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Hive Minded: Like Neurons, Honey Bees Collectively Integrate Negative Feedback To Regulate Decisions

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Abstract

Collective decision-making is essential for multicellular and self-organized society coordination, but how this occurs when most of the individuals have limited knowledge of the external environment, remains elusive. Using empirical data to inform a neuroscience-based firing rate model, we found that integration of negative feedback and network dynamics in a honey bee hive demonstrate strong similarities to the neuronal interactions of the human brain, where very brief perturbations of feedback in the system results in more rapid and accurate decisions. We show that honey bees use an inhibitory “stop” signal towards dancing honey bees that reduces both waggle dancing and waggle dance pheromone production. Stop signals were likely elicited by individuals with no individual knowledge of food quality change in the external environment. Therefore, we demonstrate that collective behaviour across different biological levels of organization, exhibits a dynamic complex system that is self-organized, but is governed by simple feedback mechanisms, facilitating efficient group decision-making by optimally aggregating individuals that have relatively limited cognitive capabilities within a society or cell in a multicellular organism. We discuss how despite being on two different levels of biological organization, both neurons in the brain and honey bee individuals, within the hive, can collectively operate, which is most likely a result of convergent evolution.

Keywords: balanced network theory, collective decision making, forager regulation, inhibitory feedback, stop signal, waggle dance

Introduction

Networks of organisms often demonstrate collective cognition, responding to changes in their environment without any one individual being fully informed. It is still unclear how an accurate and rapid collective decision is made when most of the individual cells or organisms making up the group have relatively limited knowledge of their external environment (Sasaki & Pratt 2018). Recent research has found striking similarities between the collective decision-making mechanisms used by brains and social insects (Couzin 2009; Marshall et al. 2009; Seeley et al. 2012). In both systems, mutually interacting populations, each advocating a different choice, integrate positive and negative feedback, until the accumulated positive feedback in one of the populations exceeds a threshold. This population, and its associated choice, becomes the winner (Glimcher 2003; Pratt et al. 2002; Seeley & Visscher 2004). Thus, nonlinear dynamics allows individuals with limited information to globally reach a consensus and choose the better option in less time (Atallah & Scanziani 2009; Vogels, Rajan & Abbott 2005).

A hive needs to keep track of many food sites in a complex, fluctuating environment (Real & Rathcke 1991), yet the cognitive capacity of individual bees is limited by their small brain size (Menzel & Giurfa 2001). Their nectar intake has to fulfil the energetic demands of the hive to last through the winter, yet the risks and energetic demands of foraging limits worker lifespan (Neukirch 1982; Rueppell et al. 2007). Therefore, it is of adaptive significance for the hive to preferentially send foragers to highly profitable food sites over ones with low profitability. Foragers use the waggle dance to recruit bees to a food source, in which the quality is positively associated with the likelihood of performing a waggle dance and the number of circuits made within a waggle dance (Seeley 1986; Seeley, Camazine & Sneyd 1991; Seeley, Mikheyev & Pagano 2000). Interestingly, foragers are not likely to compare waggle dances directly. Honeybee foragers instead distribute themselves among food sources proportional to the number of waggle dancers for each food site. If they had been comparing waggle dances, the relationship between bees at a site and waggle dances to that site would be nonlinear, with most bees going to the best site (Seeley & Towne 1992). It is thus likely that the collective dynamics of bee interactions, rather than individuals themselves, allow the hive to compare resource options.

In social insects, much research has been dedicated to the signals used for positive feedback, such as the waggle dance of honey bees (von Frisch 1967) and its associated waggle dance pheromones (Thom et al. 2007). Work on negative feedback, however, has focused on Implicit Negative Feedback, which is negative feedback through the absence of positive feedback. For example, honey bees returning from a poor food site are less likely to recruit more bees through waggle dancing (Seeley 1986; Seeley et al. 1991; Seeley et al. 2000). On the other hand, signals that directly convey information about detrimental changes in the environment, can be as or more important to arriving at an accurate collective decision quickly and this is known as Explicit Negative Feedback (ENF) (Plenz & Thiagarajan 2007; Sumpter 2006). Examples of Explicit Negative Feedback is relatively rare in social insects (Robinson et al. 2005; Stickland, Britton & Franks 1999), with one of the few known examples in honey bees being the stop signal. It is a vibrational signal that lasts about 150 ms with a fundamental frequency of around 350 Hz (Lau & Nieh 2010), and is accompanied by a bee (the producer) head-butting another bee (the receiver) in the hive. Honey bees have been shown to deliver stop signals to communicate predation threats and competition when foraging (Lau & Nieh 2010; Nieh 2010; Tan et al. 2016). Furthermore, during the house-hunting process, scouts advocating one nest site will deliver stop signals to bees advocating another site. These previous studies have shown that the ENF in the form of a stop signal can either come from individuals coming from the option directly and this type of negative feedback is known as “ipsi” signalling, which is in contrast to “contra” signalling that comes from individuals promoting the other options, eliciting stop signals with no direct knowledge of the first option. The two kinds of signalling are known to dictate the dynamics of honey bee collective decision making (Seeley et al. 2012).

Most theoretical work addressing the role of ENF in honeybee swarms has focused on nest site selection (Reina et al. 2017). Foraging, on the other hand, is a very different type of process because it does not require a fully binary decision. Instead, the colony allocates more foragers to better food sites, but does not necessarily need to abandon poor food sites entirely. Similar to honey bee foraging, diverging levels of activity between two neuronal clusters via attractor dynamics is well documented for decision-making in experimental settings, particularly for visual search, virtual navigation, and reaching tasks (Churchland et al. 2012; Cohen et al. 2009; Harvey, Coen & Tank 2012; Thomas & Pare 2007). Such inhibitory signals are common in brains, in which inhibitory neurons are dedicated to sending only ENF (Buzsáki, Kaila & Raichle 2007). Therefore, in this work, we explored whether honey bees, like neurons, use explicit negative feedback in the form of a stop signal in concert with positive feedback to adjust forager allocation in response to fluctuations in food availability and to thereby effectively make a collective decision.

In our experimental investigation, we trained honey bees to a profitable feeder and then replaced it with a poor quality feeder. We hypothesized that after a decline in food quality, there would be a rapid increase in the number of stop signals received by bees waggle dancing for that feeder. We predicted that this decline in quality will result in a decrease in waggle dances, waggle dance pheromones, and, on an individual and population level, lead to a decline in feeder visitations. Furthermore, because foraging is not an all-or-nothing choice, we expected that bees committed to different feeders will not try to stop each other from dancing, and thus will not exchange stop signals. Instead, we expected “ipsi” signalling, which is a form of lateral signalling, where the stop signals elicited will be coming from the bees visiting the same feeder that has declined in quality as opposed to “contra” signalling.

We then developed a modelling framework akin to firing-rate-based models for neuronal assemblies that treated honeybee foragers as leaky integrators in competition (Hopfield 1982; Patel & Rangan 2017; Shpiro et al. 2007; Wilson & Cowan 1972). Informed by our experimental observations, we investigated if a brief burst in stop signals corresponding to a decrease in food source profitability is sufficient to produce a rapid shift in model dynamics and collective reallocation of resources towards more profitable food sources.

Materials and Methods

Training & Trials

For details of the experimental setup please see the appendix. During the summer of 2016 and 2017, a free foraging, 3.5 frame observation hive of *Apis mellifera* was set up in a dark room with the windows covered. A total of 3 different bee colonies were setup in the observation hive over the 2-year period. A short tube (0.5 m) between the hive and one of the windows allowed bees to freely go outside and forage. The hive was censused and thinned roughly once a week to maintain a constant population of around 10,000 bees. Before the start of an experimental trial, bees were trained to a feeder filled with 2 M sucrose solution located 50 m from the observation hive in a grassy field. The feeder consisted of a glass jar filled and inverted on top of a 40-groove plexi-glass plate that was lined with yellow paper on the bottom and was placed on top of a blue bowl, on top of a metal stool. The plate had 40-grooves in order to prevent crowding at the feeder, which has been shown to cause an increase in stop signals (Lau & Nieh 2010). During the training, filter paper was taped on top of the jar with 2 - 3 drops of Lemon extract (McCormick, Baltimore, USA).

During training, unmarked bees that arrived to the feeder were painted with individually identifiable paint markings using Elmers acrylic paint markers. Two observers checked that all painted bees returned to the focal hive. Since competition with bees from other hives can cause stop signalling (Lau & Nieh 2010), we prevented competitors from feeding at the hive during training. To do so, we checked that all visitors to the feeder returned to the focal hive. Bees that did not do so were promptly removed upon their return to the feeder. Once 25 - 30 bees had been trained and confirmed, all additional visitors were aspirated until the time of the experimental trial. We noticed that 2 - 5 of these trained bees had stopped visiting the feeder by the time of the trial, so we only counted marked bees that visited the feeder at least once during experiments as part of the trained cohort.

Trials started between 11:00 am and 1:00 pm, and lasted about 2 hr. Right before the trial began, the feeder was replaced with a clean jar of 2.5 M sucrose solution. About 50 min into the trial this jar was replaced with another jar containing either 2.5 M or 0.75 M sucrose solution. We refer to the 2.5 M feeder as the high quality feeder and the 0.75 M one as the low quality feeder.

During 2-minute time intervals, the observer recorded the number and identity of marked bees and the number of unmarked bees visiting the feeder. In parallel, an observer at the observation hive followed a randomly chosen marked bee, one at a time, with a microphone, with a preference of following those performing the waggle dance. When a focal bee left for foraging, stopped dancing, or went out of the observation area, a new marked bee was chosen at random. A total of 9 trials were conducted in random order. All behaviours and sounds observed were narrated by the observer. The treatment of the feeder was blind to the observer at the observation hive and at the feeder. At the end of each trial, all marked bees were captured and eliminated to prevent pseudo replication.

Video Analysis

Over two-minute intervals, the number of marked bees waggle dancing and the number of stop signals produced and received by marked bees was recorded using iMovie11 (for details see the appendix for supplementary methods information).

GC-MS analysis

Waggle dance pheromones absorbed from SPME fibres were analysed using a Varian 431 Gas Chromatograph (GC) / 220 Mass Spectrometer (MS) and separated on an Agilent/J&W model VF-5ms column (30 m length, 0.25 mm column diameter and 0.25 um stationary phase thickness) (for details please see the appendix supplementary methods information). Peaks were initially identified by the retention time of the standards and then confirmed using the mass spectrophotometer data and the NIST v. 17 library.

Firing Rate Model

We used data collected from the experiment to inform a firing rate model in order to investigate if we could draw parallels between how collective feedback is used by honey bee individuals when selecting between two food sources with how neurons in the human brain integrate positive and negative feedback collectively when it is time to make a decision between two options. Therefore, we split the foragers in the hive into the following groups: (1) those dancing for the focal food source, (2) those dancing for other food sources, and (3) those that are uncommitted and not waggle dancing (Marshall et al. 2009; Seeley et al. 2012). It is important to note that the population of bees dancing for other food sources encompasses all actively dancing foragers in the hive, except for those visiting the focal feeder. The dynamics we model for this assembly thus act as an average for the recruitment intensity of bees visiting natural foraging sites. We account for this because in our experiments we could not prevent bees in our colony from visiting local flowers.

In our modelling framework, $x(t)$ quantifies the waggle dance intensity of the focal population and $y(t)$ quantifies the waggle dance intensity of the opposing population, with uncommitted bees potentially recruited to join either population via the excitatory waggle dance. The dynamics of the focal population and opposing population are thereby governed by the system of nonlinear differential equations

$$\tau \frac{dx}{dt} = -\mu x + f\left(W_x x + W_{xy} y + I_x + S_x(t)\right)$$

$$\tau \frac{dy}{dt} = -\mu y + f\left(W_y y + W_{yx} x + I_y\right),$$

where τ is the time constant for the population dynamics, μ is the decay term, quantifying the rate at which foragers spontaneously stop waggle dancing for a food source, I_j is the excitatory input from the food source corresponding to population j $(j = x, y)$, and $S_x(t)$ reflects the impact of stop signals on the focal population over time. The bees are thus considered leaky integrators, such that in the absence of sufficient positive feedback for a food source, they will become uncommitted over time.

In accounting for the experimental design, it is important to remark that since the mean quality of food sources in the local environment is approximately 1.17 M (Wykes 1952), the 2.5 M feeder used initially is of relatively high profitability whereas the 0.75 M food source used after the switch is of low profitability relative to nearby alternatives. Therefore, since before the experiment bees had been trained to know that the feeder contains a relatively high sucrose solution, the excitatory input from the respective food sources in our model, I_x and I_y, are selected so there is a bias towards population x. Reflecting this assumption, $I_x = I(1+\alpha)$ and $I_y = I(1-\alpha)$, where I is the base input level for a sugary solution and α is the bias term in which $\alpha > 0$ encodes the relatively high profitability of the feeder. The α parameter thus indicates the distributed knowledge of the hive regarding the profitability of one food site relative to the other. When the feeder only switches from high to low quality, we assume the bias changes sufficiently slowly such that it can be approximated as constant over the two hour timescale of the experiment. Hence, stop signalling should facilitate a shift in the waggle dance dynamics following the feeder switch well before the colony fully processes the change in food quality. We generally choose the base input level $I = 0.8$ for concreteness and a very small positive value for the bias, typically $\alpha = 0.01$, allowing stop signals, as opposed to knowledge at the colony level, to facilitate a response to changes in food source profitability.

Given that stop signals rapidly increased for about ten minutes after the feeder switch at time $t = 60$ minutes in our experiments, the stop signal function is modelled as $S_x(t) = \delta(H(t-60) - H(t-70))$, where δ quantifies the strength of the stop signal burst and $H(\cdot)$ denotes the Heaviside function. Note that since the feeder switch is assumed to have little impact on the number stop signals received by the opposing population, no such term is included in the y population dynamics.

The effect of dancers from assembly *j* dancing to members of assembly i is quantified by W_{ij}. The term W_i quantifies recruitment of the uncommitted population into population i. We assume that recruitment from the uncommitted population causes an increase in waggle dance activity, while waggle dances exchanged between populations act as cross inhibition; hence $W_{ij} < 0$ and $W_i > 0$. Without loss of generality, assuming the x and y populations demonstrate identical communication strategies, we set $W_x = W_y = 1$ and $W_{xy} = W_{yx} = -1$. For analogous reasons, the population time constants and decay terms are generally chosen such that $\tau = 1$ and $\mu = 1$, with the population dynamics therefore remaining in the unit interval for initial waggle dance activity between 0 and 1.

Incoming information from inputs into the focal population are integrated by gain function, $f(\cdot)$, which we choose to be sigmoidal. We use a sigmoidal gain function for three reasons. First, it is commonly used as the filter when modelling neuronal populations. Second, it bounds the dynamics, allowing the output to steeply increase only for moderately large inputs, while saturating for sufficiently small or large inputs (Dayan & Abbott 2005; Hopfield & Tank 1986). Third, previous studies have argued that social insects integrate inputs using thresholds, allowing the system to not be overly sensitive to small changes in the environment (Marshall et al. 2009). We therefore modelled the gain function as $f(z) = \dfrac{1}{1+e^{-r(z-\theta)}}$, where r determines the steepness and θ determines the midpoint of the sigmoidal curve. We selected r and θ such that the sigmoidal function takes on nearly all values in its range as I varies from 0 to 1 (Figure A1).

Statistical analysis

Feeder visits

All statistical analyses were conducted in JMP 10. A Generalized Linear Model (GLM) with a Poisson distribution corrected for over dispersion was used to analyse the effect of switching the feeder from 2.5 M (high) to a 0.75 M (low) concentration for feeder visitation rate for both the marked (previously trained) and unmarked recruited bees to the feeder during the trial. Prior to this, we determined that colony, trial, and year were non-significant as random effects, so they were removed from the model. Treatment (0.75 M vs. a 2.5 M sucrose solution feeder switch at 50 minutes into the trial) was nested within whether the bees were marked or unmarked, and this was nested within comparing whether the feeder visits were before or after the switching of the feeder.

We followed up with another GLM analysis of the feeder visitations using only data after the feeder was switched. Whether the bees were trained or recruited was nested within each of the treatments. The intra-individual foraging frequency was determined to be non-normal so a non-parametric Wilcoxon rank sum test was conducted to compare the foraging frequency after switching the feeder with either the 0.75 M or 2.5 M sucrose solution.

In-hive behaviors

We conducted a GLM to analyze the effect of the 0.75 M and 2.5 M feeder switches on the frequency of waggle dances in the observation hive. Factored into this model were time (before and after the feeder switch) and treatment (whether the feeder was switched with 0.75 M or 2.5 M) along with the interaction of time and treatment. A GLM was conducted on the number of stop signals, which compared the total number of these elicited towards waggle dancing bees, before and after the feeder switch. A chi-square goodness of fit was used to compare the number of stop signals across treatments and to compare the number of stop signals received from trained (marked) versus untrained (unmarked) bees in the hive.

Waggle dance pheromones

Waggle dance pheromones were found to be normal and analysed using a General Linear Model (GLM) where the relative abundance of the pheromone served as the dependent variable and the treatment, time, and pheromone type served as the fixed factors. All main effects and interactions were tested using this GLM.

Results

Feeder visits

Overall there was a significant difference in the number of feeder visits based on the concentration of sucrose solution used during the feeder switch (GLM Treatment: $\chi^2_1 = 74.22$, $P < 0.0001$). Within previously trained and recruited bees, there was a significant difference of feeder visits based on the sucrose concentration after the feeder switch (GLM Marking (Treatment): $\chi^2_2 = 370.40$, $P < 0.0001$). There was a significant difference of the feeder visits before and after switching the feeder within previously trained and recruited bees based on the concentration of the sucrose solution used (0.75 M or 2.5 M) (GLM Time (Treatment, Marking): $\chi^2_4 = 33.53$, $P < 0.0001$). After the feeder switch, the recruited bee visits increased more for the 2.5 M feeder switch in comparison to the 0.75 M feeder switch. In addition, the feeder visits increased significantly more for the previously trained bees after the 0.75 M switch in comparison to the 2.5 M switch (GLM Treatment (marking): $\chi^2_1 = 53.06$, $P < 0.0001$) (Table A1-A2) (Figure A2). The intra-individual foraging frequency was significantly higher for the bees already trained to forage from the feeder after the quality of it declined from 2.5 M to 0.75 M (Wilcoxon: $\chi^2_1 = 8.97$, $P = 0.003$) (Figure A3).

In-hive behaviours

The effect of the feeder switch on waggle dance behaviour depended upon whether the feeder was switched with 0.75 M or 2.5 M (GLM Treatment x Time interaction: $\chi^2_1 = 26.26$, $P < 0.0001$). Waggle dancing significantly decreased after the feeder was switched with 0.75 M solution, while there was a significant increase in waggle dances after the feeder was switched with 2.5 M (Table A3) (Figure 1).

Overall stop signal production across the entire trial was not significantly different when the feeder was switched with either 0.75 M sucrose or 2.5 M sucrose solution (GLM Treatment (time 0.75 M): $\chi^2_1 = 0.001$, $P = 0.970$; GLM Treatment (time 2.5 M): $\chi^2_1 = 0.23$, $P = 0.630$). In contrast, only in the period after the feeder was switched with 0.75 M sucrose solution, was there significantly more stop signals directed towards waggle dancers in comparison to the time period before the switch, 82 versus 47, respectively (Chi-square goodness of fit: $\chi^2_1 = 9.50$, $N = 4$, $N = 4$, $P = 0.002$). Within 50-60 minutes of the trials, immediately after the feeder was switched, there were significantly more stop signals directed towards dancing bees when the feeder was switched with the 0.75 M feeder in comparison to the 2.5 M feeder, 44 stop signals versus 10, respectively (Chi-square goodness of fit: χ^2_1 = 21.41, $N = 4$, $N = 4$, $P < 0.001$) (Figure 1). Overall there were significantly more stop signals received from untrained bees in comparison to bees that were trained to the feeder for both the 0.75 M switch and the 2.5 M feeder switch (Chi-square goodness of fit: $\chi^2_1 = 79.68$, $N = 4$, $N = 4$, $P < 0.001$; Chi-square goodness of fit: $\chi^2_1 = 105.62$, $N = 4$, $N = 4$, $P < 0.001$). This was also true 50-60 minutes after the feeder was switched ($\chi^2_1 = 26.68$, $N = 4$, $N = 4$, $P < 0.001$) (Figure 2).

Waggle dance pheromones

The level of waggle dance pheromones produced varied based on pheromone type ($F_{4,120} = 5.26$, $P = 0.001$). However, across all pheromones there was a significant interaction across time and the treatment of the feeder switch ($F_{5,120} = 3.03$, $P = 0.010$); there was stable low to no production of waggle dance production after the feeder was switched with 0.75 M sucrose solution, but in contrast the waggle dance pheromones increased across time when the feeder was switched with 2.5 M sucrose solution (Figure A4).

Firing Rate Model Dynamics

To investigate the potential decision-making mechanisms underlying the honeybee network activity, we analysed the long-time dynamics of the firing rate model. In particular, we compared the model fixed points as well as their stability in the presence and absence of stop signals, depicting the resultant waggle dance activity for the two populations in each case, respectively, in Fig. 3a-b.

With either no stop signals or bias α too large, stemming from the perceived high profitability of the feeder on the colony level, the focal bees demonstrated continued relatively high waggle dance activity for the feeder despite its diminished profitability after the sucrose solution switch at time $t = 60$ minutes. On the other hand, for small α, the short inhibitory burst of stop signals resulted in a significant relative increase in waggle dance activity in the opposing population, which remained even after the burst of stop signals ceased, suggesting that a sufficiently small bias makes the burst of stop signals communicated at the individual level sufficient for the population to make a decision to switch food sources. In this case, for the first hour, the focal population waggle dance activity x initially increased to a relatively high fixed point, reflecting the initial high profitability of the feeder, but a burst of stop signals following the time at which the feeder solution diminished in profitability caused x to decrease to a fixed point well below that of the opposing population waggle dance activity y. Once the spike in stop signals ceased, x nevertheless remained at an attracting low fixed point with y far larger, corresponding well to the now higher profitability of external food sources. These dynamics suggest that, as observed in the experiment, a brief burst of explicit negative feedback is indeed crucial to making accurate and efficient decisions. Otherwise, the focal bees would continue largely waggle dancing for the feeder despite the abundance of more profitable nearby food sources, as reflected in the model by the persistently attracting high x fixed point following the feeder switch in the absence of stop signals.

In Fig. A5a-b, we depict the corresponding bifurcation diagrams for x in the absence and presence of stop signals, respectively, showing the stable and unstable fixed points across choices of base food source input level I. Here we generally see that in the absence of stop signals x gravitates to relatively high fixed points, as depicted in Fig. A5a. However, as a result of the feeder switch, x is later attracted to a significantly lower fixed point during the subsequent burst of stop signals, as shown in Fig. A5b, and remains at a low fixed point even after the burst of stop signals is complete, where Fig. A5a again applies, since x is now far below y and is consequently attracted to a correspondingly low fixed point.

We also observed a second, smaller burst of stop signals after the first large pulse of stop signals in the experiments. To test whether this aids the decision-making process, we added a second but smaller burst of stop signals into the x dynamics from time $t = 90$ to $t = 100$ minutes, as observed experimentally, in a manner analogous to how the initial burst of stop signals was modelled. We observed that including these additional stop signals impacted the long-time dynamics when the populations integrated inputs less effectively. As shown in Fig. 4, when dynamics are slow, reflected by relatively large τ, only for a sufficiently strong second burst of stop signals does the opposing population demonstrate relatively elevated waggle dance activity in the long-run, as observed experimentally. Though incurring additional energetic costs, this second pulse of stop signals ensures the optimal feeder is chosen in more marginal cases while still not requiring as much resources from the focal colony as the initial inhibitory burst.

Discussion

This study compares the dynamics of the collective decision making across two different levels of biological organization and we are the first to empirically demonstrate that the stop signal can be used to regulate honey bee foraging recruitment based on food quality. While a previous study found no significant effect of food quality on stop signal production (Jack-McCollough & Nieh 2015), this was probably because the stop signal data was compared across long time intervals. Instead, we measured minute-by-minute stop signal dynamics. Our empirical and theoretical results demonstrate that a brief burst of stop signals within 10 minutes of food quality decline is sufficient to suppress recruitment for this particular food source. A second, smaller wave of stop signals, also appears to act as reinforcement for the first wave. In general, stop signal production towards a dancing bee appears to reach a threshold, and once it is reached, generally it causes bees to cease dancing

(Nieh 1993; Tan et al. 2016). This negative feedback is analogous to the lateral inhibition in competing neuronal assemblies that garners winner-take-all decision-making dynamics (Cannon & Miller 2016).

As indicated by our experimental observations and mathematical model, excitatory and inhibitory communication among honey bees can produce a rapid collective reallocation of recruitment to other food sources. Importantly, in our model, while there may not be fully distributed knowledge regarding changes in feeder profitability at the population level, inhibitory signals between individual bees allows the population to collectively make an effective decision about reallocating foraging resources. While previous mathematical models of bee nest selection dynamics primarily assumed inhibitory well-mixing between bee populations committed to different sites and uncommitted bees (Seeley et al 2012), our modelling framework for foraging dynamics instead reflects bee waggle dance activity akin to firing rate models of neuronal assemblies. Particularly in the large population limit, this causes signalling strength to be determined by the activity of the source population rather than the target population, assuming there are enough target bees to receive any incoming signal as in the case of large-scale neuronal networks. In the context of foraging dynamics in particular, a recent theoretical analysis using a well-mixed swarm model, incorporating bees committed to two food sources as well as an uncommitted population, corroborates the key role of explicit negative feedback in effectively realigning foraging activity in response to temporally changing environments (Bidari, Peleg & Kilpatrick 2019). The well-mixed model suggested that direct switching between feeder commitments yields particularly effective foraging in comparison to alternative inhibitory interaction schemes, with this direct switching inhibition scheme paralleling how inhibition from one population produces an immediate impact on the opposing population in our firing-rate-based model. Unlike previous models of decision-making in foraging, our model dynamics are directly motivated by brain activity as well as experimental observations of waggle dance and stop signal behaviour, and demonstrates how a brief spike in inhibition of stop signals, like what is observed for neurons in the brain during a decision making process, potentially also facilitates rapid dynamical shifts in foraging activity based on food source quality.

According to the theory of balanced networks ubiquitous in neuroscience, an ever-present bombardment of many strong excitatory and inhibitory signals causes neuronal firing events to primarily be the result of small fluctuations in the two input types, yielding high sensitivity to changes in external network inputs (Barral & Reyes 2016; Vogels et al. 2005). Consistent with this theory, honey bees, before the feeder switch, on the dance floor, were receiving an approximate constant rate of waggle dancing (positive feedback) and stop signals (negative feedback), in a balanced fashion. However, immediately after the feeder profitability was switched, a small burst of stop signals was enough input to disrupt the balance and result in a quick collective decision. Analogous to neural systems, we hypothesize that the collective behaviour of many social insect groups demonstrates self-organized criticality (De Vries & Biesmeijer 2002; Gordon 1996; Karsai & Balazsi 2002; Theraulaz, Bonabeau & Deneubourg 1995), as selected through evolution, to facilitate efficient and effective group decision making by optimally aggregating the relatively limited cognitive capabilities of each individual (Bonabeau et al. 1997; Hesse & Gross 2014).

If instead there are many alternative options and a decision needs to be made quickly, then the burst of stop signals could potentially aid in making a more accurate decision (Atallah & Scanziani 2009). Though we focused on foraging in the context of two food sources, similarly investigating foraging dynamics in the presence of many alternative food sources would mark an interesting follow-up study more representative of the natural context of honeybee foraging. A recent theoretical investigation extended the modelling framework for nest selection, as opposed to foraging, to an arbitrary number of site options, specifically addressing the interplay between inhibitory signalling, independent discovery, and abandonment (Reina et al. 2017). However, such a multi-option investigation for decision-making in foraging is qualitatively distinct because in foraging it may be beneficial to allocate resources towards several food sources whereas bees must instead decide upon a single location in nest selection.

When honeybee foragers experience an attack from a predator at a feeder, they return to the hive and deliver a large number of stop signals selectively to other foragers waggle dancing for the same feeder (Nieh 2010). In this case, the stop signals qualify as ”ipsi” signalling, because they are produced from bees that have visited the same feeder. On the other hand, when stop signals are used for choosing a new home, scout bees loyal to a potential nest site will deliver stop signals to bees waggle dancing for a different nest site, and thus use stop signals as contra-signalling, or cross-inhibition (Seeley et al. 2012). Surprisingly, our results suggest that the bees eliciting the stop signal are using contra signalling. Marked bees trained to the focal feeder rarely delivered stop signals to other marked bees. Although we cannot rule out that the unmarked bees were foragers newly recruited to the feeder, this seems to be highly unlikely given that this was a relatively small population. We suspect instead that perhaps bees following the waggle dance are tasting the food from a sample donated by the dancing bee and these bees could be making comparisons with other waggle dancing bees to determine whether or not a stop signal should be elicited. In the spirit of such comparisons, previous model investigations in the context of nest site selection demonstrate how both the relative and absolute profitability of alternatives together with cross-inhibition strength potentially influence decision-making dynamics, suggesting that changes in cross-inhibition strength facilitate adaptive decision-making over time in response to diverse decision landscapes (Pais et al. 2013).

The negative feedback we observed allowed the colony to regulate recruitment signals even though most individuals had little knowledge of the original bias to the feeder, and probably also had no knowledge of the feeder switch. Future research is needed to determine this, but mechanisms to perform complex decisions while minimizing the information load of individuals is common in the eusocial insects (Sasaki & Pratt 2012). We hypothesize that stop signals may help the hive react quickly to fluctuations in food quality and availability on a group level while minimizing the cognitive load on individual foragers (Seeley 2002; Seeley et al. 1991).

ENF from the stop signal is advantageous when maximizing food intake from variable, heterogeneous, and ephemeral food sources, as it increases the speed at which the foragers will switch from a poor quality to an energy-rich food source and thereby allocate the foraging force more efficiently. Based on previous studies (Beekman 2005; Seeley 1986; Seeley et al. 1991), we expected not only waggle dancing, but also the visitation rate by all foragers to decrease when feeder nutrition decreased. Surprisingly, marked bees foraged at the feeder more frequently, while visits by unmarked bees stayed the same after the feeder quality lowered.

There are a number of possible but divergent explanations for why bees visited the feeder more frequently after it dropped in quality. First, experiments were conducted during the height of the summer, and the trials from which we extracted visitation data occurred when there was a local dearth in water. On an individual level, the bees may have been motivated to forage on less viscous food (Nicolson et al. 2013). Second, previous studies have shown that when a colony has low nectar intake, foragers become more willing to feed at patches with low sugar levels (Seeley 1986). Third, we observed that the foragers spent significantly less time in the hive between feeder visits because they were not spending time waggle dancing, therefore they could make more foraging trips instead with this additional available time. This notion is supported by the significantly higher intra-individual foraging frequency for the marked bees visiting the 0.75 M feeder. This higher intra-individual foraging frequency was also observed previously when the energetic state of the individual was uncoupled from that of the colony (Mayack and Naug, 2013). Another possibility is that the novelty of the new 0.75 M feeder could be the cause of the increased foraging trips observed after the switch, but this is less likely as the 2.5 M treatment also involved a feeder switch as well to control for this.

In addition, the surprising increase of foraging frequency shows that regulation of foraging at the group and individual level are not necessarily coupled. The needs of the individual and the group may not necessarily align (Mayack & Naug 2013). This is an inherent property of collective decision-making - there can be a discrepancy between the action of individuals and the behaviour of the group (Couzin 2009). For example, foragers have been shown to continue revisiting a previously profitable feeder, even after they have stopped waggle dancing for this feeder, for up to ten days (Beekman 2005). This difference in individual and collective regulation may allow the colony to remember food sources that might become profitable again (Biesmeijer & Seeley 2005; Granovskiy et al. 2012), while at the same time reallocate recruitment to food sites that are currently more profitable.

Until now a negative feedback mechanism for how waggle dance pheromones would decrease in the forager recruitment process was unknown. We show that the waggle dance pheromones can be modulated by the stop signal, an explicit negative feedback signal, as all four pheromones were consistently lower after the food quality declined, indicating that stop signals have a multi-modal effect on forager recruitment. Most likely, the decline in waggle dance pheromones is an indirect result from the stop signalling, resulting from the decreased waggle dancing activity.

Importantly, the neuronal firing rate model demonstrates that - as in neuronal assemblies in the brain - negative feedback facilitates effective collective behaviour for rapid and efficient forager allocation. Furthermore, our study is one example of possible convergent evolution, in which inhibitory communication has evolved in disparate systems to aid in collective decision-making. The similarities between neuronal networks and honeybee colonies raise the possibility that knowledge of one system can be used to understand the other, and vice versa. Our ability to compare insects to neurons in the human brain emphasizes the utility of social insects as a model system to study collective decision-making and cognition, on multiple levels of biological organization.

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Experimental Setup

During experimental trials the window by the observation hive was opened to provide natural light for the video filming of bee behavior on the bottom frame of the hive. The bottom frame of one side of the hive was blocked with wood such that the bees would enter and dance on only one side of the frame. During the experimental trials the observation hive door would be gently opened to obtain a view of the entire frame and so that we could take audio-recordings of focal foragers (Lau & Nieh 2010).

A camcorder (MS Canon Vixia HF R500) was placed on a tripod far enough away to capture the entire bottom bee frame and the majority of the dance floor of the hive within the video frame. To record the audio of the stop signals, a small electric condenser microphone (RadioShack omnidirectional tie-clip microphone, no. 33-3013) was connected to the video camera through a mini-amplifier (Radioshack no. 2771008). The audio cable connected to the amplifier was split such that one cable was connected to head phones for the observer and the other was fed into the camera for recording. The microphone contained a 40 mm long, 8 mm internal diameter Tygon tubing that was added to the end of the microphone in order to focus the audio-recordings made by focal bees (Visscher & Seeley 2007). This was attached to a 1 m wooden dowel rod using a wire and Parafilm that allowed the observer to point the microphone at a focal honey bee from a distance with minimal disturbance to the hive. Throughout the experimental trials the microphone was held by the observer 1 cm above a focal bee, as in Lau and Nieh's (2010) study. In the second half of the experimental trials, in the summer of 2017, after the feeder was switched, a solid phase microextraction (SPME) portable field sampler with a Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) fiber coating (Sigma-Aldrich, Milwaukee, USA) was attached underneath the microphone, in 10 min intervals, until the end of the trial, to measure waggle dance pheromones (Thom et al. 2007). A total of 3 trails for each treatment (0.75 M and 2.5 M) was conducted in which multiple bees were chosen at random within the 10 minute absorption periods per SPME fiber. These field samplers were stored at 4 ºC until the end of the trial and then analyzed immediately. Six of these were conditioned and re-used randomly throughout the summer. Over the two summers, the bee colony was replaced twice such that at least two trials were conducted with each of the three colonies.

Video analysis

Video analysis focused on instances of waggle dancing and stop signaling. A waggle dance was defined as a bee dancing in a figure-eight pattern while waggling in one direction on the straight part of the figure eight (von Frisch 1967). A stop signal was defined as a high pitch piping noise that was associated with a brief pause in movement of the producer and receiver (Nieh 2010). If the producer of the stop signal received food within one second after it was produced, then we considered it to be a begging call and this was not counted as a stop signal in the final analysis (Nieh 1993; Pastor & Seeley 2005).

GC-MS analysis

After sampling, SPME fibers were desorbed in a Varian 431 Gas Chromatograph (GC) / 220 Mass Spectrometer (MS) for 5 minutes at 40 ºC. All four waggle dance pheromones were separated on an Agilent/J&W model VF-5ms column (30 m length, 0.25 mm column diameter and 0.25 um stationary phase thickness) with a split ratio of 100:1 at 6 min, an injection temperature of 250 ºC, and helium carrier gas at a constant flow of 1 mL per min.

The GC oven had an initial temperature of 40 °C that was held for 5 min, which was then ramped at 50 °C per min to 150 °C with no hold. Next, it was then ramped to 260 ºC at 15 ºC per min with a 10.5 min hold until the end. Individual waggle dance pheromones were identified and quantified using standards that were purchased from Sigma-Aldrich except for Z-(9)-Pentacosene, which was synthesized. The MS was set to electron impact (EI) mode, auto-tuned to 70 eV, and had a scan range of 40 – 650 m/z. Peaks were initially identified by the retention time of the standards and then confirmed using the mass spectrophotometer data and the NIST v. 17 library. The treatment was blind to the operator and analyzer of the instrument and the data, respectively.

Appendix tables

Table A1. Parameter estimates resulting of the nested GLM comparing feeder visitations before and after the feeder switch.

Term	**Estimate**	**Std Error**	**Chi-Square**	**P-value**	**Lower CL**	**Upper CL**
Intercept	0.789	0.031	382.775	< 0.0001*	0.727	0.849
Treatment(Low): Marking(Marked): Time(After)	0.104	0.037	8.156	0.0043*	0.0327	0.176
Treatment(Low): Marking(Unmarked): Time(After)	0.235	0.056	17.986	< 0.0001*	0.126	0.347
Treatment(High): Marking(Marked): Time(After)	-0.010	0.047	0.045	0.832	-0.103	0.0828

Treatment(High): Marking(Unmarked): Time(After)	0.250	0.094	7.34	0.0067*	0.0684	0.438
Treatment(Low)	0.257	0.0312	74.220	< 0.0001*	0.197	0.319
Treatment(Low): Marking(Marked)	0.417	0.0336	169.076	< 0.0001*	0.352	0.483
Treatment(High): Marking(Marked)	0.666	0.0526	201.324	< 0.0001*	0.565	0.771

These were nested within the treatment of the feeder switch and within previously trained and recruited bees.

Table A2. Parameter estimates resulting from the nested GLM of feeder visits.

Term	**Estimate**	**Std Error**	**Chi-square**	**P-value**	**Lower CL**	**Upper CL**
Intercept	0.934	0.040	333.653	< 0.0001*	0.853	1.012
Marking(Marked)	0.444	0.040	135.325	< 0.0001*	0.365	0.524
Marking(Marked): Treatment(Low)	0.190	0.043	20.507	< 0.0001*	0.107	0.275
Marking(Unmarked): Treatment(Low)	0.375	0.0688	32.557	< 0.0001*	0.242	0.512

This includes data only after the feeder was switched with either 2.5 M or 0.75 M sucrose solution at the 50 minute mark half way through the trial. The effect of treatment (2.5 M or

0.75 M) was nested within whether the bees were previously trained (marked) versus recruited during the 120 minute trial (unmarked).

Table A3. Parameter estimates from the nested GLM of waggle dance frequency from within the observation hive dance floor area.

Term	**Estimate**	**Std Error**	**Chi-square**	**P-value**	**Lower CL**	**Upper CL**
Intercept	0.266	0.0475	28.472	< 0.0001*	0.171	0.357
Treatment(Low)	-0.192	0.0475	16.380	< 0.0001*	-0.285	-0.0989
Treatment(Low): Time(After)	-0.272	0.0699	15.744	< 0.0001*	-0.411	-0.137
Treatment(High): Time(After)	0.207	0.0643	10.610	0.0011*	0.0819	0.334

The comparison of waggle dances before and after the feeder switch at the 50 minute mark was nested within the treatment, whether the feeder was switched with a 2.5 M sucrose solution or a 0.75 M sucrose solution.

Figure legends

Figure 1. The frequency of waggle dances inside the observation hive on the dance floor area (the bottom frame) represented with a dashed line for each of the 2 minute intervals measured throughout the 110-minute duration of the trial. Data across the 9 total trails is represented by means with standard errors from Poisson-transformed data. The total number of stop signals produced in the hive are represented by blue bars for the control and red bars for the

experimental trails. For clarity only the 20 – 80-minute period is displayed. The dotted line at the 50-minute mark represents when the feeder was switched from a 2.5 M sucrose solution to either a control 2.5 M (blue line) or experimental 0.75 M (red line) sucrose solution about half-way through the trial.

Figure 2. Total number of stop signals produced from either trained (marked) bees (green) versus recruited (unmarked) bees (orange), for example bees not initially trained to the feeder but recruited at some point, pooled together across time, treatment, and trials for both the control (2.5 M feeder switch) and the experimental (0.75 M feeder switch) groups.

Figure 3. The effect of one burst of stop signals on the waggle dance activity for the focal population, x (dashed), and opposing population, y (solid) . For each panel, the strength of stop signals received by the focal population, $S_x(t)$, with time is depicted on the bottom and the resultant waggle dance activity on the top. Panel (a) depicts the dynamics if there are no stop signals and panel (b) depicts the dynamics with stop signals of strength $\delta = 0.4$ following the feeder switch for time around $60 \leq t \leq 70$. Parameters are chosen such that $\tau = 1, \mu = 1, I = 0.8, \alpha = 0.01, r = 3,$ and $\theta = 1$.

Figure 4. The waggle dance activity of the focal population, x (dashed), and opposing population, y (solid), in the presence of an additional smaller, second burst of stop signals. For panels (a) and (b), the strength of stop signals received by the focal population, $S_x(t)$, with time is depicted on the bottom and the resultant waggle dance activity on the top. In each, the initial burst of stop signals has strength $\delta = 0.4$ and the dynamics are slow with $\tau = 10$. Panel (a) depicts the dynamics with a second stop signal burst of strength $\omega = 0.8\delta$ and panel (b) depicts the dynamics for $\omega = 0.9\delta$. Panel (c) shows the difference in population

activities in the long-time limit, given by $x - y$, across choices of ω for several time scales prescribed by τ.

Appendix figure legends

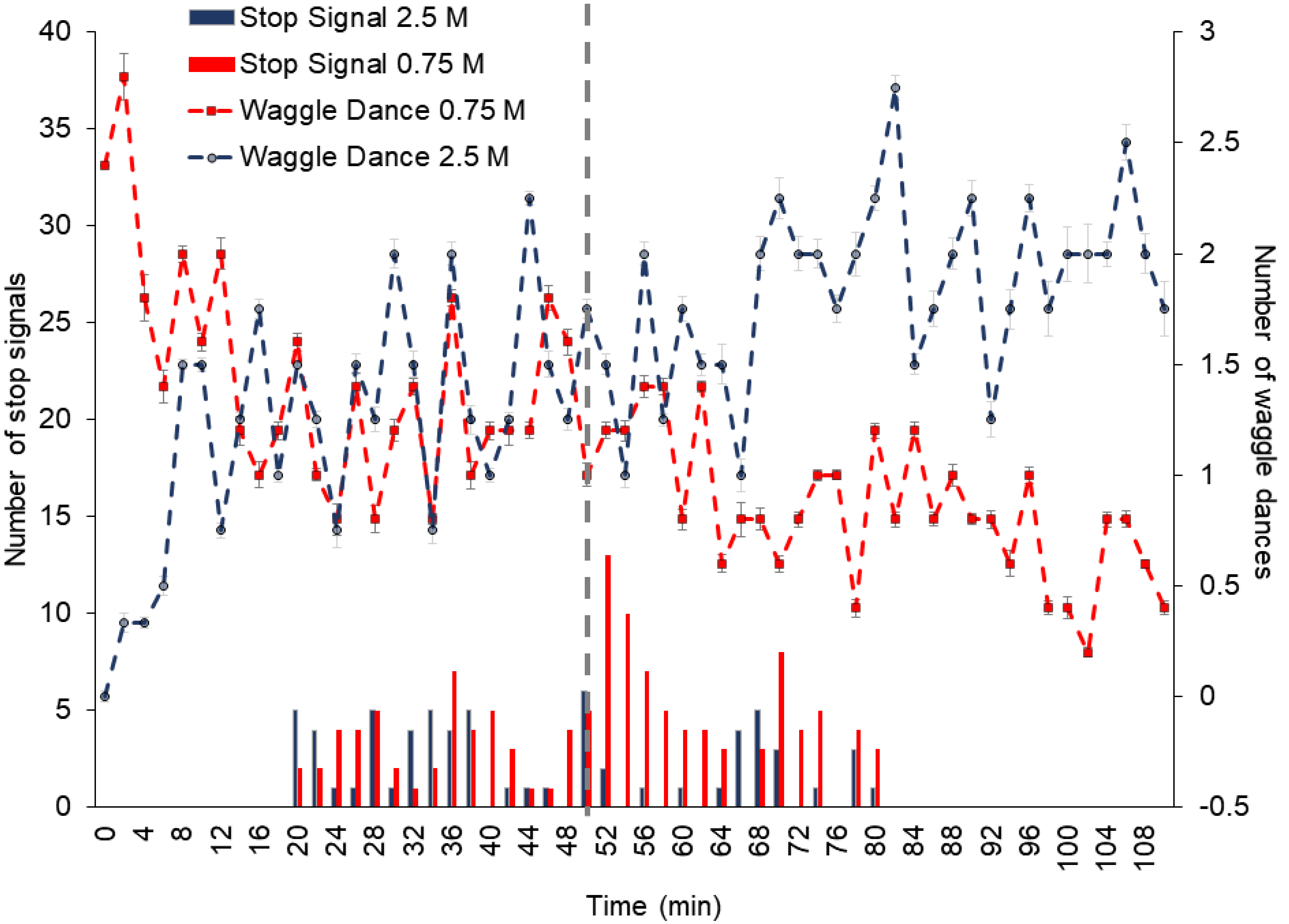
Figure A1. A diagram of the relationships between the populations in the model. The x population is the focal population, the y population represents bees dancing for natural forage, and the u population consists of all uncommitted foragers. Arrows represent interactions, and the associated parameters are their weights. A pointed arrow head indicates positive feedback to the target of the arrow, while a square end indicates inhibition to the target of the arrow. The δ arrows do not come from any one population since we could not ascertain the source of stop signals from our data.

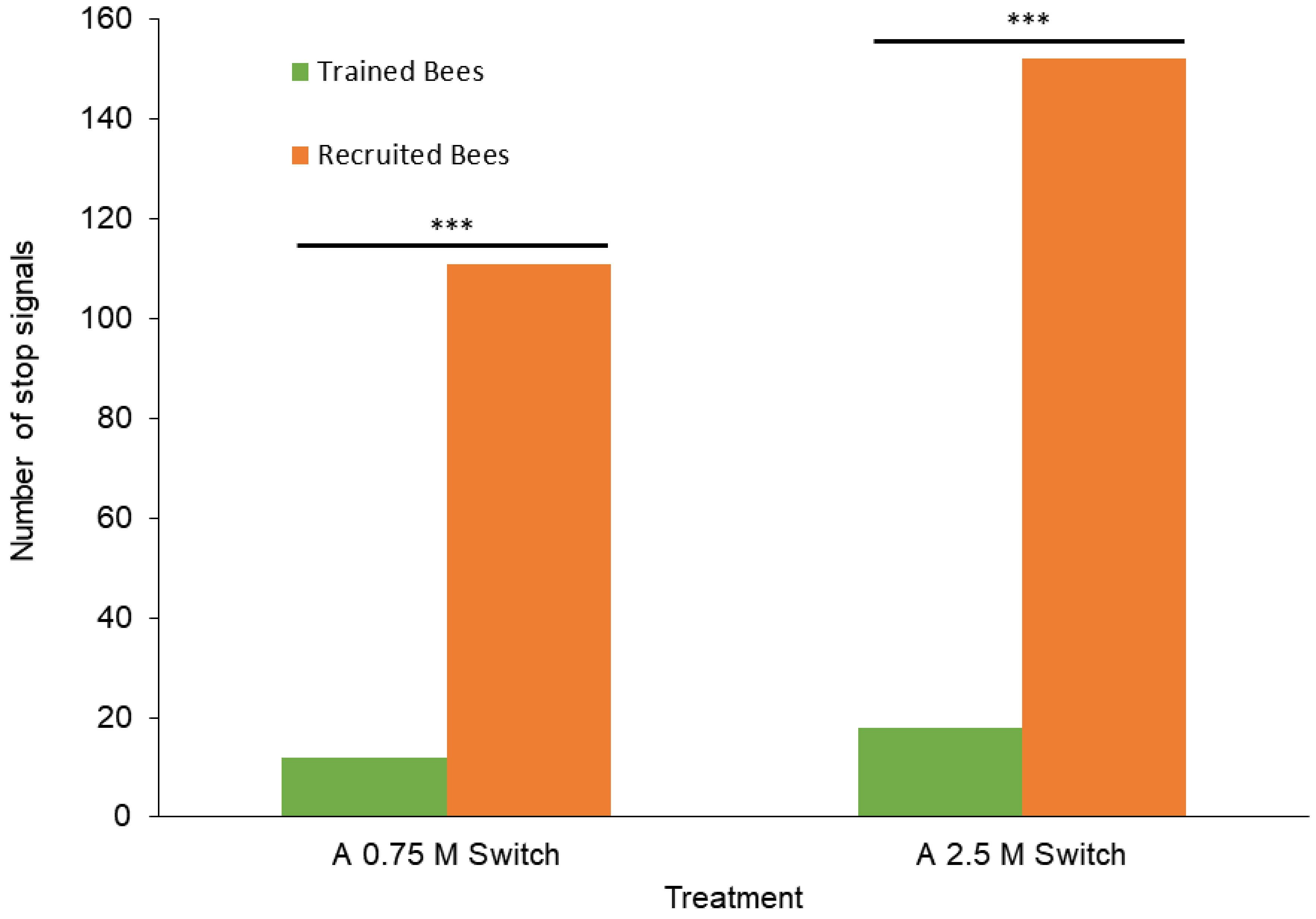
Figure A2. Frequency of feeder visitations for the forager bees (a) previously trained (individually paint marked) and (b) recruited (unmarked bees) to the artificial feeder 50 m away from the observation hive during the 110-minute trial. The number of feeder visitations was recorded at 2-minute intervals for the entire duration of the trial. Data represents means and standard errors of Poisson transformed data across the 9 trials, conducted during the summer of 2016 and 2017. The dotted line at the 50-minute mark represents when the feeder was switched from a 2.5 M sucrose solution to either a control of 2.5 M (blue line) or the treatment of 0.75 M (red line) sucrose solution about half way through the trial.

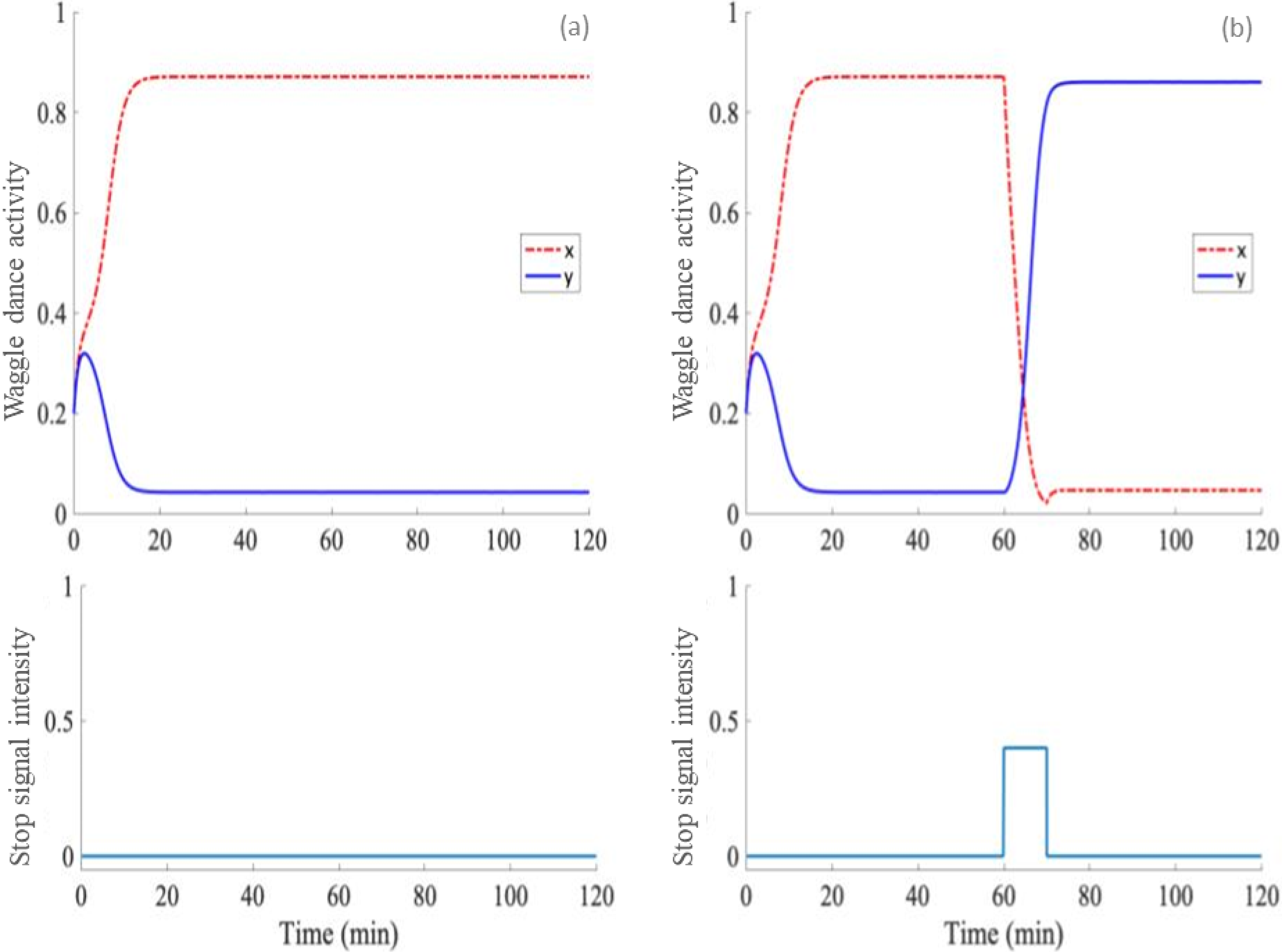
Figure A3. A box plot representing the medians and interquartile ranges of the intra-individual foraging frequency during the 60 minutes after the 2.5 M feeder was switched with either the control 2.5 M (N = 92) or experimental 0.75 M (N = 94) sucrose solution. All trained bees were uniquely paint-marked so individual foraging frequencies could be identified. Therefore, the intra-individual foraging frequency of unmarked recruited bees to the feeder during the trial were unable to be monitored. ** indicates a highly significant difference below the alpha = 0.01 level.

Figure A4. Gas chromatography mass spectrometry of mean relative abundances across time in 10-minute intervals of the four waggle dance pheromones after the feeder was switched with (a) the control of 2.5 M sucrose solution and (b) the experimental 0.75 M sucrose solution. The error bars are represented by standard deviations. Three of the previously identified waggle dance pheromones were verified using standards that were commercially available and Z-(9)-Pentacosene was synthesized for verification. Each pheromone was measured using SPME fiber that was held over 1 cm above the focal bee in the observation hive for 10 minute intervals after the feeder was switched in each trial.

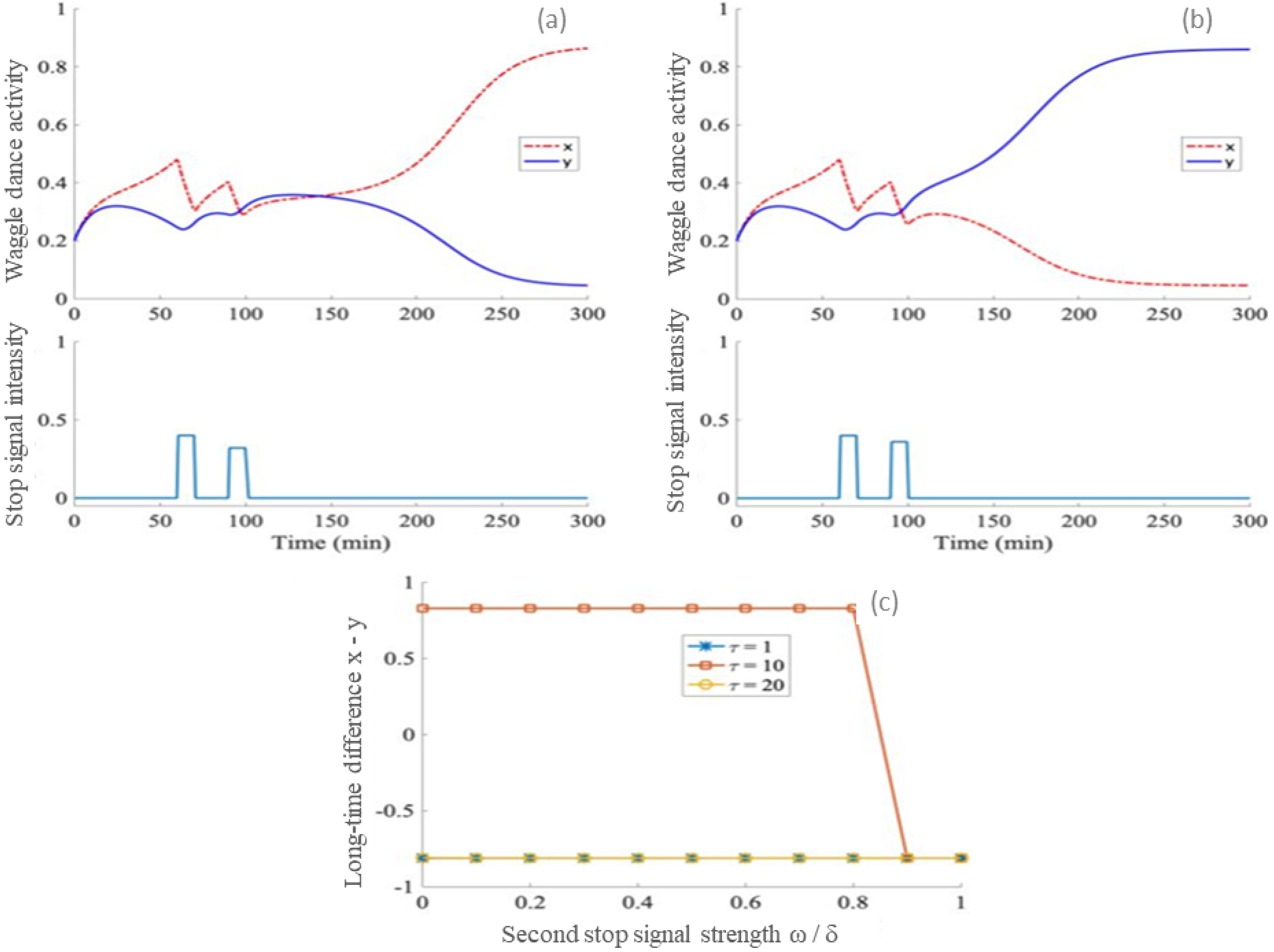
Figure A5. Bifurcation diagrams for the model dynamics, showing the stable (blue dots) and unstable fixed points (red stars) for x across choices of base input level I (a) without stop signals and (b) with stop signals. For these diagrams, fair initial conditions were selected, such that $x(0) = y(0) = 0.2$ for concreteness, though similar dynamics are evoked over a spectrum of fair initial conditions in which $x(0) = y(0)$.


Stop Signal 2.5 M
Stop Signal 0.75 M
Waggle Dance 0.75 M
Waggle Dance 2.5 M
Number of stop signals
Number of waggle dances
Time (min)
0
5
10
15
20
25
30
35
40
-0.5
0
0.5
1
1.5
2
2.5
3
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76
80
84
88
92
96
100
104
108


160
140
120
100
80
60
40
20
0
Number of stop signals
🟩 Trained Bees
🟧 Recruited Bees
***
***
A 0.75 M Switch
A 2.5 M Switch
Treatment



(a)
(b)
Waggle dance activity
1
0.8
0.6
0.4
0.2
0
x
y
Stop signal intensity
1
0.5
0
0
20
40
60
80
100
120
Time (min)


(a)
Waggle dance activity
x
y
Stop signal intensity
Time (min)
(b)
Waggle dance activity
x
y
Stop signal intensity
Time (min)
(c)
Long-time difference x - y
τ = 1
τ = 10
τ = 20
Second stop signal strength ω / δ