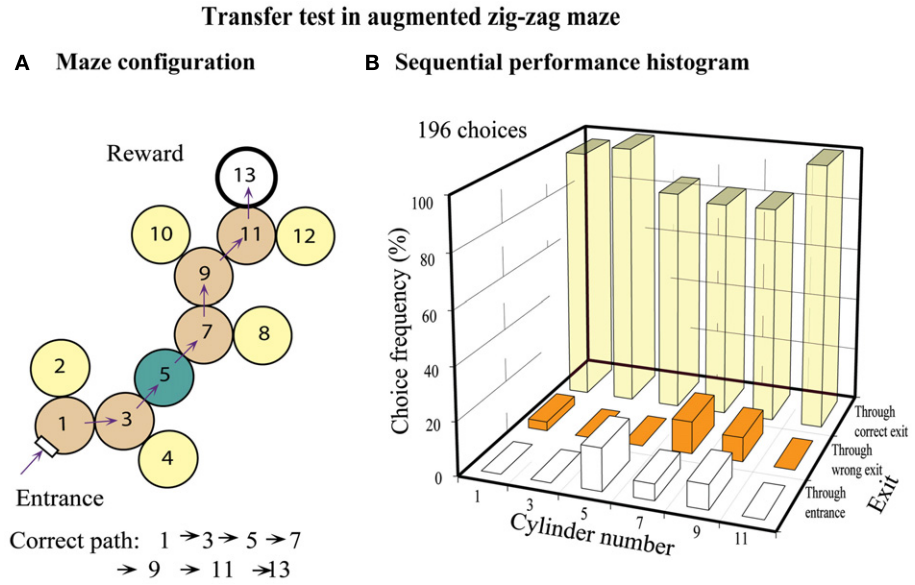

FIGURE 7 | raining in a ig ag ma e and transfer test in an augmented ig ag ma e. (A) Configuration of the augmented zig-zag maze, with an additional cylinder. **(B)** Histogram showing performance at each decision cylinder. Adapted from Zhang et al. (2000). Details in text.

stage) offered a choice between a blue square (Stimulus B) and a green one (Stimulus B′), and the third cylinder a choice between a pattern consisting of a sectored disk (C) or of concentric rings (C′; **Figure 8B**). When the sample stimulus was the horizontal grating, the feeder could only be reached if the bee chose blue in the second cylinder and the sectored disk in the third. However, when the sample was the vertical grating, the bee could reach the reward only if she chose green in the second cylinder and the ring pattern in the third.

After training, the bees were tested not only on the training sequences ABC and A′B′C′ (learning tests; **Figure 8B**), but also in transfer tests which presented five other permutations of the training sequences. The results of tests on one of the permuted sequences (BAC and B′A′C′) are illustrated in **Figure 8C**.

The results showed that bees are indeed capable of learning SDMTS tasks. Clearly, viewing the sample stimulus (horizontal or vertical grating) triggers recall of the stimulus that should be chosen in each of the subsequent stages (**Figure 8B**). Furthermore, the trained bees continued to choose the appropriate stimulus at each stage of the maze even in the transfer test (**Figure 8C**), as well in tests using other sequence permutations (Zhang et al., 1999). These findings indicate that, in general, exposure to any one of the stimuli that were encountered in the training (A,B,C, A′,B′,C′) was sufficient to trigger associative recall of all of the other stimuli belonging to that set. In all of the tests, changing the sample stimulus (from A to A′, B to B′, or C to C′) caused the bees to change (and reverse) their preference for the stimuli that they encountered at subsequent stages of the maze. It should be noted that, in this experiment, the bees were not specifically trained to distinguish between A and A′, which were the sample stimuli in the training. Nevertheless, the bees distinguished between them in the transfer tests because they associated them with the stimulus sets ABC and A′B′C′, respectively. It is also clear from this set of tests that the bees were capable of treating the stimulus pairs (B, B′; **Figure 8C**) as well as (C, C′; not shown in the figure) as sample stimuli, even though these were never encountered as sample stimuli in the training.

The above findings suggest that bees solve the SDMTS task by mapping the six visual stimuli that they encounter in the training into two distinct sets (A, B, C) and (A′, B′, C′), as illustrated in **Figure 9** After training, exposure to any stimulus belonging to a member of one of these sets triggers recall of the other two members belonging to that set. Thus exposure to B, for example triggers recall of A and C; whereas exposure to C′ triggers recall of A′ and B′.

Learning the Symbolic-Delayed-Matching-To-Sample task across sensory modalities

Can bees learn an SDMTS task when they are required to make associations that span different sensory modalities? Clearly, humans display impressive cross-modal associative recall. It is a common experience that a smell or a sound can trigger a vivid recollection of an associated event in the past – even if it involves a different sensory modality, and even if the episode occurred a long time ago (Baddley, 1983).

Srinivasan et al. (1998) explored this capacity by asking whether bees could learn to associate specific scents with specific colors. The apparatus consisted of a compound Y-maze, as in the above experiments, but with a single decision stage (**Figure 10A**). The sample stimulus, presented in the first cylinder, was a scent that was either lemon or mango. The decision stage offered a choice of two colors, blue or yellow. When the bees encountered lemon at the entrance, they had to learn to choose blue in the decision stage; when they encountered mango, they had to choose yellow. The bees learned this task very well (**Figure 10B** Experiment 1). The scent of lemon evidently evoked recall of blue, whereas mango

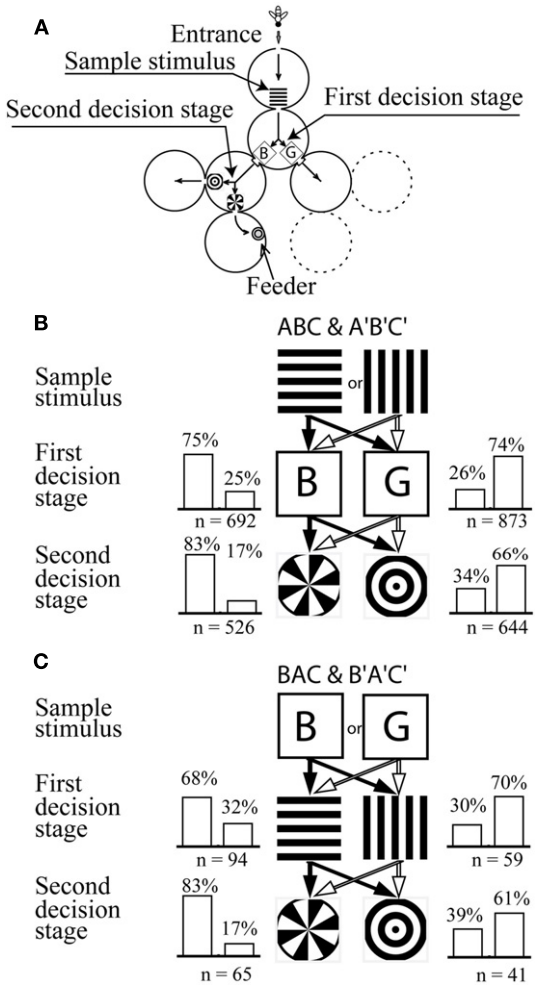

FIGURE 8 | Learning a symbolic delayed matching to sample task in the visual modality. (A) *Y*-maze setup with two decision stages. **(B)** Training stimulus configuration and training performance. The bees learned to choose horizontal-blue-sector or vertical-green-ring associations in order to get a reward. **(C)** Stimulus configuration and performance on the transfer test. Modified from Zhang et al. (1999). Details in text.

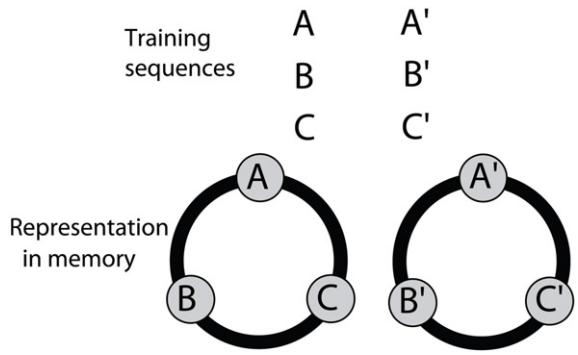

FIGURE 9 | A model for associative grouping, derived from the results of the symbolic delayed match to sample experiments. Details in text.

triggered recall of yellow. Bees could also be trained to make the opposite associations: lemon with yellow, and mango with blue (**Figure 10B**, Experiment 2), as well as symbolic matches in the opposite direction (**Figures 10C,D**).

The evidence presented here clearly shows that honeybees are able to learn SDMTS tasks, not only in the visual modality, but also across sensory modalities. Learning an SDMTS task requires that the bee be able, when presented with a sample stimulus, to recall other stimuli that are associated with the sample stimulus. For a foraging honeybee, cross-modal associative recall can facilitate the search for a food source. For example, detecting the scent of lavender could initiate a search for purple flowers.

Learning the concepts of "sameness" and "difference"

A related question is whether honeybees are able to group stimuli according to certain rules, or concepts, such as "sameness" or "difference." In vertebrates, the capacity to acquire such concepts has been studied using two experimental procedures, the DMTS task and the *delayed non-match-to-sample* (DNMTS) task (Zentall and Hogan, 1978; Holmes, 1979). The DNMTS task is similar to the match-to-sample task except that the animal is required to respond to the stimulus that is different from the sample. It should be pointed out, however, that an ability to learn the concept of "sameness" or "difference" would be proven only if the animal is able transfer the ability to correctly choose the matching (or the non-matching) stimulus to a completely novel set of stimuli, which it had not experienced during training.

Giurfa et al. (2001) examined whether honeybees could learn the concepts of "sameness" and "difference." The apparatus used in the experiments was similar to that used for the SDMTS tasks. Bees were trained on sectored and ring patterns, as shown in **Figure 11A**. That is, they had to learn to choose the sectored or the ring pattern in the decision chamber, according to whether the sample stimulus at the entrance was the sectored or the ring pattern. The bees learned this task well, showing a clear ability to choose the matching stimulus in each case (**Figure 11C**, left-hand panel). The trained bees were then subjected to a transfer test, as shown in **Figure 11B**, where the stimuli were two colors, blue and yellow. The bees were immediately able to transfer the matching task to the colors, despite the fact they had never been trained on them (**Figure 11C**, right-hand panel). They were also able to transfer the matching ability to other novel stimuli, such as gratings oriented at $+45^\circ$ and -45° (data not shown).

Bees can also be trained to match odors, and can immediately transfer the learned matching ability to colors. Thus, the concept of "matching," once learned, can be transferred even across sensory modalities.

Finally, bees can also learn the concept of "difference." That is, they can be trained to choose the non-matching stimulus, rather than the matching one. **Figure 12A** shows learning curves obtained in two experiments investigating this capability. In one experiment, the training stimuli were colors (blue and yellow). Here, bees had to learn to choose yellow in the decision chamber when they encountered blue at the entrance, and vice versa. In another experiment, the training stimuli were linear gratings, oriented

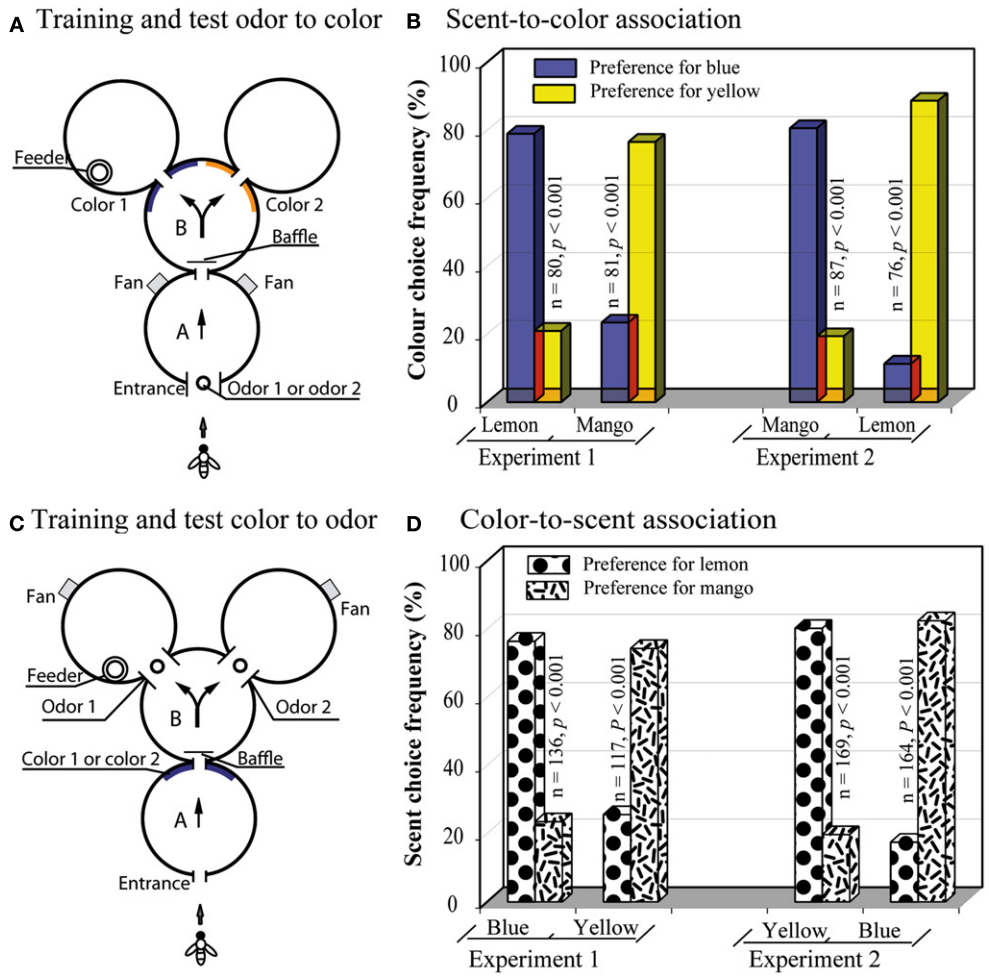

FIGURE 10 | Learning a symbolic delayed matching to sample task across the sensory modalities. (A) *Y*-maze setup with odor presentation in the first chamber. **(B)** Results of the scent-to-color association tests. **(C)** *Y*-maze setup with color presentation in the first chamber. **(D)** Results of the scent-to-color association tests. Modified from Srinivasan et al. (1998). Details in text.

horizontally and vertically. There, bees had to learn to choose the vertical grating in the decision chamber when they encountered a horizontal grating at the entrance, and vice versa. It is evident from **Figure 12A** that the bees learned both non-matching tasks well. Furthermore, in each case the trained bees were immediately able to transfer the learned, non-matching concept to novel stimuli. Bees trained on the colors were able to perform non-matching on the gratings, and vice versa (**Figures 12B,C**).

These findings demonstrate that bees can indeed learn rather abstract concepts, such as "sameness" and "difference," and apply them to novel situations – situations on which they have not directly been trained.

CONTEXTUAL CUES IN DECISION MAKING

Beside the aforementioned ability of bees to learn abstract rules and categorize objects, bees can use the context in which a stimulus appears to produce an appropriate response. Contextual cues are dependent on the external environment, and the animal's internal motivation. They can facilitate memory retrieval, when the context in which the memory was encoded is replicated. Thus, context cues help to carve up the world into distinct regions, and help animals cope with possible confusions (Colborn et al., 1999; Fauria et al., 2002; Cheng, 2005; Dale et al., 2005). Collett and Kelber (1988) found in their study that honeybees can retrieve the right landmark memory by the context in which the landmark is placed. Bees can also change their response to a visual pattern according to whether the stimulus provides access to the hive or the feeder (Gadagkar et al., 1995). Dale et al. demonstrated that honeybees and bumblebees can learn to treat the same visual and olfactory target in different ways in various spatial, temporal, or motivational contexts. Such contextual influences are important because they allow bees to flexibly adapt to many different situations (Dale et al., 2005). Context learning can be seen as the complementary strategy to categorization: While categories contain different objects or situations that elicit the same behavioral response, the context in which an object or situation is encountered can alter the behavioral response to it. A bee can learn, for example, that dandelions contain nectar in the morning, but not in

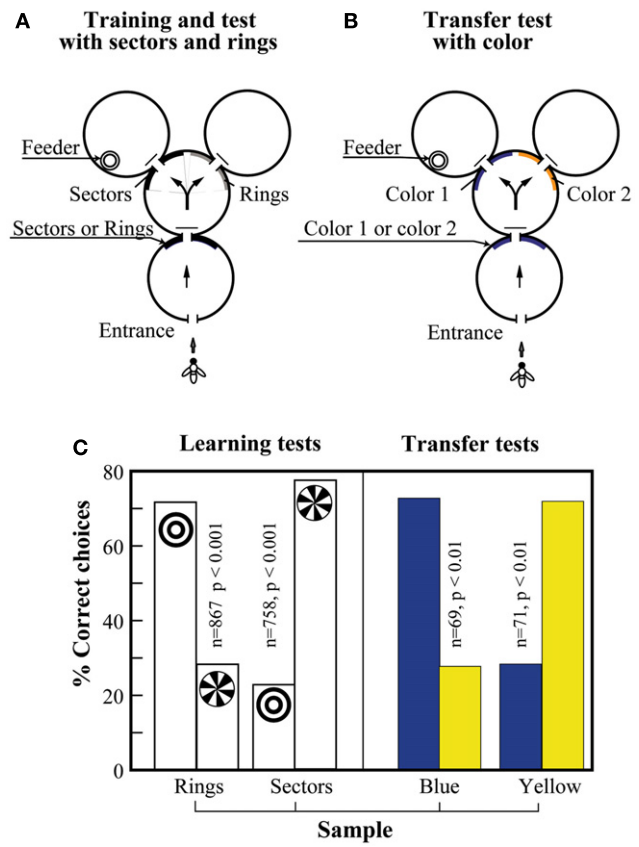

FIGURE 11 | Learning the concept of sameness in the visual modality. (A) Maze setup with geometric patterns as sample and matching stimuli. **(B)** Maze setup with colors as sample and matching stimuli. **(C)** After learning geometric pattern matching (left panel), the bees were immediately able to solve the color matching task as well (right panel). Modified from Giurfa et al. (2001). Details in text.

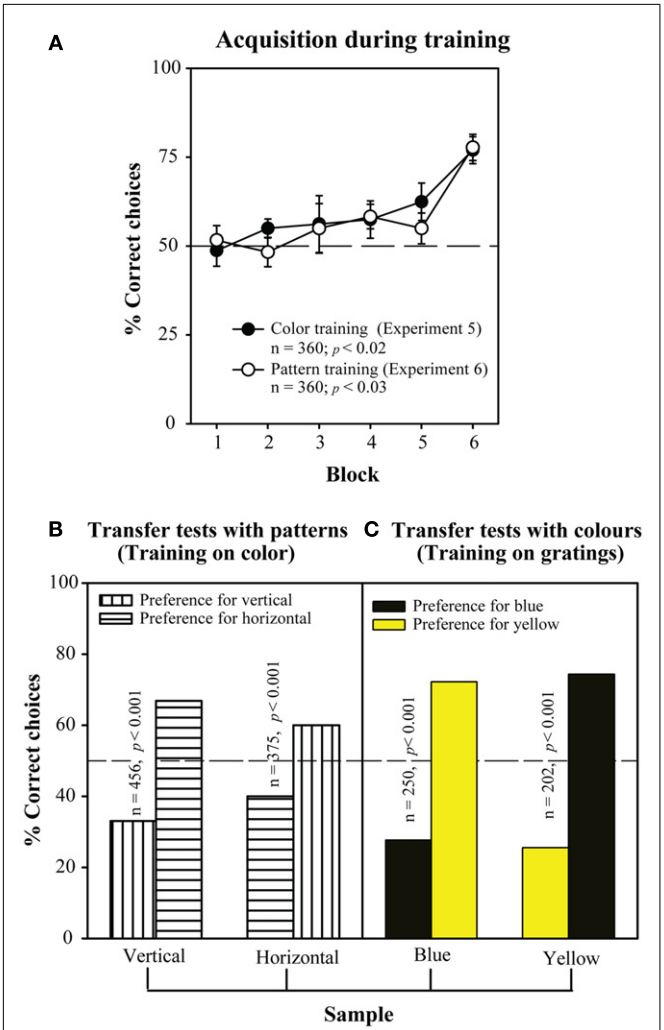

FIGURE 12 | Learning the concept of difference Setup similar to **Figure 11**, but the bees are rewarded for choosing the non-matching pattern. **(A)** Acquisition curve during the training phase. **(B)** Results of the transfer tests, after the bees were trained on color, and **(C)** after the bees were trained on patterns. Modified from Giurfa et al. (2001). Details in text.

the afternoon. Thus, using the time of day as a context, a honeybee forager will land on a dandelion flower in the morning, but ignore it in the afternoon, and keep searching for clover, which provides nectar in the afternoon but not in the morning.

Honeybees know what to do when

How time and motivation can act as contextual cues was investigated by Zhang et al. (2006). In this study, bees were trained to forage in a Y-maze, where they had to choose between two competing visual stimuli in order to collect a sugar reward. When returning to the hive, the bees had to make another decision between two stimuli in order to gain entry to the nest and deliver the sugar they had collected.

In a first series of experiments, the bees learned to reverse their stimulus preference between the morning and the afternoon, i.e., following a midday break and an overnight break. They learned this quickly in two configurations: with identical and also with dissimilar stimuli at the hive and the feeder, demonstrating that the time of day can act as a contextual cue, so that a bee can treat the same stimulus differently according to the time at which it is encountered.

In the second series of experiments, the bees were trained to choose a yellow stimulus in the maze to get a sugar reward, and a blue stimulus in order to enter the hive. Since maze and hive were less than 10 m apart, the time between a decision for yellow in the maze, and the subsequent decision for blue at the hive was just about 2 min. This demonstrates that bees can use task as a context as well: when foraging, the bee prefers yellow. On the way home, however, she changes her stimulus preference within just a few minutes, and preferentially chooses blue at the hive. This experiment was repeated with reversed colors, and showed the same result.

In the third experimental series, the bees were trained to choose a horizontal grating stimulus in the rewarded maze in the morning, and a vertical grating stimulus in the afternoon. At the same time, in order to find access to the hive, the foragers had to decide for

the opposite configuration at the hive: the vertical grating granted access in the morning, and in the afternoon, the horizontal grating marked the open entrance. **Figure 13** shows that the bees could solve even this very complicated task: they reversed their stimulus preference based on the time of day between morning and afternoon. At the same time, the task at hand acted as contextual cue, and enabled the bees to make opposing decisions within just a few minutes, when foraging and returning to the hive.

This study shows that bees can use time as a contextual cue, setting two competing visual stimuli in different contexts, while simultaneously observing a task-dependent rule (i.e., choosing A at the feeder and B at the hive in the morning, and B at the feeder and A at the hive in the afternoon). The experimental bees learned to treat the two stimuli differently in the morning and in the afternoon, as well as when flying to the feeder and returning to the hive. The training imposed a learnt stimulus preference on the bees' circadian rhythm (**Figure 13**), demonstrating that honeybees possess a sophisticated memory which is able to memorize tasks within a temporal context (Zhang et al., 2006). They could use this ability to treat stimuli differently during navigation to a food source and on the way back to the hive, as well as during for aging on at least two different times of the day, in order to be at the right place at the right time. "Planning" activities within a temporal and spatial frame of reference could enable foragers to use resources more efficiently.

Circadian timed episodic-like memory: how to do the right thing in the right place at the right time

Pahl et al. (2007) further investigated how bees use context cues to separate conflicting stimuli, in order to produce efficient foraging behavior. To study how the color, shape, and location of stimuli could be memorized within a time frame, bees were trained to forage at two Y-mazes at equal distances, but in different directions, from the hive. Maze A presented blue horizontal (rewarded) vs vertical gratings in the afternoon, while maze B presented yellow vertical (rewarded) vs horizontal gratings in the morning (the stimuli are shown in **Figure 14**). The bees quickly learned to fly to the active maze at the right time, and chose the rewarded stimulus with an accuracy of about 83%. With this as a baseline, several transfer tests were carried out, in which color and shape properties of the stimuli were removed, and the location of the test maze was changed systematically. In this way, the relative importance of different stimulus properties could be investigated. During training, the bees memorized information about the color and shape of stimuli, but also about the location of the maze and the time of day when it provided a reward. In transfer test 1 in the mazes' original locations A and B, the color cues were removed by presenting the bees with black-and-white gratings. The bees chose the previously rewarded grating orientations without the color cue, according to the maze location, and the time of day, in about 75% of the visits. In the next step, the location cue was removed by dismantling mazes A and B, and setting up a new maze at a neutral point C between the training mazes. When the bees visited the new maze and the training stimulus configuration was presented (transfer test 2, **Figure 14A**), the foragers chose the yellow vertical grating in the morning and the blue horizontal stimulus in the afternoon with an accuracy of 83%. In transfer test 3, the orientation and location cues were eliminated by presenting the bees with vertical blue and yellow gratings in the morning, and horizontal blue and yellow gratings in the afternoon. The bees chose the color according to the time of day, with high accuracy of about 91% (**Figure 14B**). In the last test, color and location cues were removed by presenting black gratings in the neutral maze C. In this situation, the bees chose the orientation according to the time of day, at a frequency of correct choices of about 72%.

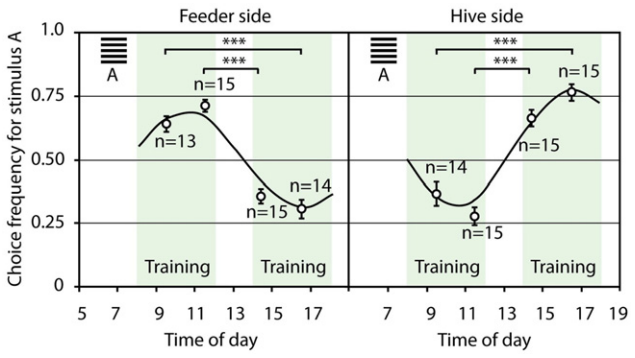

FIGURE 13 | ime and task as contextual cues. During training, the horizontal grating stimulus in the maze and the vertical grating at the hive were rewarded in the morning, whereas in the afternoon, the vertical grating in the maze and the horizontal grating at the hive were rewarded. Shown are the choice frequencies for the horizontal grating stimulus. The trained bees reversed their stimulus preference in the maze and at the hive entrance following midday breaks, as well as overnight breaks. At the same time, the bees reversed their stimulus preference within a few minutes, and made opposing decisions between foraging and homing. The modulation of the average choice frequency, with reference to the horizontal grating, could be approximated very well by a sinusoidal curve with a frequency of 0.52, i.e., a period of 12 h. The phase of the sinusoidal curve at the hive was shifted 180° with reference to the feeder. ***Denotes $p < 0.001$. Modified from Zhang et al. (2006).

The results suggest that color and shape are the most important visual cues when bees decide between flowers. The absence of the spatial cue did not impair the bees' performance; they still showed a significant preference for the rewarded stimulus according to the time of day (**Figures 14A–C**). When visiting different feeding sites, or even when a new flower patch is discovered, previous experience enables bees to choose the most profitable flower according to the time of day (Pahl et al., 2007).

Visual and olfactory properties are not the only cues separating different flower species. Flowers open and close their blossoms at regular times during the day, as the Swedish taxonomist Carl von Linné observed more than 250 years ago (Linné, 1751). Moreover, it is not only the opening and closing times of blossoms that follow a circadian pattern. Beutler and Kleber found that the amount and concentration of nectar varies over time in a species-typical way (Beutler, 1930), and the same is true for pollen (Parker, 1925). Thus, time is a factor of great importance for nectar and pollen collectors (von Frisch, 1967). Bees would profit from a time sense not only to compensate for the sun's movement during the waggle dance, but also in order to visit the flowers during their peak nectar- and pollen-production times. This sense of time was first described by August Forel, who found bees waiting at his coffee

table just before breakfast and afternoon tea in anticipation of sweet marmalade (Forel, 1910). His observation inspired further investigation of the bees' biological clock by von Frisch's student Behling (1929) and later by Koltermann (1971), who found that he could train bees to remember up to nine different times during a day when he presented a scented sucrose feeder.

The ability of honeybees to integrate elements of circadian time, place, and visual information shown in Pahl et al.'s (2007) study is akin to the episodic-like memory demonstrated in food caching scrub-jays (Clayton and Dickinson, 1998), and has therefore been named circadian timed episodic-like memory.

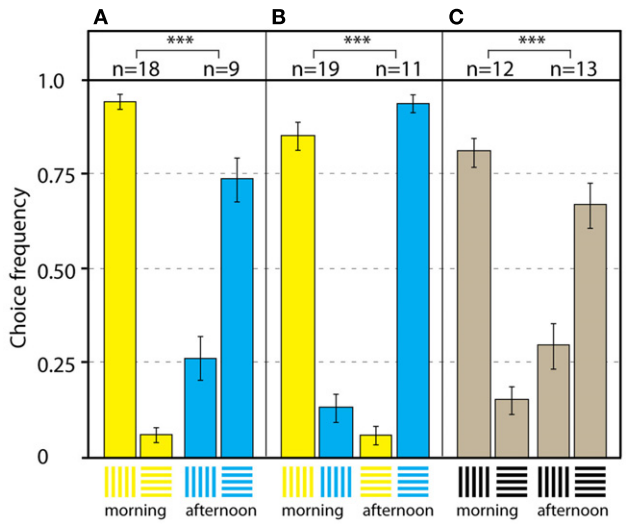

FIGURE 14 | hat to do where and when. Results of the transfer tests in the neutral location. **(A)** Transfer test 2 with yellow and blue training patterns. The bees preferred the yellow vertical stimulus in the morning, and the blue horizontal stimulus in the afternoon. **(B)** Transfer test 3 with yellow and blue patterns in the same orientation. The bees preferred the yellow stimulus in the morning, and the blue stimulus in the afternoon. **(C)** Transfer test 4 with black patterns. The bees preferred the vertical grating in the morning, and the horizontal grating in the afternoon. *n* Denotes number of individual bees in each test; bars are means ± SEM. ***Denotes $p < 0.001$. Modified from Pahl et al. (2007).

NUMBER-BASED DECISION MAKING IN HONEYBEES

Numerical abilities are an important marker in the cognitive abilities of an animal. So far, mainly vertebrate species like pigeons (Koehler, 1941) and monkeys (Brannon and Terrace, 2000) have been tested for the ability to make number-based decisions, and few convincing accounts for invertebrates exist so far (Chittka and Geiger, 1995; Franks et al., 2006; Dacke and Srinivasan, 2008). Gross et al. (2009) set out to shift the balance more in favor of the invertebrates. Honeybees, by virtue of their other impressive cognitive features, are prime candidates for investigations of this nature. Using the DMTS paradigm, the limits of the bees' ability to match two visual stimuli solely on the basis of the shared number of present elements were tested. After the experimental animals had learned the basic DMTS task in a modified Y-maze, they were able to discriminate patterns containing two or three elements. To make sure that the experimental bees were indeed using the amount of objects on a stimulus to make a decision, a series of experiments was carried out. Firstly, to exclude direct visual matching of the stimuli, the positions of the objects in sample and matching stimuli was randomized. The bees could still match two and three in all configurations (**Figure 15A**). The next step was

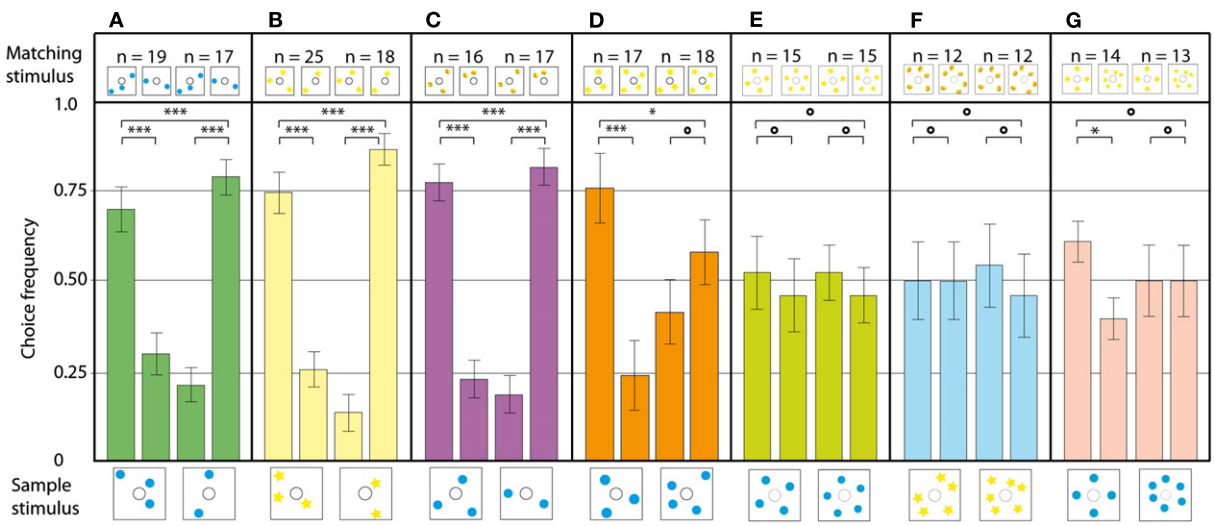

FIGURE 15 | Number based decision making in a delayed match to sample task. The stimulus below each pair of bars is the sample, and that above each bar is the respective choice stimulus. The data present the pooled first choices of individual bees. **(A)** The configuration of dots on the sample and choice stimuli is randomized. **(B)** The blue dots are replaced with yellow stars, to see if bees can transfer their matching ability to different, unknown stimuli. **(C)** The sample and choice stimuli are composed of two different elements. **(DF)** Bees trained to discriminate between two and three are tested on stimuli with **(D)** three and four elements, **(E)** four and five elements, **(F)** five and six elements, **(G)** four and six elements. *n*, Number of bees per condition. Error bars show standard error. ***Denotes statistically significant difference at $p < 0.001$, **Denotes $p < 0.01$, *denotes $p < 0.05$, and O denotes $p > 0.05$. Modified from Gross et al. (2009).

to present the bees with novel objects, which they had never seen before in training. The animals solved this task very well, demonstrating the ability to transfer the matching rule even to novel objects (**Figure 15B**). In the following experiment, the bees' ability to generalize over different objects was tested by using blue dots in the sample stimulus, and yellow lemons in the matching stimulus (**Figure 15C**). Even in this configuration, the bees had no trouble matching the stimuli based on number. When the bees were tested on a novel numerosity, a four to four match, the performance dropped significantly. In the three vs four configuration shown in **Figure 15D**, the bees could still do the three to three match, but their decisions in the four to four match were randomly distributed in two out of three experiments. In the experiments on the discrimination of higher object numbers, such as four vs five (**Figure 15E**) and five vs six (**Figure 15F**), the decisions were randomly distributed. Interestingly, when the bees were tested in a four vs six discrimination task, they were able to do the four to four match, but not the six to six match (**Figure 15G**). Thus, the bees' numerosity discrimination ability does not follow Weber's law, indicating that the animals are using absolute number, and not relative amount of objects, to discriminate between the stimuli. A series of control experiments confirmed that the bees were not using lower order cues such as the color or configuration of elements, combined area, or edge lengths of elements, spatial frequency of the stimuli, or illusory contours formed by the elements.

There are two basic mechanisms to assess the exact number of objects in a stimulus: subitizing and true counting. True counting requires subjects to (1) produce a standard sequence of number tags, (2) apply a unique number tag to each item to be counted, (3) remember what already has been counted, and (4) know that the last number tag tells how many objects are there (von Frisch, 1971). This is clearly not what the bees were doing in our experiment. Subitizing is the instant recognition of the number of objects without sequential counting. Stanley Jevons found, in his paper from 1871, that the maximum number of beans in a box he could estimate correctly, after a brief presentation not allowing for sequential counting, was 4. With higher numbers, the amount of errors increased rapidly (Jevons, 1871). The honeybee is the first invertebrate species where a numerical ability has been convincingly demonstrated, and the process by which it achieves numerical discrimination is most likely subitizing (Gross et al., 2009). The fact that its sense of number has a similar extent as the subitizing abilities reported for many vertebrate species, including human infants (Linnel and Fluck, 2001), hints toward a highly conserved mechanism; one quite separate from real counting (Gross, 2011). The results from this study indicate that numerosity is treated by the bees as one more primary visual feature of a scene, along with color, contrast, size, and speed (Burr and Ross, 2008; Gross et al., 2009). Data on the adaptive value of numerical competence are rare, because most studies were conducted in laboratory situations. However, there are some examples of field studies: food-hoarding robins have been shown to use information about the number of food items in a cache in the wild (Hunt et al., 2008). Lyon reported a spontaneous use of numerical information (egg counting) in a natural context, reducing the fitness costs of conspecific brood parasitism in American coots (Gallistel, 1988). Lions base the decision to attack or retreat from a group of intruders on the number of roaring individuals (McComb et al., 1994). Honeybees could use their sense of number to recognize flowers by the amount of petals (Leppik, 1953), to navigate by the number of landmarks encountered (Chittka and Geiger, 1995; Dacke and Srinivasan, 2008), or to make foraging decisions according to the number of bees already present on a blossom (Gross et al., 2009).

CONCLUSION

The experiments described above give an indication of the range of environmental cues and cognitive processes that can be used by foraging honeybees in deciding what to do in particular contexts. Bees can easily learn a cue or a rule that leads to a reward, and generalize that cue or rule to novel situations in order to continue accessing that reward. However, bees are far from being hard-wired automats, and can flexibly and adaptively fine-tune their decision-making process to cope with radically different contexts and situations. Stimuli – even from different sensory modalities – that tend to co-occur in a bee's experience are grouped together and associatively recalled, while abstract concepts such as "sameness" and "number" can be readily assimilated, as possible solutions, into an individual's decision-making repertoire. Finally, bees can also deal with multiple contexts, first making one decision in one context, and then flexibly switching to the opposing decision in a different context. The resulting picture of honeybee decision making is therefore a complex one, involving not only the interpretation of environmental cues and context-dependent choices, but also input from the stored memories of past experiences.

A NOTE ON INTER-INDIVIDUAL VARIABILITY IN DECISION MAKING

In decision-making experiments with honeybees, the animals usually reach a peak at 75–85% decisions for the rewarded stimulus, while in 15–25%, they choose the unrewarded stimulus. This is often seen as a failure to reach the perfect score of 100%. In the bees' natural foraging environment, however, rewards are not as predictable as in behavioral experiments in the lab. In the course of a nectar-gathering season, different flowering plants are in bloom successively. Even in the course of 1 day, the profitability of resources may change between morning and afternoon. Thus, honeybees (and all other animals) constantly face the decision between foraging at a well-established, but finite resource, and searching for a new, potentially richer, but uncertain one (March, 1991). In maze experiments with honeybees, the costs of choosing the "wrong," previously unrewarded stimulus are low: the bee finds an empty feeder, is released and can re-enter the maze for another trial in a matter of minutes. Foraging in a natural environment, a previously unrewarding flower may well start producing nectar or pollen later in the day, and thus justify the occasional visit by a bee. The costs of scouting for a novel flower, however, are a lot higher: considering the uncertainty of a reward, as well as the increased risk of predation, we would expect a lower rate of behavioral variability in the bees' ecological context. Indeed, flower constancy is a well-known behavioral trait in honeybees. It was described for the first time some 2300 years ago by Aristotle, but the reason for bees to stick with one type of flower at a time is still a matter of debate (Chittka et al., 1999; Raine and Chittka, 2007; Grüter and Ratnieks, 2011). Brembs (2011) argues that animals need to

balance the efficiency of their behaviors with variability, in order to prevent predictability. In this line of research, honeybees can be a useful model to investigate the adaptiveness of behavioral variability, because reward situations and the costs of "wrong" decisions can be easily manipulated in experimental setups with free-flying bees.

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Key factors for the emergence of collective decision in invertebrates

Raphaël Jeanson[1,2], Audrey Dussutour[1,2] and Vincent Fourcassié[1,2]*

[1] Centre de Recherches sur la Cognition Animale, Université de Toulouse, Université Paul Sabatier, Toulouse, France
[2] CNRS, Centre de Recherches sur la Cognition Animale, Toulouse, France

Edited by:
Björn Brembs, Freie Universität Berlin, Germany

Reviewed by:
Benjamin Hayden, University of Rochester, USA
Nathalie Stroeymeyt, Université de Lausanne, Switzerland

***Correspondence:**
Vincent Fourcassié, Centre de Recherches sur la Cognition Animale, Université de Toulouse, Université Paul Sabatier, 118 route de Narbonne, F-31062 Toulouse Cedex 9, France.
e-mail: vincent.fourcassie@univ-tlse3.fr

In many species of group living invertebrates, in particular arthropods, collective decisions can emerge from the combined actions of individuals and the direct or indirect interactions between individuals. These decisions allow groups of individuals to respond quickly and accurately to changes that occur in their environment. Examples of such decisions are found in a variety of invertebrate taxa and in many different contexts, e.g., exploring a new territory, foraging for food, finding a suitable location where to aggregate or to establish a nest, defending oneself against predators, etc. In this paper we review the collective decisions that have been documented in different invertebrate taxa where individuals are known to live temporarily or permanently in social or gregarious groups. We first present some simple examples of collective decisions involving the choice between two alternatives. We then define the fundamental rules required for these collective decisions to emerge throughout the invertebrate taxon, from simple organisms such as caterpillars, to animals endowed with highly developed perceptive and cognitive capacities such as ants and bees. The presentation of these rules gives us the opportunity to illustrate one of the pitfalls of the study of collective choice in animals by showing through computer simulations how a choice between two alternatives can be misinterpreted as the result of the action of self-organized mechanisms. In the second part, we discuss the peculiarities of collective decisions in invertebrates, their properties, and characteristics. We conclude by discussing the issue of individual complexity in collective decision-making process.

Keywords: collective decision, emergence, insect, invertebrate, non linearity, self organi ation, social interactions

HISTORICAL BACKGROUND

Ever since the first description of their behavior, from the classic naturalist literature to that of the mid twentieth century, invertebrates have been considered as simple organisms, endowed with limited cognitive capabilities. For example, despite detailed observations and clever experimental tests proving the contrary, the French entomologist Fabre (2000) clung to the belief that insects were unable to learn and were moved solely by instinct. Early behaviorists placed most invertebrates (with cephalopods as a notable exception) at the lower rungs of the ladder of animal intelligence and for a long time invertebrates were considered as the ideal model organisms for the study of behavioral reflex systems (Loeb, 1901; Kühn, 1919; Kandel, 2001). Yet, in the past 70 years, there has been a wealth of studies demonstrating that invertebrates, and particularly insects, are endowed with cognitive capabilities of the same level, or even superior, to those of many vertebrates. The impetus for these studies was certainly given by Karl von Frisch with his work on honeybees. The discovery that honeybees are able to learn flower locations, odors, colors, shape, and to communicate in an abstract way with their nestmates (Frisch, 1967) was the start of a huge research effort that allowed to unravel and to fully appreciate the complexity of the cognitive mechanisms underlying decision-making processes in bees (reviews by Menzel and Giurfa, 2001, 2006; Giurfa, 2007) and other insects (review by Menzel et al., 2007; Dornhaus and Franks, 2008; Wehner, 2009).

In many invertebrate species however, individuals do not live in isolation but form groups whose degree of sociality can be extremely variable, going from simple seasonal gathering of individuals at a favorable location (Waldbauer, 2001) to the highest form of sociality (Wilson, 1971; Hölldobler and Wilson, 1990, 2008; Costa, 2006; Seeley, 2010). Social insects in particular are made famous by their collective behaviors, as opposed to their individual performances. These collective performances are expressed in a variety of contexts, e.g., nest construction and maintenance, colony emigration, foraging, colony defense, and division of labor. For many years, scientists have sought to identify the "ghost in the machine" that gives rise to these collective performances but the explanations they provided were more of a poetic than of a scientific nature. The research that came later showed that collective performances of social insects were neither explained by a single all-powerful individual (termed "queen" by Réaumur) giving orders to her vassals, nor to a "spirit" (Maeterlinck, 1902) or a "soul" (Marais, 2009). At the beginning of the twentieth century, the great American myrmecologist Wheeler (1911) coined the term super organism to describe the structure and function of social insect societies. However, although appealing to biologists, this notion did little to further the understanding of the

mechanisms allowing the transition from individual to collective performances in these insects. Wheeler (1928) later introduced the important notion of emergence in his book *The social insects: their origin and evolution*. He noted that "social insect colonies as a whole are not equivalent to the sum of their individuals but represent a different, emergent level," Fifty years later, in the early 1980s, Jean-Louis Deneubourg, a student of Ilya Prigogine at the Free University of Brussels, was one of the first to give a reappraisal of Wheeler's notion of emergence in social insects by including it within the framework of self-organization theory. Together with biologist Jacques Pasteels, they showed through computer simulations and mathematical models how social insect societies could be considered as complex systems in which the transition from the lower level components (individual workers) to the higher level component (colony) could be explained by self-organized mechanisms based on the use of simple rules by individuals relying solely on local information, and on the direct or indirect interactions among these individuals (Deneubourg et al., 1986; Pasteels and Deneubourg, 1987). So far this approach has proved to be extremely successful in accounting for a variety of behaviors observed at the collective level in social insects (review by Detrain and Deneubourg, 2006, 2008), as well as in other invertebrates (**Table 1**).

DEFINITION AND EXAMPLES OF COLLECTIVE DECISIONS IN INVERTEBRATES

DEFINITION OF COLLECTIVE DECISIONS

Self-organization allows a group of animals to make consensus decisions, i.e., to make a choice between two or more mutually exclusive alternatives without losing the cohesion of the group (Conradt and Roper, 2005; Sumpter and Pratt, 2009). Consensus decisions contrast with combined decisions in which individuals are influenced by each other but do not aim at reaching a unique decision. For the sake of convenience, we will solely be employing the term collective decisions from here on in. In some instances, the choice the group must make is critical for its survival, as when a swarm of honeybees chooses a cavity in which to settle (Seeley, 2010) or when a colony of house-hunting ants chooses a location in which to install its new nest (Franks et al., 2002). In most cases, although each member of the group only has access to partial information, and thus is unable to compare among the different alternatives offered, the properties of the mechanisms underlying these collective decisions are such that the best choice is made by the group, i.e., the choice of the highest quality food source (Beckers et al., 1990), the shortest path (Goss et al., 1989), or the best location at which to aggregate or settle a colony (Canonge et al., 2011). This has led to the notion of "swarm intelligence" (Bonabeau et al., 1999; Garnier et al., 2007; Blum and Merkle, 2010) and has been a source of inspiration for scientists working in other disciplines than biology, opening in particular new avenues of research in computer science (Ant Colony Optimization Algorithms: review by Blum, 2005) and robotics (swarm robotics: Pfeifer et al., 2007; Trianni, 2010).

A common misconception about collective decision-making is that it necessarily implies some sort of consultation among individuals within the group, the weighing of each other's opinion, and the sharing of all the information available about all possible choices until a consensus is reached and all the members of the group adhere to a single decision. Although this type of collective decision can be found in non-human vertebrates (Conradt and Roper, 2005; Conradt and List, 2009), it is relatively rare in animals, particularly in invertebrates and, when present, always involves the intervention of self-organized mechanisms in the form of positive or negative feedbacks (Sumpter and Pratt, 2009). The reason for this lies in the fact that the species of invertebrates in which collective decisions arise generally form large groups and/or are distributed over a large and discontinuous space, e.g., nest chambers, which impedes communication between group members. It is worth noting that all individuals in the group have the same weight in the final decision, independent of the presence of one or several informed leaders in the group. By employing the word leaders we do not mean that the members of the group make allegiances to particular individuals; when informed leaders are present, their leadership character only lies in the fact that they possess more information than other members of the group at the start of the process and that they initiate the decision-making process. For example, consider the case of food recruitment in ants. When a scout ant has found a food source that it judges worth exploiting, it returns to the nest and simultaneously lays a pheromone trail. Once the scout has arrived in the nest, its nestmates are alerted either by the odor of the trail and/or by specific motor displays of the scout. In mass recruitment the action of the scout stops at this stage and recruited workers follow the trail until reaching the food source, while in group recruitment (de Biseau et al., 1994; Cerdá et al., 2009; Collignon and Detrain, 2010) recruited workers need to be guided by the scout. In both cases however, when several food sources are advertised at the same time, scouts have the same weight than other workers in the final decision as to which source is exploited. This also holds true for nest emigration in bees (Seeley and Visscher, 2004; Visscher, 2007; Sumpter and Pratt, 2009) and house-hunting ants (Franks et al., 2002; Sumpter and Pratt, 2009), at least as long as some scouts do not have prior knowledge of potential nest locations before the initiation of the emigration process. In the latter case, knowledgeable scouts can be disproportionally influential in the final decision (Stroeymeyt et al., 2011a).

EXAMPLES OF COLLECTIVE DECISIONS IN INVERTEBRATES

When a group of animals is offered a choice between two identical options, it randomly selects one alternative, which can lead over many replicates to a U-shaped distribution of choices. The emergence of such asymmetrical distributions in a uniform environment is a characteristic of collective decisions (Pasteels et al., 1987; Deneubourg and Goss, 1989; Camazine et al., 2001) and has been reported across various behavioral contexts and taxa (**Table 1**). In the following we give some examples of asymmetrical distributions observed in binary choice experiments in invertebrates.

Figure 1A illustrates the collective behavior of ants in a panic situation. After being introduced in a circular arena with two similar exits, ants preferentially use a single door when a panic is induced by the addition of a strong repellent (**Figure 1A**). **Figure 1B** shows the collective defensive behavior of honeybees. When faced with two similar lures at the entrance of their hive,

able 1 | Collective decisions arising by self organi ation mechanisms in binary choice experiments in invertebrates.

Collective behavior studied	axon	pecies	ype of information	Identical options	Different options	Reference
Choice of a shelter or of an aggregation site	Cockroaches	*Periplaneta* sp.	Tactile/chemical	Y	Y	Halloy et al. (2007), Sempo et al. (2009), Canonge et al. (2011), Leoncini and Rivault (2005), Said et al. (2005)
		Blattella germanica	Tactile/chemical	Y	Y	Ame et al. (2006), Rivault and Cloarec (1998), Ame et al. (2004, 2006), Jeanson and Deneubourg (2007)
	Isopods	*Porcellio scaber*	Tactile/chemical?	Y	Y	Devigne et al. (2011), Broly et al. (2012)
	Ants	*Messor barbarus*	Tactile/chemical	Y	Y	Jeanson et al. (2004a)
Choice of a new nest site	Ants	*Temnothorax* sp.	Tactile	N	Y	E.g., Pratt et al. (2002), Franks et al. (2002, 2003b, 2006)
		Monomorium pharaonis	Chemical	N	Y	Evison et al. (2012)
	Honeybees	*Apis mellifera*	Tactile/visual	Y	Y	Visscher (2007), Seeley et al. (2012), Seeley (2010)
Choice of a path during migration	Spiders	*Larinioides cornutus*	Silk (tactile/chemical)	Y	NA	Jeanson et al. (2004b)
		Anelosimus eximius	Silk (tactile/chemical)	Y	NA	Mailleux et al. (2008), Saffre et al. (1999)
Choice of a path during foraging	Annelids	*Eisenia fetida*	Tactile	Y	NA	Zirbes et al. (2010)
	Mites	*Dermatophagoides pteronyssinus, Tetranychus urticae*	Silk (tactile/chemical)	Y	Y	Yano (2008), Mailleux et al. (2010)
	Cockroaches	*Blattella germanica*	Chemical	Y	Y	Jeanson and Deneubourg (2006)
	Ants	*Linepithema humile*	Chemical	Y	Y	Goss et al. (1989), Vittori et al. (2006), Garnier et al. (2009)
		Lasius niger	Chemical	Y	Y	Beckers et al. (1992), Detrain et al. (2001), Dussutour et al. (2004, 2005, 2006)
		Oecophylla longinoda	Tactile	Y	NA	Lioni and Deneubourg (2004), Lioni et al. (2001)
Choice of a food source during foraging	Lepidopterans	*Malacosoma disstria*	Silk (tactile/chemical)	Y	Y	Dussutour et al. (2007), Dussutour et al. (2008)
	Cockroaches	*Malacosoma americanum*	Silk (tactile/chemical)	Y	Y	Fitzgerald (1995)
	Ants	*Blattella germanica*	Chemical	Y	NA	Lihoreau et al. (2010)
		Lasius niger	Chemical	Y	Y	Pasteels et al. (1987), Beckers et al. (1990, 1993), Portha et al. (2002)
		Myrmica sabuleti	Chemical	NA	Y	de Biseau et al. (1991)
		Monomorium pharaonis	Chemical	Y	Y	Sumpter and Beekman (2003)
		Pheidole megacephala	Chemical	Y	Y	Dussutour et al. (2009a)
		Tetramorium caespitum	Chemical/tactile	Y	Y	Collignon and Detrain (2010)
	Bees	*Apis mellifera*	Tactile	NA	Y	Seeley et al. (1991), Seeley (1995)
		Trigona recursa	Chemical	NA	Y	Schmidt et al. (2006)
		Melipona fasciata	?	NA	Y	Biesmeijer and Ermers (1999)
Choice of a path during exploration	Ants	*Linepithema humile*	Chemical	Y	NA	Deneubourg et al. (1990)
		Lasius niger	Chemical	Y	NA	Devigne and Detrain (2002)
		Pheidole megacephala	Chemical	Y	NA	Dussutour et al. (2009b)
Choice of two targets during colony defense	Bees	*Apis mellifera*	Visual/chemical	Y	NA	Millor et al. (1999)
Choice of two exits in a panic situation	Ants	*Atta insularis*	?	Y	NA	Altshuler et al. (2005)

The type of choice and the behavioral context in which it occurs is indicated in the first column. The second and third column gives the taxon and the species in which the collective decision has been observed, respectively. The fourth column gives the nature of the information used by animals. ? means that no information has yet been provided on the type of information used. The fifth and sixth columns indicates whether experiments with choices between identical or different options have been performed (Y= choice observed, N= no choice observed, NA= experiments not performed or not reported).

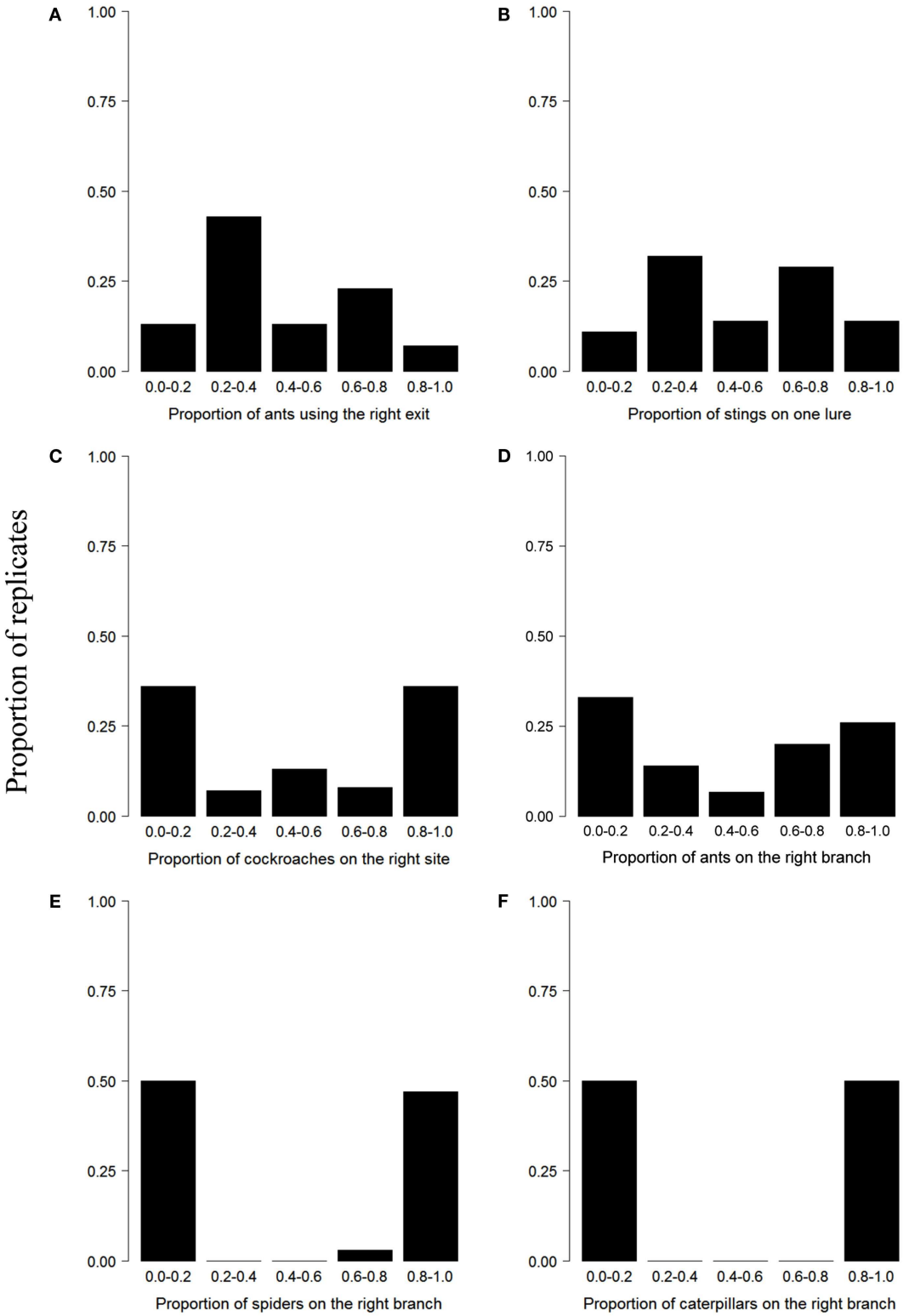

FIGURE 1 | Examples of U shaped choice distributions across different behavioral contexts and taxa. In all experiments, groups were faced with two identical options. **(A)** Selection of an exit during panic in the ant *Atta insularis* (30 replicates), adapted from Altshuler et al. (2005), **(B)** selection of a target by attacking honeybees *Apis mellifera* (31 replicates), from Millor et al. (1999), **(C)** selection of a shelter in the cockroach *Blattella germanica* (49 replicates), from Ame et al. (2004), **(D)** selection of a branch of a diamond-shape bridge in the ant *Lasius niger* (15 replicates), from Dussutour et al. (2005), **(E)** selection of an aggregation site in the spider *Larinioides cornutus* (30 replicates), from Jeanson et al. (2004b), **(F)** selection of a food source in the caterpillar *Malacosoma disstria* (20 replicates), from Dussutour et al. (2008). Published data or data provided by the authors were used to plot the histograms.

most bees focus their attack on one single target after their colony has been heavily disturbed (**Figure 1B**). The explanation lies in the fact that the barbed stingers left in place by honeybees emit an alarm pheromone that is attractive to nearby individuals. Initial fluctuations in the number of stings induce a small difference in the attractiveness of the targets that is amplified as the number of stings increased. Although bees preferentially focus their attacks on a single target, a second target can also be attacked (**Figure 1B**). This phenomenon is in part explained by the fact that some experiments are characterized by a low level of attacks, which prevents the initiation of an amplification process (Millor et al., 1999). **Figure 1C** represents the collective selection of a refuge by nymphs of the cockroach *Blattella germanica*. In presence of two identical shelters, groups of nymphs aggregate mostly at one site (Ame et al., 2004). In this case, amplification is mediated by a modulation of the individual resting time: the probability of leaving a shelter decreases with the number of conspecifics already present at the shelter. In the ant *Lasius niger*, foragers mostly use a single branch of a diamond-shaped bridge giving access to a food source from their nest (**Figure 1D**). This collective choice emerges from the trail-laying and trail-following behavior of the foraging workers. Small initial fluctuations of the relative concentration of pheromone on each branch are amplified by the successive passages of ants that eventually lead to the selection of a unique path. During migration, spiders lay down silk draglines that are attached discretly to the substrate. When they are given access to a bifurcated escape route, this pattern of silk attachment creates silk shortcuts that are followed by conspecifics (**Figure 1E**). In this system, the multiplication of silk strands laid by previous individuals serves as an amplification mechanism. When groups of tent caterpillars of the genus *Malacosoma* are offered a binary choice between two similar food sources, they massively exploit one resource and disregard the other (**Figure 1F**). During their displacement, caterpillars lay down silk threads impregnated with pheromones. The first caterpillar leaving a bivouac chooses a direction at random and the conspecifics that follow then reinforce the trail laid by the first individual (Dussutour et al., 2007). The similarity of the choice distributions observed for spiders and social caterpillars suggests that silk has very strong amplifying properties and that the initial activity of a few individuals is sufficient to give rise to clear-cut decisions. In these examples, the asymmetry of choices varies and depends notably on the strength of the underlying amplification mechanisms. In the following section, we will emphasize the contribution of feedbacks, the type of interactions, and noise in the emergence of collective decisions.

REQUIREMENTS OF COLLECTIVE DECISIONS

POSITIVE AND NEGATIVE FEEDBACKS

The selection of a single option out of many alternative ones relies on the implementation of positive feedbacks or quorum response in which the probability of an individual of exhibiting a behavior is a non-linear function of the number of individuals already engaged in this behavior (Sumpter and Pratt, 2009). These feedbacks arise through a multitude of direct or indirect interactions among individuals and lead to amplification of random fluctuations (DeAngelis et al., 1986; Thomas, 1998; Jeanson and Deneubourg, 2009). Their contribution is critical for the expression of a clear-cut choice and the maintenance of the social cohesion of the group. They can be launched by the combination of positive interactions so that the change in the direction of the initial deviation is reinforced (**Figure 2**). For instance, the presence of a pheromonal trail increases the probability that an individual follows a path and reinforces it. Positive feedbacks can also arise from the combination of an even number of negative interactions. Hence the probability of leaving a group can decrease with group size and, consequently, favor large group formation. The action of positive feedbacks is generally counterbalanced by the existence of negative feedbacks that participate to the stabilization of emerging collective patterns (Camazine et al., 2001). For instance, crowding under a shelter in cockroaches or at a food source in ants, the exhaustion of a food source, and the existence of a limited number of foragers in a colony all constitute negative feedbacks. The emission of specific signals can also counteract positive feedbacks (Robinson et al., 2005). In honeybees for example, foragers experiencing attacks at a food source produce stop signals which causes the cessation of the waggle dances and thus decreases the recruitment to the food source under attack (Nieh, 2010). The same kind of signals are used during swarming by nest site scouts in order to inhibit other scouts from dancing and advertising other sites than their own. A mathematical model shows that this cross-inhibition between population of scouts advertising for different sites actually allows colonies to avoid potential deadlocks when they have to choose between two sites of equal quality (Seeley et al., 2012).

TYPE OF INTERACTIONS INVOLVED IN COLLECTIVE DECISIONS AND THEIR IMPACT

In order for positive feedbacks to function in social groups, it requires individuals to modulate their behaviors in response to interactions with conspecifics. In other words, the probability of an individual adopting a specific behavior depends on its ability to assess the number of conspecifics already engaged in that behavior or to detect traces of their earlier activities. This implies a direct or indirect exchange of information between group members.

Indirect interactions involve the perception of some trace of the earlier activities of conspecifics. Pheromonal trails in ants and silk strands in caterpillars are good examples of indirect interactions whose efficiency for the emergence of collective decisions depends upon their longevity in the environment. The latter can be strongly affected by abiotic factors and the physical structure of the environment. For instance, the persistence of trail pheromones strongly depends on ambient temperature or on the nature of the substrate on which they are deposited (Jeanson et al., 2003). In the ant

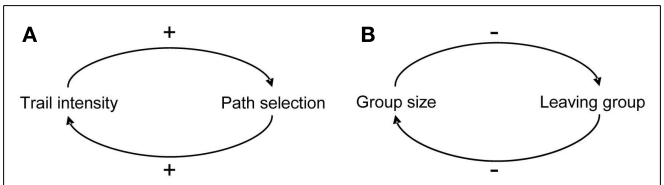

FIGURE 2 | ositive feedback loops in (A) the selection of one of two paths in ants (B) the selection of an aggregation site in cockroaches, + and − signs represent positive and negative influence respectively, from eanson and Deneubourg (2009) .

L. niger, foragers usually select one branch of a diamond-shaped bridge connecting their nest to a foraging area (**Figure 1D**). However, in presence of two branches varying in their physico-chemical properties (quality of the paper covering each branch) ants preferentially follow the branch covered by the lighter paper (Detrain et al., 2001). The difference in the physico-chemical characteristics of the two substrates influences the accessibility of the pheromone trail to foragers and, through amplifying processes, leads to the choice of one branch over another. Therefore the characteristics of the environment can alter the outcome of collective decisions without altering individual behaviors.

On the other hand, direct interactions require the simultaneous presence of individuals. The best example of direct interactions in collective decision-making is given by the process called tandem-running that occurs at one stage of nest emigration in the ant *Temnothorax albipennis*. During tandem-running a single informed worker that knows of the location of a suitable new nest guides its nestmates to this location. The act of guiding is made through a tactile contact between the leader and the follower that keeps antennating the abdomen of the ant in front of him (Richardson et al., 2007). Tactile information also allows emigrating ants to assess the number of ants in a new nest and thus to judge if a quorum has been reached which determines the decision to stay in a candidate nest (Pratt, 2005). Direct interactions can also be mediated by odor as in cockroaches in which group formation relies on the perception of cuticular hydrocarbons (Rivault and Cloarec, 1998; Said et al., 2005). The combination of direct and indirect interactions, which are not mutually exclusive, can further enhance amplification giving rise to collective decisions.

NOISE

In non-linear systems, fluctuations at the individual level, even small ones, can lead to profound changes at the collective level, highlighting the fact that noise and stochasticity are intrinsic to any collective decision (Detrain and Deneubourg, 2008). For instance, in mass-recruiting ants, a well-known source of fluctuation or "noise" is related to the ability of foragers to faithfully follow a chemical trail. Recruited workers may lose the trail they follow or make "wrong" choices at trail junctions. When several food sources of identical quality are concurrently available in the nest surroundings, any slightly unbalanced distribution of workers and/or amount of trail marks over the different foraging paths leading to the food sources can lead the whole colony to select only one resource. The choice of one foraging path or one food source is therefore probabilistic and unpredictable. This has lead Deneubourg et al. (1983) to argue that noise or errors could be adaptive in the sense that they could offer ants the opportunity to discover a better alternative, e.g., a higher-quality food source or a shorter path. Therefore an optimal error level could exist which could minimize the time needed to discover better food sources and maximize foraging efficiency. It should be noted that noise may be induced by a large variety of sources in collective decision-making processes. Generally, the behavior of individuals never conforms exactly to the statistical average, rather, it exhibits variations over time, both within and between individuals. For example, both the amount of pheromone deposited per trip and the recruitment threshold, i.e., the amount of pheromone required to elicit the recruitment of a worker, may differ between individuals (Mailleux et al., 2000, 2003, 2005). Unlike "lost foragers," such fluctuations do not directly favor the discovery of alternative sources. Instead, they simply introduce a small amount of variability (noise) into the decision-making process. Such undirected noise can be sufficient for the system to behave adaptively by facilitating quick transitions to more advantageous solutions in changing environments (Dussutour et al., 2009a). Similarly, in house-hunting ants, noise in the acceptability threshold of searching ants or in nest quality assessment by scouts during the selection of a new nest site allows flexibility and efficient decision (Marshall et al., 2006; Robinson et al., 2009).

The elucidation of the dynamics of collective decisions requires the characterization of the link between both the individual and collective levels. Modeling is a relevant approach because it allows to test whether the mechanisms that are predicted to act at the lower scale level (individuals) are able to generate the phenomenon observed at the level just above (group or colony; Camazine et al., 2001; Sumpter and Pratt, 2003). Models also aid in making predictions for conditions that are difficult to reproduce experimentally, e.g., large colony size in social insects or decision-making processes extending over a long time period. In the following section, we employ the modeling approach to emphasize the need to achieve experiments in which the group faces two identical alternatives.

MODELING COLLECTIVE DECISIONS

In order to illustrate the utility and benefit of the modeling approach in the study of collective decisions, we will employ a simple model developed in the context of shelter selection in cockroaches. An experimental group of first-instar *B. germanica* larvae were offered the choice between two identical resting sites (Ame et al., 2004). The empirical results indicated that cockroaches aggregated mostly at a single site after 24 h (**Figure 1C**). The authors identified a single behavioral rule that is sufficient to account for the choice pattern they observed: the individual probability of leaving a shelter decreases as the population in the shelter increases. This established, they went on to propose a straightforward mathematical model demonstrating that a collective decision can arise through this simple modulation. Formally, the individual probability Q_1 of leaving shelter 1 as a function of the number of individuals X_1 under shelter 1 is defined by:

$$Q_1 = \frac{\sigma}{k_1 + X_1^{\eta}} \quad (1)$$

with $\sigma = 0.06$, $k_1 = 6$, and $\eta = 2$. For values of $\eta > 0$, this function indicates that the probability of leaving a site decreases with the number of conspecifics X_1 already at the site. The parameter η controls the steepness of the response to conspecifics, i.e., the degree of non-linearity: the higher the value of η, the greater the influence of conspecifics on the individual decision to move. The constant k_1 represents the intrinsic attractiveness of shelter 1: the higher the value of k_1, the lower the probability of leaving shelter 1.

Using Monte Carlo simulations, we explore how variations in the values of η and k in Eq. 1 can influence the spatial distribution of individuals between shelters. In our simulations the values of η range between 0 and 2 and for $\eta = 0$, the individual decision to move does not depend on the presence of conspecifics,

i.e., individuals behave as if they were alone. We also compare the collective patterns obtained by the simulations when groups of individuals are facing two identical ($k_1 = k_2$) or two different shelters ($k_1 = 10k_2$, i.e., the individual preference for shelter 1 is ten times greater than for shelter 2). At the beginning of each simulation run, individuals are randomly distributed between shelters. Then, at each time step, the individual probability of changing sites is determined by Eq. 1. An individual moves from one shelter to the other if a number drawn randomly between 0 and 1 is inferior or equal to Q_1, otherwise it stays on its site. The individual probabilities Q_1 and Q_2 are updated at each time step as a function of the number of individuals X_1 and X_2 at each site. A thousand simulation runs with groups of 26 individuals are performed for each condition (time step: 1 s, simulation duration: 12 h). Each shelter can accommodate all individuals and cockroaches move immediately from one shelter to the other. The results are reported on **Figure 3**.

For $k_1 = 10k_2$ and values of η ranging between 0 and 2, the spatial distributions of individuals are qualitatively similar: the proportion of simulations as a function of the number of individuals at site 1 is highly right-skewed, i.e., most individuals aggregate at site 1. If one were looking at the spatial distribution of cockroaches without *a priori* knowledge about the underlying rules, one could erroneously conclude that a collective decision has arisen in all situations. However, our simulations show that the summation of the individual preferences for environmental heterogeneities (i.e., $k_1 = 10k_2$, $\eta = 0$) is sufficient to produce an asymmetrical distribution of cockroaches at the two sites, without the need to invoke the contribution of amplification processes.

Now, consider the situations where $k_1 = k_2$. For $\eta = 0$, individuals are evenly distributed between both sites, i.e., 50% of the population on average is found at each site. For $\eta = 1$, the individual decision to move depends on the presence of conspecifics but the strength of amplification is too weak to induce the collective selection of a single shelter. For $\eta = 2$ however, a dramatic change occurs: individuals strongly aggregate at a single site. The asymmetrical distribution observed provides strong evidence that a collective choice arose through interattraction and the implementation of positive feedback loops. A rigorous quantification of individual behaviors would then be required to identify the fundamental rules supporting amplification loops and driving the emergence of collective choice.

This model is a good illustration of the fact that binary choice experiments between two different alternatives are unable to provide insights into the mechanisms underlying a collective choice. In fact, without performing the crucial test where animals are given the choice between two strictly identical options it is impossible to know whether the asymmetric distribution of choice observed in tests with two different alternatives arises from social interactions between group members amplified by positive feedbacks or from the addition of individual responses to environmental heterogeneities. Only after having performed a test with two equal alternatives can one achieve experiments in which the group faces resources of different quality to disentangle the relative contribution of social interactions and individual responses in the collective decision observed.

PECULIARITIES, PROPERTIES, AND CHARACTERISTICS OF COLLECTIVE DECISION

INFLUENCE OF GROUP SIZE

In collective decision, the intensity of amplification processes, and thereby the degree of choice asymmetry, strongly depends on the number of individuals (or interactions) involved. For instance, small colonies of the ant *Monomorium pharaonis* cannot form efficient foraging trails because they do not generate high enough traffic to compensate for the evaporation of the trail pheromone (Beekman et al., 2001). Likewise, although ants offered a choice between two identical paths between their nest and a food source generally follow only one path (Beckers et al., 1992; Sumpter and Beekman, 2003; Dussutour et al., 2009b), models, and experiments show that when the flow of ants exiting the nest is too low the system is characterized by a unique unstable equilibrium in which both paths are used more or less equally. When the flow of ants exiting the nest increases a bifurcation occurs and the system reaches a stable equilibrium, with most ants using either the first or the second path. On the other hand, in the house-hunting ants *T. albipennis*, a collective decision can be reached even in small size colonies because of the peculiar mechanism used by ants when deciding to commit to a new nest. The workers will stay in a new nest only if a certain number of individuals, i.e., a quorum, have settled in that nest. This quorum however is not an absolute number but depends on the size of the colony so that a decision can be reached even in colonies containing less than 50 workers (the largest colonies of *T. albipennis* can contain more than 400 individuals; Dornhaus and Franks, 2006).

The influence of group size on collective decision is also illustrated by the experiments in which a group of cockroaches were made to choose between two food sources (Lihoreau et al., 2010) or two shelters (Ame et al., 2004). Whereas groups of 50 individual cockroaches exploit both food sources equally (50% individuals forage on each source), an asymmetry emerges in groups of 200 individuals, with the majority of individuals feeding on one of the food source only (**Figure 4**). From an experimental perspective, it is worth nothing that an absence of asymmetry in the exploitation of several resources does not necessarily imply that a group of animals is unable to achieve collective decisions; it could be simply explained by the fact that the conditions (e.g., critical group size) for them to emerge are not met.

It should be noted that the effect of group size on collective decisions strongly depends on the behavioral context in which they are expressed. In cockroaches, retention effects have been identified as responsible for both the selection of a shelter or the exploitation of a food source. Specifically, the average duration of feeding bouts increases with the number of individuals feeding at a food source (Lihoreau et al., 2010) and sheltering time increases with the number of individuals already present (Ame et al., 2004). In both situations, the probability of leaving a resource decreases with the number of individuals already present on it. However, the critical group size for the emergence of an asymmetrical choice is different in the two situations: although groups of 20 cockroaches are able to achieve a collective decision and select a single aggregation site, groups of 50 cockroaches are unable to choose between two equivalent food sources (**Figures 1C** and **4**).

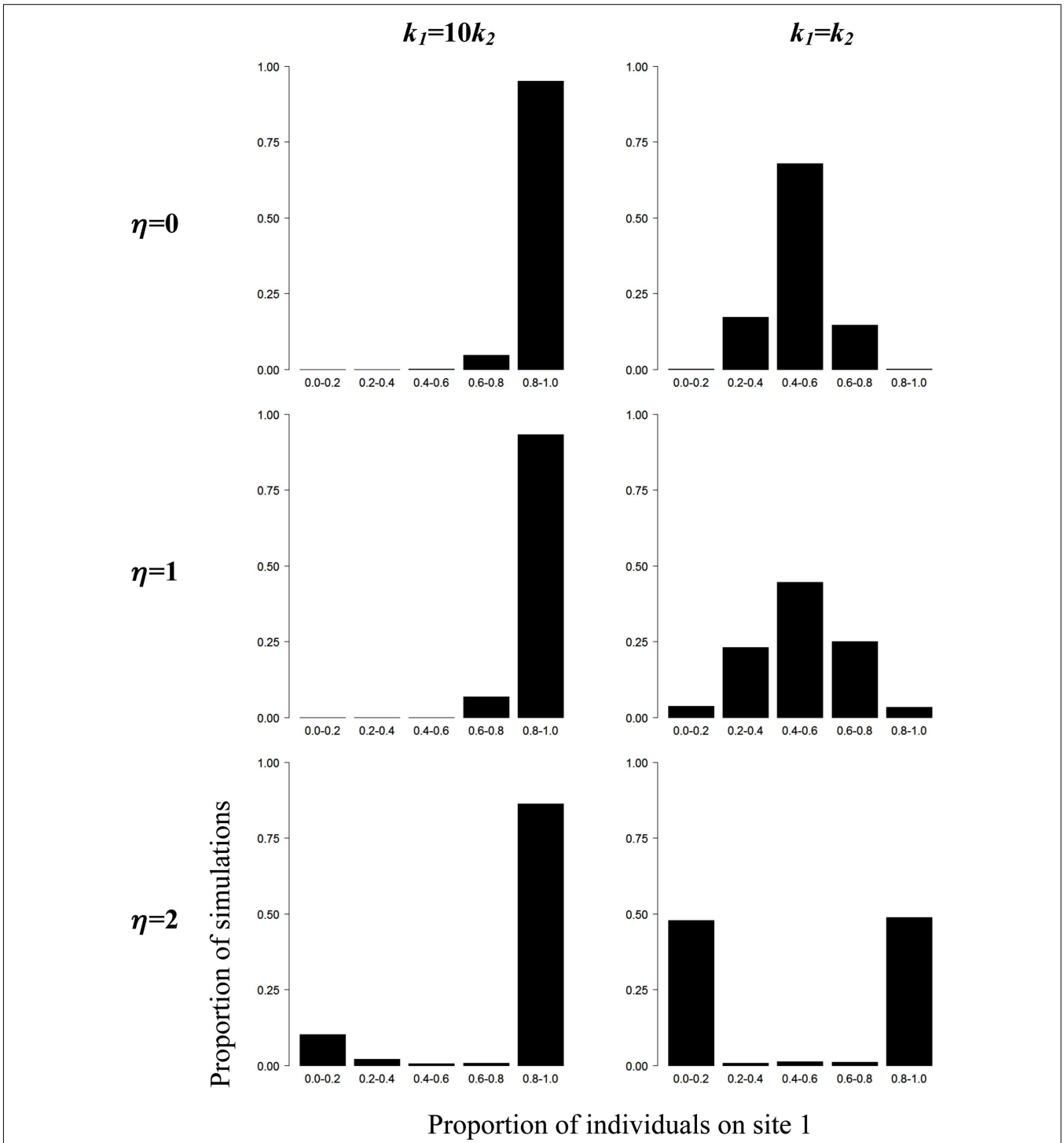

FIGURE 3 | imulations of collective aggregation behavior at one of two sites in the cockroach. Proportion of simulations ($n = 1000$) as a function of the proportion of individuals ($N = 26$) on shelter 1 in presence of two different ($k_1 = 10k_2$) or two identical ($k_1 = k_2$) sites and for different values of η (see text for details).

ACCURACY

In decision-making, speed and accuracy are often in opposition. Much time may be required to make an accurate decision between alternatives, because gathering, processing, and evaluating information may be a lengthy process. If an animal has to make a swift decision it may therefore be less discriminating. This link between speed and accuracy is so widespread that it has been termed the speed–accuracy trade-off paradigm

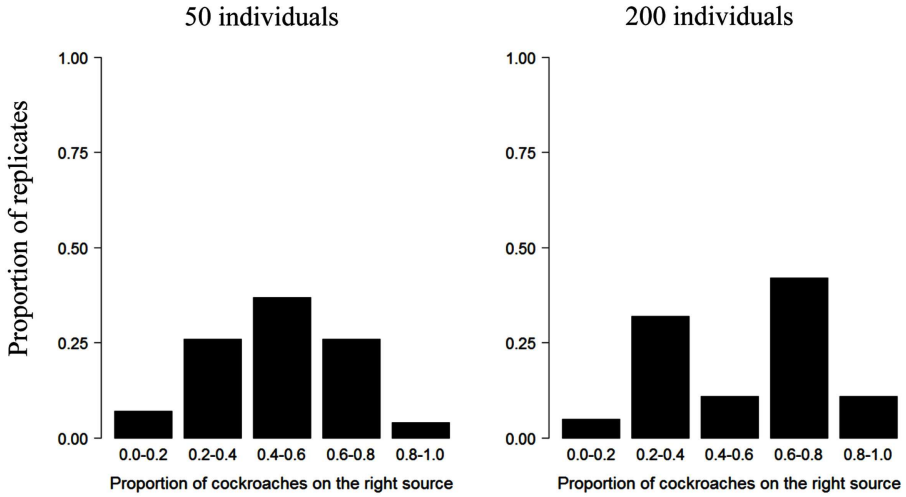

FIGURE 4 | Collective choice of one of two identical food sources in the cockroach *Blattella germanica*. Proportion of replicates of the experiment as a function of the number of cockroaches feeding at one of the two sources from Lihoreau et al. (2010), Lihoreau, Deneubourg, and Rivault (pers. com.). The asymmetry in the exploitation of the food sources is more pronounced in larger groups.

(Busemeyer and Townsend, 1993; Osman et al., 2000; Franks et al., 2003a; Sumpter and Pratt, 2009; review by Chittka et al., 2009).

Although choosing collectively can lead to non-adaptive decisions in some notable exceptions (see Beckers et al., 1990 for the choice of a food source in foraging ants and Stroeymeyt et al., 2011b for the choice of a nest in house-hunting ants), on average it allows greater accuracy than do completely independent choice or weak responses to the behavior of conspecifics. The accuracy of groups of individuals in decision-making is typically predicted to be greater than that of isolated individuals; it initially increases with group size before leveling off (Krause et al., 2010). This phenomenon is driven by the fact that larger groups of individuals are more effective at gathering information than smaller groups or than solitary individuals, whereas the integration of the information gathered by different group members allows more accurate decisions to be made by larger groups (Couzin, 2009). Therefore collective decisions allow effective averaging of information without the need of complex comparison between options (Robinson et al., 2009).

The gathering of information prior to making a collective decision can be a way to circumvent the speed–accuracy trade-off. Hence in house-hunting ant *T. albipennis* colonies can gather and store information about available nest sites well before emigration, while their nest is still intact. This information is later retrieved and used during emigration and allows to improve simultaneously both speed and accuracy in the choice of candidate nests (Stroeymeyt et al., 2010).

Interestingly, steep threshold responses can sometimes amplify random fluctuations and lead to mass adoption of incorrect choices. This may lead animals in groups to make decisions that they would not make alone. In forest tent caterpillars for instance, isolated individuals show a high preference for a nutritionally balanced food source when offered the choice between a nutritionally balanced and a nutritionally unbalanced food source (Dussutour et al., 2007). In contrast, groups of caterpillars randomly choose one resource and are trapped at the first resource discovered. This results from an excessively strong and rapid amplification due to the silk laid down by caterpillars during foraging. While such amplification allows the maintenance of cohesion between group members, it prevents the group from achieving optimal diet choices.

ROBUSTNESS

Because distributed coordination does not depend on a specific subset of individuals, groups are inherently robust to perturbation (Camazine et al., 2001). Failure of one or several individuals usually does not put the group at risk. If an individual fails to carry out its task, another one promptly replaces it. Ants provide good examples of such robustness. Hence, in mass recruitment the removal of a scout ant does not affect recruitment since recruited workers "interact" mainly with the trail that has been laid by the scout and thus are kept continuously informed about the food location. Conversely, for decision based on direct interaction such as group recruitment, removing one individual from the population early in the decision process could have an important impact on the decision outcome because the mere presence of individuals is required to initiate the process. Worker interaction rates have been demonstrated to be robust to changes in group size or density (Pacala et al., 1996). Hitherto however, the robustness of collective decision in invertebrates has mainly been investigated through models (see e.g., Marshall et al., 2009).

FLEXIBILITY

It is currently agreed upon that collective decisions lack flexibility, i.e., that many species in which collective decisions are observed are unable to adapt to dynamic environments, such as switching to exploiting a newly discovered high-quality food source when the foraging effort of the colony is already concentrated on a food source of lesser value (Beckers et al., 1990). The apparent inability of a group of animals to adapt to changing conditions is

supported by laboratory experiments (Goss et al., 1989; Beckers et al., 1992; Traniello and Robson, 1995) and mathematical models (Goss et al., 1989; Nicolis and Deneubourg, 1999; Camazine et al., 2001). For example, in ants, pheromone trails allow a rapid collective choice for one alternative, but they also impose constraints on the overall foraging efficiency. Some ant species however are able to circumvent these constraints because of the properties of the trail pheromone they use. In particular, the decay rate of these pheromones plays an important role in the flexibility of collective foraging decisions: short-lived, volatile trails are more suited to the recruitment to ephemeral food sources because they can be rapidly modulated, whereas long-lived trails are more suited to the recruitment to persistent, or recurrent, food sources. When foraging in their natural environments, species of ant using a single pheromone trail experience a trade-off between efficient recruitment and flexibility in their response to the changes in the environment. For example, Goss et al. (1989) first provided Argentine ants (*Linepithema humile*) with a long path between their nest and a food source and after some time introduced a second, shorter path. When the short path was added after the ants had established their trail on the long path, the majority of ants continued to travel on the long path. Similar results have been reported with *L. niger* (Beckers et al., 1992). Mathematical models predict that ants will remain on an established trail for periods longer than the evaporation rate of the pheromone because ants continue to reinforce the trail on the long path (Goss et al., 1989; Nicolis and Deneubourg, 1999; Sumpter and Pratt, 2003). Pheromone trails can thus result in ants becoming "trapped" in suboptimal solutions.

That being said, there are ways to escape the deadlocks of suboptimal solutions however. For example, theoretical models on food recruitment usually consider that ants use just one single trail pheromone (e.g., Pasteels et al., 1987; Nicolis and Deneubourg, 1999); in practice however, many species of ants use a variety of pheromones to mark the path to food discoveries (Jeanson et al., 2003; Wyatt, 2003; Jackson et al., 2007) and the interplay of two pheromones has been demonstrated to be important under dynamically changing foraging conditions (Dussutour et al., 2009a; Reid et al., 2011). This is the case of the ant species *Pheidole megacephala* which uses two different pheromones, a long-lasting pheromone during exploration and a short-lasting exploitation pheromone during recruitment to a food source. Theoretical models and experiments indicate that the combination of these two pheromones allows *P. megacephala* colonies to track changing foraging conditions more effectively than would a single pheromone. When colonies of this species were provided first with a long path between their nest and a food source and then with a shorter path after some time, the majority of ants were able to select the short path, even if ants had already established a chemical trail on the long branch (Dussutour et al., 2009b). In the same way, species using positive feedback loops channeled by direct interactions such as tandem-running may be more flexible than species using mass pheromone recruitment and may prevent the colonies from locking to poor choice. For example, *T. albipennis* colonies are able to correct errors by continuing to survey potential nest sites during the last stage of the emigration process or even after they have settled altogether in a new nest (Dornhaus et al., 2004; Franks et al., 2007). If a nest site of better quality is discovered ants are able to switch nest.

COLLECTIVE DECISIONS AND INDIVIDUALITY

Many studies on collective decision-making processes consider that social groups are composed of identical and interchangeable individuals. This assumption has proved to be valid in many contexts and has provided relevant insights for the understanding of the global dynamics and properties of the systems under study. Under some circumstances however, considering inter-individual variability can further improve our comprehension of whole-group functioning. For instance, individuals in groups of animals can differ in sex, body size, and/or the morphological/temporal caste they belong to. This may lead to differences in their response threshold to the signals involved in collective decisions, e.g., trail pheromone (Detrain and Pasteels, 1991; Morgan et al., 2006; Kleineidam et al., 2007). Groups can also contain individuals with differing behavioral tendencies, i.e., "personalities" (Sih et al., 2004, caterpillars: Dussutour et al., 2008; Nicolis et al., 2008; bumblebees: Burns, 2005, spiders: Pruitt and Riechert, 2011, honeybees: Burns and Dyer, 2008; ants: Chapman et al., 2011). Inter-individual differences may have important consequences for collective decisions; for instance, these differences have been shown to lead to colony decisions that are dependent upon the ratio of the different categories of individuals in the group. Dussutour et al. (2008) have shown that in social caterpillars, individuals within a group fall into two clearly distinguishable behavioral categories: inactive and active. Active caterpillars spend considerable time exploring the environment and relatively little time feeding, whereas inactive caterpillars have longer meals and explore less. At the collective level, when given a choice between two equal low quality food sources, active caterpillar-biased colonies are less cohesive than colonies comprised of proportionately fewer active caterpillars. They do not focus their activity on one source but split and exploit two sources at the same time. In contrast, inactive caterpillar-biased colonies focus their activity on one source only. In the case of social caterpillars collective behavior patterns can thus be explained by individual differences. By the same token, social insect colonies can benefit from having workers with different behavioral types. For example, studies on foraging honeybees show that co-existing strategies, where some individuals place more emphasis on accuracy and others on speed, can be advantageous to the colony in a variable environment (Burns and Dyer, 2008). The importance of inter-individual differences in collective decisions has not been fully investigated. Yet, individuality is recognized as an intrinsic character of all biological systems in which no two individuals are the same. This question thus appears critical for the understanding of collective behaviors and collective decisions in animals, including invertebrates.

COLLECTIVE DECISIONS AND INDIVIDUAL COMPLEXITY

Studies on collective decisions generally assume that complex structures or behaviors at the collective level can be explained by simple behavioral rules at the individual level. This issue has been the subject of some misinterpretation in the early history of the studies of self-organization in collective behaviors (Deneubourg et al., 1999; Camazine et al., 2001) and to the rejection of this

approach by some biologists. It should be emphasized, however, that the self-organization approach does not deny individual complexity, particularly from a cognitive point of view. In fact, the degree of individual complexity found in animal groups can be extremely variable and even the tenant of self-organization will acknowledge that individual complexity can also be a source of collective complexity (Anderson and McShea, 2001). Self-organization studies simply apply a principle that is universally used in science: that of parsimony. There is no need to invoke the contribution of complex individual behaviors when models of collective decisions show that simple behavioral rules are able to account for the production of complex collective patterns. For instance, the selection of the more rewarding of two food sources in ants does not require any active comparison between the food sources at the individual level but instead only relies on a modulation of the trail-laying behavior of individual ants in response to the quality of the food sources. In the same way, the modulation of individual behavior (staying or leaving) by cockroach nymphs as a function of the number of conspecifics in the neighborhood when aggregating at a single site does not require that these insects explicitly count the number of individuals in their surroundings. To do so they may just rely on an assessment of the overall quantity of aggregation pheromones (i.e., cuticular hydrocarbons) perceived at a site, which is a function of both the number and the sex of the individuals in their surroundings (Jeanson and Deneubourg, 2007). Of course, this does not mean that insects are unable to make direct and subtle comparisons between alternatives, as has been reported for a long time in parasitoids (Wajnberg et al., 2007) or more recently in house-hunting ants (Sasaki and Pratt, 2011). Moreover, complex cognitive processes such as learning and memory can also be involved in collective decisions. Private navigational information (memory) can either override social information (trail pheromone in ants or waggle dance in honeybees) and thus reduce the flexibility of collective decisions by counteracting amplification process (Grüter et al., 2008, 2011) or, on the contrary, it can contribute to enhance the amplification process (trail-following behavior in ants: Czaczkes et al., 2011). For example, workers of the ant *L. niger* are able to memorize rapidly the spatial location of a food source on the basis of the visual cues they find in their surroundings (Aron et al., 1993; Evison et al., 2008; Czaczkes et al., 2011; Grüter et al., 2011). Initially, recruited workers follow the chemical trail laid down by their nestmates, but they are rapidly able to orient on the sole basis of the visual cues they have memorized. In red wood ants, such a process leads foraging workers to develop a fidelity to a particular trail (Rosengren, 1971) and, on a longer time scale, explains the stability of the network of foraging trails around their nest over successive years (Rosengren and Fortelius, 1986; Salo and Rosengren, 2001). On the other hand visual memory can also act as a constraint that restrains amplification processes and thus limits the flexibility allowed by this latter when rapid changes occur in the localization of the resources exploited (Fewell, 1990; Grüter et al., 2008; Grüter and Ratnieks, 2011). Memory may also play an important role in house-hunting ants. Knowledgeable workers that had the possibility to gather information about potential nest sites prior to the emigration event allow to enhance the collective performance of the colony during the choice of a new nest and play a disproportionate role in this decision (Stroeymeyt et al., 2011a).

A last detail that needs to be emphasized is that although the behavioral rules at the basis of collective decisions can be simple, it is rarely true of the underlying mechanisms (physiological, neural, sensorial, perceptual, or cognitive) that allow these rules to be expressed (Seeley, 2002). For example, the statement that dancing in bees or pheromone deposits in ants are simple behaviors does not imply that complex mechanisms occurring upstream the behavioral performance are not involved. In ants, a scout that has discovered a food source must integrate several parameters before deciding if and how it will recruit nestmates to the food source. These parameters depend on both the characteristics of the food source (quality, novelty, accessibility, transportability; review by Detrain and Deneubourg, 2002) and on the nutritional needs of its colony (Mailleux et al., 2006). In the same way, in the context of nest-moving in honeybees, a scout's decision to perform a waggle dance for recruiting additional scouts to a cavity depends on its capacity to assess and weigh multiple parameters, e.g., the cavity volume, shape or temperature, and its exposure to sunlight or wind (Seeley, 1977, 2010; Seeley and Morse, 1978).

CONCLUSION

The ability to organize collectively and to make collective decisions is generally assumed to be the hallmark of highly social species. Our review shows however that collective decisions in invertebrates are not only found in social insects, such as ants or bees, but also in other organisms that, at least at some point in their life cycle, show some form of sociality. Generally, collective decisions immediately benefit all individuals in a group by allowing the rapid selection of the best of several different options. Whether this ultimately can lead to increase their fitness depends on a variety of factors related to the organisms' biology and their environment. In ants for example, in which the greatest part of the colony is constituted by sterile individuals that share a common interest, the ability to use a collective exploration strategy and to choose and monopolize the best among several food sources through chemical mass recruitment have probably been determinant in the evolution of the dominant character of some species. In fact, both of these features happen to be common to most invasive ant species (Lach et al., 2010). In other invertebrate organisms, collective decisions may not always be ultimately advantageous and are probably subject to a cost-benefit trade-off, much alike that which has been discussed in behavioral ecology for group living (Krause and Ruxton, 2002). For example, the collective choice of a single favorable site at which to aggregate in cockroach can be advantageous on the short-term but can lead on the long-term to an increase in the competition for food (which can generate cannibalism) or to the rapid spread of potentially lethal pathogens. In any case, collective decisions have so far been studied more from a mechanistic than from a functional point of view and, although collective decisions are generally assumed to benefit animals, their functional properties would certainly deserve to be investigated in dedicated studies in order to understand the selective forces acting on them (Boomsma and Franks, 2006). This can be achieved only by examining the outcome of collective decisions in the natural environment of the species under study (Traniello and Robson, 1995).

One of the objectives of this review was to highlight the importance of comparative studies for the study of collective decisions in invertebrates. Studies on different organisms allow to delineate the generic rules underlying collective decisions and the key factors required for their emergence. They could also be useful in developing scenarios for the evolutionary steps that have lead to the emergence of collective decisions within some groups of invertebrates like ants or bees in which they play a major role for the cohesion of the colonies. It appears that one of the key factors required for the emergence of collective decisions is the ability of an individual to modulate its behavior as a function of the quality of the resource it has found (e.g., trail-laying in ants) or as a function of its conspecifics' behavior (e.g., staying at a site or leaving it in cockroaches). It could thus be predicted that any species of invertebrates in which individuals are endowed with such capabilities would potentially be able to make collective decisions. Further studies on a more extended range of invertebrate organisms will allow to investigate whether this prediction is true or not.

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