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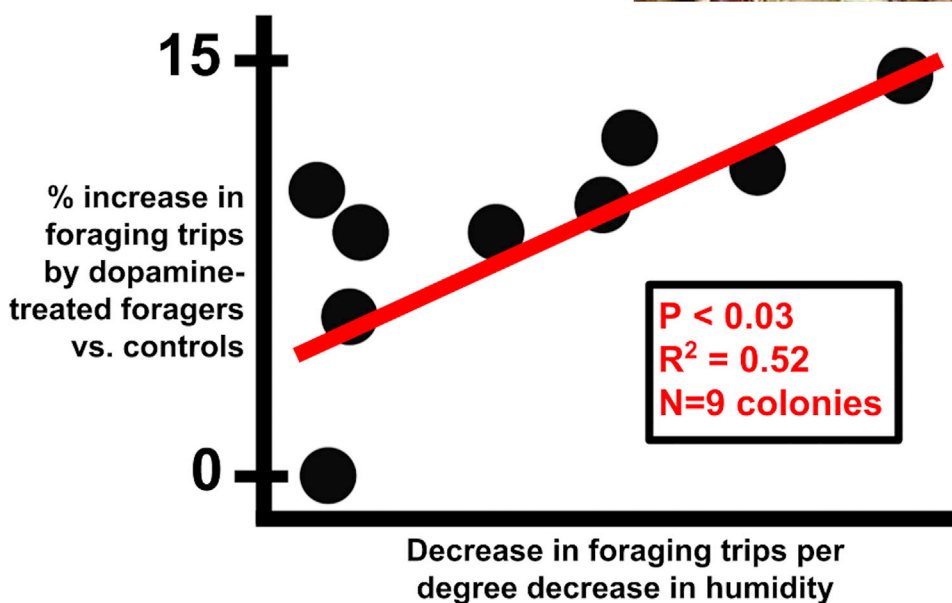
The Role of Dopamine in the Collective Regulation of Foraging in Harvester Ants

RNA-seq:

Persistent behavioral differences among harvester ant colonies are associated with transcriptomic differences in forager brains in neurohormonal signaling & biogenic amine neurophysiology.

Dopamine & Foraging:

Pharmacological increases in forager brain dopamine increase foraging activity relative to control-treated nestmates. The effect of dopamine is strongest in colonies that decrease foraging most in dry conditions.



Daniel A. Friedman, Anna Pilko, Dorota Skowronska-Krawczyk, Karolina Krasinska, Jacqueline W. Parker, Jay Hirsh, Deborah M. Gordon

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HIGHLIGHTS

Red harvester ant colonies vary in how they adjust foraging effort in dry conditions

Colonies that differ in behavior significantly differ in forager brain transcriptome

Pharmacological increases of dopamine increased foraging in field experiments

Foragers from colonies more sensitive to humidity were more stimulated by dopamine

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Article

The Role of Dopamine in the Collective Regulation of Foraging in Harvester Ants

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SUMMARY

Colonies of the red harvester ant (*Pogonomyrmex barbatus*) differ in how they regulate collective foraging activity in response to changes in humidity. We used transcriptomic, physiological, and pharmacological experiments to investigate the molecular basis of this ecologically important variation in collective behavior among colonies. RNA sequencing of forager brain tissue showed an association between colony foraging activity and differential expression of transcripts related to biogenic amine and neurohormonal metabolism and signaling. In field experiments, pharmacological increases in forager brain dopamine titer caused significant increases in foraging activity. Colonies that were naturally most sensitive to humidity were significantly more responsive to the stimulatory effect of exogenous dopamine. In addition, forager brain tissue significantly varied among colonies in biogenic amine content. Neurophysiological variation among colonies associated with individual forager sensitivity to humidity may reflect the heritable molecular variation on which natural selection acts to shape the collective regulation of foraging.

INTRODUCTION

Many biological systems, from brains to insect colonies, are regulated by distributed processes based on local interactions. Variation among groups in collective behavior (Pruitt et al., 2017) can arise from differences between groups in individual response to local interactions or from differences in group composition (Bengston and Jandt, 2014; Jandt et al., 2014). In social insects, such as ants and honeybees, collective behavior is regulated through olfactory interactions among workers (Dornhaus and Franks, 2008; Feinerman and Korman, 2017; Gordon, 1996, 2010; Gordon and Mehdiabadi, 1999). Ants are a globally distributed clade of social insect species (Gibb et al., 2017; Parr et al., 2017; Ward, 2014), and ecological factors shape the evolution of ant collective behavior (Gordon, 2013, 2014; Lanan, 2014). Rapid advances in high-throughput sequencing technologies are providing insight into the genomic, transcriptomic, and epigenomic differences among social insect species (Boomsma et al., 2017; Favreau et al., 2018; Toth and Rehan, 2017). Molecular studies have characterized various mechanistic aspects of division of labor among workers within social insect colonies (Friedman and Gordon, 2016; Kamhi and Traniello, 2013; Linksvayer, 2015; Simola et al., 2016), building on a long history of diverse research into social insect behavior (Detrain and Deneubourg, 2006; Gordon, 1992; Hölldobler and Wilson, 2009; Seeley, 2010; von Frisch, 1974). However, much less is known about the molecular variation among social insect colonies associated with heritable variation in collective behavior (Bengston and Jandt, 2014; Jandt et al., 2014; Jandt and Gordon, 2016).

Across ant and bee species, variation among nestmates in reproductive status and behavioral performance are associated with tissue-specific physiological and transcriptomic differences (Chandra et al., 2018; Gordon, 2016a; Jeanne, 2016; Johnson and Linksvayer, 2010; Toth and Dolezal, 2017). Worker brain biogenic amine and neurohormonal signaling pathways are especially important in regulating the foraging activity of social (Friedman and Gordon, 2016; Gospocic et al., 2017; Kamhi and Traniello, 2013; Yan et al., 2014) and solitary insects (Kamhi et al., 2017; Perry and Barron, 2013; Waddell, 2013). Changes in brain biogenic amine content influence individual worker behavior by altering their sensitivity to certain stimuli such as foraging cues (Bubak et al., 2016; Kamhi and Traniello, 2013; Muscedere et al., 2012; Scheiner et al., 2017). Natural variation among nestmates in sensitivity to stimuli can be adaptive for colony function, for example, by allowing for dynamic task allocation (Gordon, 1989; Gordon and Mehdiabadi, 1999). Dopamine appears to be central to the regulation of individual foraging activity in social insects (Friedman and Gordon, 2016; Kamhi and Traniello, 2013) and other animals (Barron

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et al., 2010; Calhoun et al., 2015; Clark and Dagher, 2014; Friston et al., 2012; Perry and Barron, 2013; Waddell, 2013). In honeybees, brain dopamine titers are higher in foragers than in non-foragers (Tedjakumala et al., 2017; Wagener-Hulme et al., 1999), and pharmacological increases in brain dopaminergic signaling increase foraging activity (Mustard et al., 2012; Perry et al., 2016; Scheiner et al., 2017; Søvik et al., 2014, 2015b). In many ant species, foragers have higher brain dopamine titer than workers of other task group (Kamhi et al., 2017; Seid and Traniello, 2005; Smith et al., 2013), although in some species this pattern appears to be reversed (Penick et al., 2014). Laboratory pharmacological studies in carpenter ants confirm a role for dopaminergic signaling in the regulation of individual foraging activity (Entler et al., 2016). In ants, dopamine is also involved in trophallaxis (Wada-Katsumata et al., 2011) and reproduction (Okada et al., 2015; Penick et al., 2014). Recent pharmacological experiments in the field have been used to examine ant behavior (Malé et al., 2017), but not the biogenic amine neurophysiology of foraging.

Colonies of the red harvester ant, *Pogonomyrmex barbatus*, forage in the desert for seeds that provide both food and water. Foragers lose water while out in the desert sun, and the rate of water loss is higher in dry conditions (Lighton and Bartholomew, 1988; Lighton and Feener, 1989). To manage the tradeoff between food accumulation and water loss, colonies adjust foraging activity to changes in ambient conditions, especially humidity (Gordon, 2013; Gordon et al., 2013; Prabhakar et al., 2012). Colony foraging activity is regulated in a distributed fashion by brief olfactory interactions when one ant assesses the cuticular hydrocarbons of the other (Greene et al., 2013; Greene and Gordon, 2003): an outgoing forager is stimulated to leave the nest by its rate of interaction with incoming foragers with food (Davidson et al., 2016; Greene et al., 2013; Pinter-Wollman et al., 2013; Pless et al., 2015). Since a forager continues to search until it finds a seed, the rate of forager return is related to food availability (Gordon, 1991). Colonies of *P. barbatus* significantly vary in how strongly they reduce foraging activity in dry conditions (Gordon, 1991, 2013; Gordon et al., 2011, 2013), meaning that colonies differ in their sensitivity to humidity. These behavioral differences among colonies of *P. barbatus* persist year after year despite total worker turnover (Gordon, 1991, 2013; Gordon et al., 2011; Gordon and Hölldobler, 1987), and daughter colonies resemble their mothers in the thresholds for dry conditions that lead them to reduce foraging (Gordon, 2013). This variation among colonies of *P. barbatus* in foraging behavior is ecologically important and associated with differences in colony lifetime reproductive success (Gordon, 2013; Ingram et al., 2013). Colony differences in collective behavior could be due to stable colony differences in how foragers adjust their sensitivity to interactions in dry conditions (Davidson et al., 2016; Paggiara et al., 2018).

Here we examine the neurophysiological basis of variation among red harvester ant colonies in how they regulate their collective foraging behavior. Because differences among *P. barbatus* colonies in sensitivity to humidity persist year after year, it appears that successive cohorts of nestmate foragers inherit genetic or epigenetic factors, which bias their foraging activity in dry conditions. Molecular variation among foragers from different colonies may lead to differences in individual forager decisions about whether to forage in dry conditions. This would produce the observed variation among colonies in the collective regulation in foraging. The specific molecular mechanisms that might underlie forager sensitivity to humidity are not known.

To investigate this, we first used RNA sequencing (RNA-seq) to assess transcriptomic differences in forager brain tissue between 2 sets of colonies that naturally varied in how strongly they reduced foraging activity in dry conditions. Patterns of transcript differential- and co-expression between the 2 sets of colonies included significant changes in biogenic amine and neurohormonal pathways. To test the role of dopamine signaling in the regulation of individual foraging activity, we manipulated the brain dopamine titer of foragers in field experiments during 2 consecutive years. Foragers with increased brain dopamine titer made significantly more foraging trips than control-treated nestmates, and foragers treated with a metabolic inhibitor of dopamine synthesis significantly decreased foraging activity relative to control-treated nestmates. In the set of 9 colonies used in pharmacological experiments, we also characterized natural patterns of behavioral variation and forager brain biogenic amine content. Colonies that were naturally more sensitive to humidity tended to be more responsive to the stimulatory effect of exogenous dopamine. This suggests that a forager's decision whether to leave the nest on its next trip may be influenced by dopamine, so that variation among colonies in the regulation of foraging in response to conditions may be due to differences among colonies in forager biogenic amine neurophysiology.

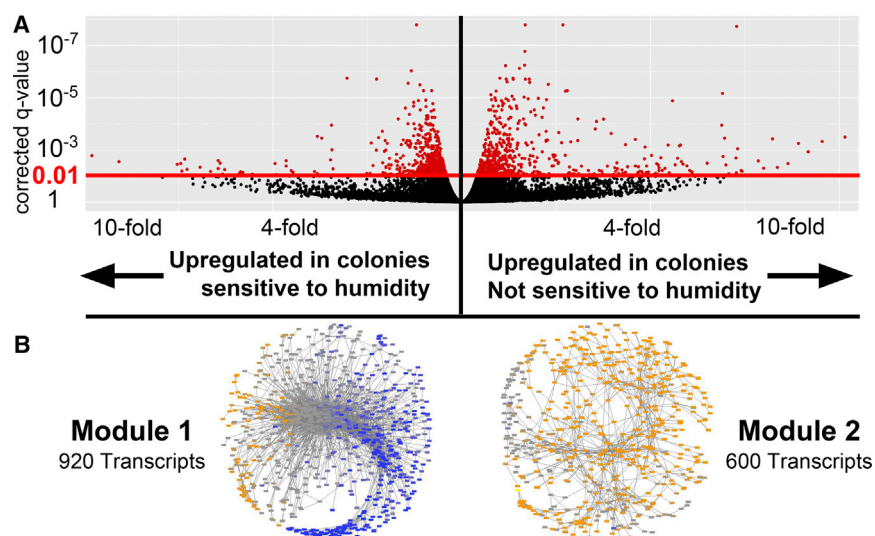


Figure 1. Forager Brain Transcriptomic Differences Are Associated with Differences in Colony Behavior

(A) Volcano plot representing forager brain gene expression differences between groups of colonies that vary in their sensitivity to humidity (see [Transparent Methods 1A](#)). Red transcripts are significantly differentially expressed at FDR-corrected q-value < 0.01.

(B) Transcript co-expression graph. Nodes are transcripts that are colored according to mean fold-change between the 2 sets of colonies. Edges connecting nodes represent correlated expression levels across all 18 libraries. Blue nodes are transcripts upregulated in foragers from colonies that strongly reduce foraging on dry days. Orange nodes are transcripts upregulated in foragers from colonies that do not strongly reduce foraging on dry days. Gray nodes are transcripts that were evenly expressed between the 2 groups of colonies.

RESULTS

Forager Brain Transcriptomic Differences Are Associated with Differences in Colony Behavior

Forager brain transcriptomes differed between 2 sets of *P. barbatus* colonies that differed in how strongly they reduced foraging activity in dry conditions (see [Transparent Methods 1A](#)). We collected active foragers on the same morning from 6 colonies, of which 3 strongly reduced foraging in dry conditions and 3 did not. For each colony, 3 replicate RNA-seq libraries were sequenced from the mRNA extracted from 3 pooled dissected forager brains. We used the kallisto/sleuth RNA-seq analysis pipeline (Bray et al., 2016) to quantify transcript expression against the *P. barbatus* reference transcriptome (Smith et al., 2011). Of the 20,387 transcripts in the reference transcriptome 273 were significantly differentially expressed in whole forager brains between the 2 sets of colonies (Figure 1A). A total of 113 transcripts were upregulated in colonies that do not reduce foraging on dry days, and 160 transcripts were upregulated in colonies that strongly reduce foraging on dry days. Across the whole transcriptome, the per-transcript mean expression levels were correlated between the 2 sets of colonies (Pearson $r^2 = 0.99$). A linear principal component analysis in sleuth (Pimentel et al., 2016) showed that colony transcriptomes did not cluster clearly by behavioral type.

Overall, the list of 273 transcripts significantly differentially expressed in either direction was enriched in the terms “hormone activity,” “oxidoreductase activity,” and “copper ion binding” (p value < 0.0005, Fisher’s exact test, false discovery rate [FDR] < 0.25). The 160 transcripts upregulated in colonies that did not reduce foraging in dry conditions did not show any GO term enrichment with FDR < 0.9. The 113 transcripts upregulated in the colonies that strongly reduced foraging in dry conditions were enriched in GO terms “neuropeptide signaling pathway,” “catecholamine metabolic process,” and “receptor binding” (all with p value < 0.005 and FDR < 0.3). The enrichment in biogenic amine signaling and metabolism GO terms was reflected in the higher expression of the neurometabolic enzymes phenylalanine hydroxylase (3.17-fold change, XM_011648879.1, q-value = 0.0049) and tyramine β -hydroxylase (1.55-fold change, XM_011649732.1, q-value = 0.00011, alternate transcript from same locus XM_011649733.1 upregulated 1.44-fold, q-value = 1.60×10^{-7}). The enrichment of the GO term

“neuropeptide signaling pathway” was driven by increased expression of transcripts from pathways involved in the regulation of insect foraging behavior, including the FMRFamide receptor (1.69-fold change, XM_011639920.1, q-value = 0.0036), an allatostatin peptide hormone (1.2-fold change, XM_011640492.1, q-value = 1.77×10^{-5}), and the hypertrehalosaemic prohormone (1.82-fold change, XM_011643332.1, q-value 1.60×10^{-7}), all of which are important in insect neurohormonal signaling in solitary insects (Caers et al., 2012; Orchard and Lange, 2013; Verlinden et al., 2015). In addition, foragers from colonies that strongly reduced foraging on dry days had significantly higher expression of an inositol monophosphatase (3.1-fold change, XM_011632239.1, q-value = 5.38×10^{-5}), a phosphoinositide phospholipase (1.33-fold change, XM_011632265.1, q-value = 0.0021), and the glycogen synthase kinase 3 β interaction protein (1.54-fold change, XM_011646061.1, q-value = 0.0002). These 3 protein products are involved in the inositol phosphate signaling pathway (Berridge, 2009), implicated in the transcriptional changes between nurse and forager honeybees (Lutz et al., 2012; Whitfield et al., 2003).

To further examine the functional relationships among brain-expressed transcripts in *P. barbatus* foragers, we performed a transcriptome-wide co-expression analysis (Mikheyev and Linksvayer, 2015; Morandin et al., 2016), using Cytoscape (see Transparent Methods 1A) (Su et al., 2014). The final transcript co-expression network consisted of 1,933 correlated transcripts across 167 isolated subnetworks. Only 2 of the 167 subnetworks had more than 50 transcripts (920 and 600 transcripts, respectively, hereafter referred to as “Module 1” and “Module 2,” Figure 1B). The 3 next-largest subnetworks had between 10 and 50 transcripts, and the remaining 162 connected subnetworks all had fewer than 6 connected transcripts. The 2 large connected transcript co-expression modules described above were biased in their mean expression values between the 2 groups of colonies. Of the 920 transcripts in Module 1, 656 (71%) had higher, but not necessarily significantly different mean expression levels in colonies that strongly reduced foraging activity in dry conditions. Of the 600 transcripts in Module 2, 545 (91%) had higher mean expression values in colonies that did not strongly reduce foraging activity in dry conditions. In addition, the modules were functionally enriched in several GO categories of neurophysiological relevance. Module 1 was enriched in GO terms “G-protein-coupled amine receptor activity,” “regulation of neurotransmitter levels,” and “postsynaptic signal transduction” (all p values < 0.001, FDR < 0.05). Module 1 was strongly depleted in transcripts relating to “odorant binding” and “olfactory receptor activity.” Module 1 was significantly depleted in the GO term “cellular nitrogen compound metabolic process,” whereas this exact term was significantly enriched in Module 2. In addition, Module 2 was enriched in GO term “cellular response to stress,” and multiple GO terms relating to metabolism (all p values < 0.001, FDR < 0.05).

Manipulation of Forager Brain Dopamine Titer Alters Foraging Activity

Based on the aforementioned transcriptomic results, we hypothesized that differences among colonies in forager brain neurophysiology could lead to colony differences in behavior. To test this hypothesis, we observed the behavior of foragers with altered brain dopamine titers in the field.

First, we used mass spectrometry to determine that oral administration of dopamine to *P. barbatus* workers significantly raises single brain dopamine titers in a dose- and time-dependent fashion (see Transparent Methods 1B–1D). We measured single forager brain dopamine titer at 2 time points (18 and 66 hr after treatment), using two concentrations of oral dopamine solution (3 mg/mL and 30 mg/mL), with 6–9 ant brains measured per biological group. At the 18-hr time point used for later behavioral experiments, brain dopamine levels in single *P. barbatus* workers in the lower-dose (3 mg/mL dopamine) treatment group were significantly increased by 2.67-fold relative to controls (Figure 2) ($t = 2.61$, $df = 15$, $p = 0.0199$). In the higher-dose (30 mg/mL dopamine) treatment group, brain dopamine titers were increased by 19.91-fold relative to controls ($t = 4.82$, $df = 15$, $p < 0.0002$). Brain dopamine titers were still significantly increased in both treatment groups relative to controls on the third day after treatment (both $p < 0.005$).

Next, we tested the hypothesis that increasing forager brain dopamine titer would increase foraging activity in the field (see Transparent Methods 1E). Foragers treated with 3 mg/mL dopamine made significantly more foraging trips compared with their control-treated nestmates in field experiments over 2 years (Figure 3, Data S1). In 2016, dopamine-treated foragers made on average 20.5% more foraging trips than control-treated nestmates ($N = 9$ colonies, effect $\neq 0$, $p < 0.001$, $t = 5.60$). In the same 9 colonies in 2017, dopamine-treated foragers made on average 10.3% more foraging trips than control-treated nestmates (effect $\neq 0$, $p < 0.001$, $t = 6.09$). A colony’s response to dopamine in 2016 was not significantly correlated with its response to dopamine in 2017 (Kendall rank correlation $p > 0.5$).

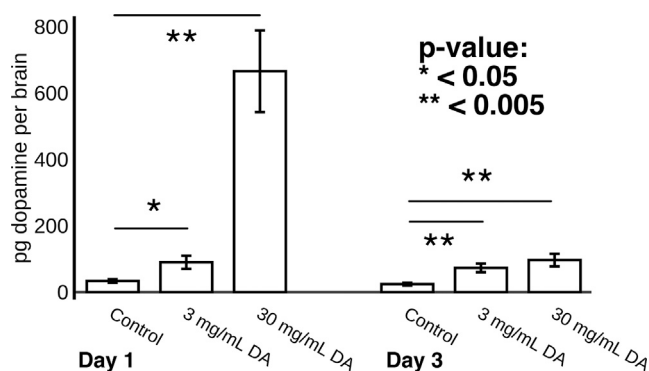


Figure 2. Oral Administration of Dopamine to *P. barbatus* Workers Significantly Raises Single Brain Dopamine Titers in a Dose- and Time-Dependent Fashion

Data shown on y axis are mean \pm SEM of dopamine titers in picograms per single dissected *P. barbatus* brain. Dopamine titer was measured 1 and 3 days after oral administration of 0 mg/mL, 3 mg/mL, and 30 mg/mL dopamine in water solution. No. of single brains measured per group is 6–9. Dopamine titer was measured by mass spectrometry with a labeled internal dopamine standard (see [Transparent Methods 1B–1D](#)).

To test the hypothesis that decreasing forager brain dopamine would lead to a decrease in foraging activity, we tested the effect of 3-iodotyrosine (3IY) on foraging activity. 3IY is a metabolic inhibitor that reduces brain dopamine titer in insects ([Neckameyer, 1996](#)) however, we did not use mass spectrometry to quantify brain dopamine titer change due to 3IY. In the same colonies used in the aforementioned dopamine experiments, foragers treated with 3 mg/mL 3IY made significantly fewer foraging trips than control-treated nestmates ([Figure 3, Data S1](#)). In 2017, 3IY-treated foragers made on average 19.1% fewer foraging trips than control-treated nestmates (effect \neq 0, $p < 0.001$, $t = 13.60$).

Colonies Naturally More Sensitive to Humidity Are More Responsive to Dopamine

Next we asked how variation in colony response to pharmacological manipulation was associated with natural variation in how strongly colonies reduced foraging activity in dry conditions. Colony reduction of foraging activity in response to humidity was quantified by estimating the decrease in daily foraging trips made by the colony per percent decrease in humidity (see [Transparent Methods 1F](#)). As in previous studies, the 9 colonies strongly differed in how much they reduced foraging activity in dry conditions. The estimated reduction in foraging trips made per colony per 1% reduction in humidity ranged from 27 to 266.

Colonies that more strongly reduced foraging activity in dry conditions were more responsive to the stimulatory effect of exogenous dopamine ([Figure 4](#)) ($N = 9$ colonies, Kendall's rank correlation test, $\tau = 0.44$, $p = 0.013$, Pearson's correlation $r^2 = 0.52$, $p = 0.028$). There was no significant relation between a colony's response to 3IY and how strongly it reduced foraging activity in dry conditions (Pearson's correlation test $p > 0.7$).

Colonies Significantly Vary in Forager Brain Biogenic Amine Content

Brain dopamine and serotonin titers were quantified from active foragers from the 9 colonies used in the aforementioned pharmacological experiments (see [Transparent Methods 1G](#)). Colonies significantly varied in their average forager brain dopamine to serotonin ratio ([Figure 5](#)) ($N = 9$ colonies, $N = 5$ samples/colony of 2 pooled brains, ANOVA for effect of colony, $p < 0.001$). There was no significant relationship between the colony's average forager brain dopamine to serotonin ratio and how strongly the colony reduced foraging activity in dry conditions earlier in the season (Spearman's $R = -0.13$, $p = 0.74$). Colonies with higher dopamine to serotonin ratios tended to be less responsive toward the stimulatory effects of dopamine, but this trend was not significant (Spearman's $R = -0.48$, $p = 0.19$).

DISCUSSION

We used transcriptomic, pharmacological, and physiological experiments to assess the molecular basis of variation among colonies of *P. barbatus* in foraging behavior.

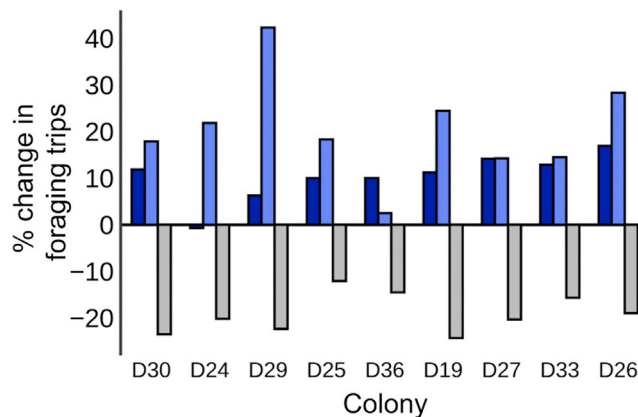


Figure 3. Manipulation of Forager Brain Dopamine Titer Alters Foraging Activity

The x axis shows colony ID. The y axis shows the percent change in foraging trips made by drug-treated foragers relative to controls (see [Transparent Methods 1E](#)). Top bars show percent increase in foraging trips made by foragers treated with 3 mg/mL dopamine; light blue bars, 2016; dark blue bars, 2017. Bottom bars show percent decrease in foraging trips made by foragers treated with 3 mg/mL 3IY, 2017.

Colonies that differed in the regulation of foraging activity significantly differed in forager brain gene expression ([Figure 1A](#)). These transcriptomic changes were enriched in biogenic amine and neurohormone-related signaling transcripts. In addition, colonies that differed in how they regulate foraging in dry conditions appeared to differ in the use of two large modules of co-expressed transcripts related to neural signaling and metabolism ([Figure 1B](#)). This suggests that differences among colonies in foraging activity may be due to differences in how foragers evaluate foraging-related stimuli, reflected in transcriptomic changes in their neural signaling pathways (e.g., as in [Lucas and Sokolowski \[2009\]](#)). To our knowledge, this is the first reported measurement of brain transcriptome from foraging ants in their natural context. Several of the same pathways differentially expressed between foraging and non-foraging nestmates in other social insects, such as neuropeptides and inositol phosphate metabolism ([Friedman and Gordon, 2016](#); [Kamhi and Traniello, 2013](#); [Yan et al., 2014](#)), were differentially expressed in the brain tissue of foragers from colonies that vary in foraging activity. These pathways are deeply conserved and often involved in regulating foraging and feeding behavior across insect species ([Barron et al., 2010](#); [Gospocic et al., 2017](#)). Here we extend these results to suggest that the neuromolecular mechanisms involved in behavioral variation among solitary insects and social insect nestmates may also play a role in generating collective behavioral differences among colonies ([Jandt et al., 2014](#)).

Within a colony, variation among social insect workers in foraging activity has been linked to changes in the neuromodulatory biogenic amines dopamine, tyramine, and octopamine ([Kamhi et al., 2017](#); [Scheiner et al., 2006, 2017](#)), apparently by altering the sensitivity to foraging-related cues ([Kamhi and Traniello, 2013](#)). Our transcriptomic results from forager brains, showing differential expression of the biogenic amine metabolic genes phenylalanine hydroxylase and tyramine β -hydroxylase, were consistent with alterations in either dopamine or octopamine metabolism. In studies of behavioral variation among nestmates, dopamine has consistently been associated with foraging activity in ants ([Kamhi and Traniello \[2013\]](#), although see [Penick et al. \[2014\]](#)). In addition, the role of dopamine in regulating the foraging activity of ant and bee workers has been confirmed with pharmacological experiments ([Entler et al., 2016](#); [Perry and Barron, 2013](#); [Søvik et al., 2014](#)), but such experiments have not yet been done to investigate the role of octopamine or tyramine in the regulation of ant foraging. Here, we tested only the role of dopamine, and further work is needed to examine the role of other biogenic amines in the regulation of foraging in harvester ants.

We found differential expression of key biogenic amine metabolic loci in forager brain tissue from colonies that differed in their sensitivity to humidity. In addition, biogenic amine metabolism-related transcripts were significantly enriched in the list of transcripts upregulated in colonies sensitive to humidity. This suggests that colony differences in sensitivity to humidity may be related to differences in forager brain biogenic amine metabolism, although transcriptomic differences alone are not sufficient to demonstrate physiological impact. Biogenic amine metabolic loci have a well-known role in insect cuticle sclerotization and tanning ([Kramer et al., 2001](#); [Rebeiz and Williams, 2017](#)) and may influence desiccation tolerance or

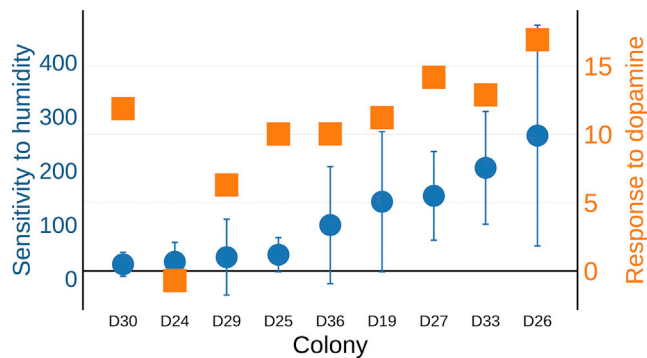


Figure 4. Colonies Naturally More Sensitive to Humidity Are More Responsive to Dopamine

x Axis represents colony. The left y axis shows sensitivity to humidity, the estimated number of fewer foraging trips made by the colony per percent decrease in humidity (see [Transparent Methods 1F](#)). The right y axis shows response to dopamine, the estimated number of fewer forager trips (\pm SEM) made by the colony per percent decrease in relative humidity (See [Transparent Methods 1F](#)).

coloration in *P. barbatus*. However, our RNA-seq data were generated from dissected brain tissue without residual head cuticle, so we cannot determine whether forager cuticle expression of biogenic amine metabolic loci is associated with variation among colonies in foraging behavior. Whole-brain biogenic amine titers vary consistently between foraging and non-foraging workers in ant and bee colonies ([Kamhi and Traniello, 2013](#)), but previous transcriptomic studies have not identified differential expression of biogenic amine metabolic genes between nestmates ([Feldmeyer et al., 2014](#); [Manfredini et al., 2014](#); [Mikheyev and Linksvayer, 2015](#)). This may be because brain-specific transcriptomic changes important for biogenic amine metabolism are obscured when whole-body or whole-head gene expression profiles are measured ([Johnson et al., 2013](#)). Alternatively, associations between worker task and brain biogenic amine content may be driven by mechanisms other than the brain-specific differential expression of metabolic loci, for example, by changes in metabolite transport from the hemolymph.

Pharmacological increases of forager brain dopamine significantly increased foraging activity in foragers relative to their nestmates the following day ([Figure 3](#)). Conversely, ostensible reductions of forager brain dopamine significantly reduced foraging activity the following day ([Figure 3](#)). To our knowledge this is the first behavioral pharmacological manipulation of biogenic amine neurophysiology in ants in the field. These experiments link dopamine signaling and foraging activity in ants, showing a positive association that is consistent with the results of previous pharmacological studies on the role of dopamine signaling in the regulation of insect foraging ([Perry and Barron, 2013](#); [Søvik et al., 2015a](#)).

There are several non-exclusive behavioral mechanisms that may explain how changes in dopamine signaling influence an individual forager's decision to leave the nest on its next trip. First, changes in dopamine signaling could change how a forager perceives interactions with nestmates. Previous work has suggested that increases in brain dopamine titer increase an ant's sensitivity to foraging cues such as pheromone trails ([Kamhi and Traniello, 2013](#)). Foragers of *P. barbatus* are not stimulated to leave the nest by pheromone trails ([Prabhakar et al., 2012](#)). Instead, olfactory interactions between outgoing and returning foragers with food stimulate outgoing foragers to leave the nest ([Davidson et al., 2016](#); [Pinter-Wollman et al., 2013](#)). We suggest that increases in dopamine signaling may increase forager sensitivity to these olfactory interactions, whereas decreases in dopamine signaling may decrease forager sensitivity to interactions. In this way, increased dopamine signaling might override the negative influence of low humidity. Second, changes in dopamine signaling could alter the forager's perception of its own physiological state or the harshness of the environment, including low humidity. Self-evaluation of physiological state is important in the regulation of individual foraging activity in other ant species ([Robinson et al., 2012](#); [Silberman et al., 2016](#)), and dopamine can influence the evaluation of environmental stimuli and organismal state ([Barron et al., 2015](#); [Friston et al., 2012](#); [Scaplen and Kaun, 2016](#)). Thus, increases in dopamine signaling may lead dopamine-treated *P. barbatus* foragers to overestimate their physiological readiness for foraging given the perceived humidity, and vice-versa for 3IY-treated foragers. Third, dopamine signaling influences the light-dependent circadian rhythm of insects ([Hirsh et al., 2010](#); [Nall and Sehgal, 2014](#)), and changes in dopaminergic signaling could interact with circadian patterns of gene expression in the brain of *P. barbatus*.

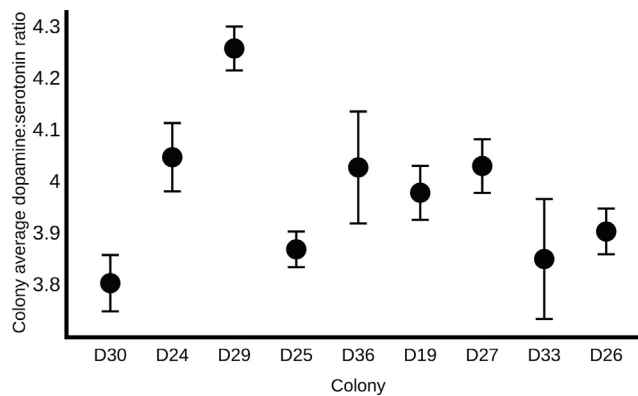


Figure 5. Colonies Significantly Vary in Forager Brain Biogenic Amine Content

The x axis represents colony. The y axis is the average forager brain dopamine to serotonin ratio \pm SEM, as measured by high-performance liquid chromatography (see [Transparent Methods 1G](#)). N = 5 samples of 2 pooled forager brains per colony.

foragers ([Ingram et al., 2005, 2016](#)) and stimulate foraging despite low humidity. Finally, our pharmacological treatments may alter foraging activity by changing the brain titer of some neurotransmitter other than dopamine. By the time of the behavioral observation, some of the ingested dopamine may have been metabolized into related compounds such as tyramine or octopamine. Both tyramine and octopamine regulate some aspects of foraging in bees ([Kamhi et al., 2017](#); [Scheiner et al., 2006, 2017](#)), although less is known about their role in ant foraging. Similarly, 3IY may modulate the brain titers of biogenic amines other than dopamine, or act directly on aminergic receptors.

Colonies that more strongly reduced foraging activity in dry conditions were more sensitive to the stimulatory effects of dopamine on foraging activity ([Figure 4](#)). This significant correlation suggests that exogenous dopamine may improve forager perception of daily conditions, generating a more positive response to the cues that stimulate foraging in the colonies that reduce foraging activity most when conditions are poor. Alternatively, elevated forager brain dopamine titers may simply override the forager's ability to detect that environmental conditions are poor, eliciting a higher stimulatory response in colonies most sensitive to changes in ambient humidity. Variation among colonies could arise from shared genetic or epigenetic factors that influence dopaminergic neurophysiology. Colonies of *P. barbatus* may show stable differences in how they regulate foraging activity in dry conditions ([Gordon, 2013](#)) due to persistent factors that modulate the influence of humidity on forager behavior.

Colonies significantly differed in average forager brain dopamine to serotonin ratio ([Figure 5](#)). To our knowledge, this is the first measurement of brain biogenic amine titers in an ant species outside of the laboratory. Differences among colonies in forager brain biogenic content were not correlated with variation among colonies in sensitivity to humidity or response to pharmacology. Thus variation among colonies of *P. barbatus* in foraging activity may be due to changes in neurophysiology at a finer scale than the whole brain. For example, biogenic amine metabolic differences between foraging and non-foraging honeybees occur within specific subregions ([Schulz et al., 2003](#)). The expression of dopamine receptors or dopamine-activated neural signaling pathways may also influence behaviorally important dopaminergic neurophysiology ([Landayan et al., 2018](#); [Yamamoto and Seto, 2014](#)). Such non-metabolic effects in biogenic amine signaling pathways would not lead to observable differences among colonies in their average level of forager whole-brain dopamine to serotonin ratio. Our transcriptomic results from forager brains showed that differences between colonies in foraging behavior are associated with the expression of genes related to neural signaling aspects of biogenic aminergic neurophysiology. Forager brain octopamine or tyramine titers may also be important in foraging activity ([Kamhi et al., 2017](#); [Perry and Barron, 2013](#); [Scheiner et al., 2017](#)) and were not measured here. Further analyses of forager neurophysiology are needed to explore how differences among colonies in brain biogenic amine signaling and metabolism are associated with differences among colonies in behavior.

Here we link variation among colonies in sensitivity to humidity with variation among colonies in forager dopaminergic neurophysiology. Transcriptomic results suggested that natural variation among colonies

in forager neurophysiology may be the source of differences among colonies in sensitivity to humidity, implicating biogenic amine and neurohormonal signaling. Pharmacological experiments found that foragers from colonies who reduced foraging most in dry conditions were most stimulated by exogenous dopamine. This further supports a role for dopamine signaling in the variation among colonies in sensitivity to humidity.

Our study leaves open several questions to be pursued in future research. First, our transcriptomic results implicate a variety of molecular pathways that are associated with behavioral differences among colonies. The transcriptomic differences were enriched in loci related to biogenic amine metabolism and signaling. This result is consistent with a role not only for dopamine in behavioral differences among colonies but also for other neuromodulators such as tyramine or octopamine. Although our pharmacological treatments demonstrated a clear influence on foraging behavior of altered brain dopamine titers, it would be interesting to measure the influence of drug treatments on the metabolism of biogenic amines other than dopamine, or on other neurotransmitter receptors. Second, our results do not specify whether the increase in foraging activity due to dopamine treatment is because a few foragers dramatically increase foraging activity, or whether most increase activity slightly. We are currently investigating this in experiments with individually marked ants. Finally, there is much to learn about how dopamine affects a forager's decision to leave the nest on its next foraging trip and how this is related to the stimulation of foraging by the rate of olfactory encounters with returning foragers (Davidson et al., 2016; Pinter-Wollman et al., 2013).

Molecular studies in social insects have primarily examined differences between workers performing different tasks within the same colony (Johnson and Linksvayer, 2010; Kamhi and Traniello, 2013; Linksvayer, 2015; Robinson, 2003). However, it is the variation among colonies in task performance that leads to variation in reproductive success and thus the evolution of collective behavior (Gordon, 2011, 2013; 2016b). To understand the evolution of colony behavior, we need to learn how patterns of molecular variation among colonies are shaped by the interaction of genetic, epigenetic, and environmental factors (Abouheif et al., 2014; Rehan and Toth, 2015; Toth and Rehan, 2017; Toth and Robinson, 2007; West-Eberhard, 2003).

METHODS

All methods can be found in the accompanying [Transparent Methods supplemental file](#).

DATA AND SOFTWARE AVAILABILITY

Reads are available in the Short Read Archive (BioProject: PRJNA277638).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Transparent Methods, one table, and one data file and can be found with this article online at <https://doi.org/10.1016/j.isci.2018.09.001>.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.M.G. and D.A.F.; Field experiments and observations, D.A.F.; RNA-seq molecular biology, A.P., D.S.-K., and D.A.F.; RNA-seq analysis, D.A.F.; Mass spectrometry measurements, K.K.; HPLC measurements, J.H. and J.W.P.; Writing, D.M.G. and D.A.F.; Funding acquisition, D.M.G. and D.A.F.; Supervision, D.M.G.

DECLARATION OF INTERESTS

The authors state no competing interests.

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Supplemental Information

The Role of Dopamine in the Collective

Regulation of Foraging in Harvester Ants

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4. Supplemental References

1. Transparent Methods

A. Transcriptomic Methods

Foragers of *Pogonomyrmex barbatus* were collected into liquid nitrogen between 06:00-08:00 on 8/20/2014, from colonies at a long-term field site near Rodeo NM, at which all colonies have been identified and censused since 1985 (Ingram et al., 2013). Foragers were collected as soon as they left the nest entrance, not carrying anything, and moved off the nest mound onto a foraging trail or fan. Foragers were collected from 6 mature colonies in which foraging behavior had been monitored in previous work. Three of the colonies strongly reduced foraging on dry days relative to humid days, in counts of foraging activity made in in 2011 and 2012 (Gordon, 2013), while the other 3 colonies did not strongly reduce foraging activity on dry days. There were similar differences between some of the colonies in each group in foraging activity measured in 2009 (Gordon et al., 2011). Other work shows that colonies are consistent from year to year in foraging activity (Gordon, 1991; Gordon et al., 2013). No ethical precautions were required for this study.

Samples were shipped from the field site to the laboratory in liquid nitrogen (Cryoship), and stored at -80C. Whole brains were cleanly dissected away from muscular, glandular, and connective tissue in cold RNAlater buffer. Dissected brains were frozen in Triazol at -80C until RNA extraction. Total RNA was extracted from dissected brains using a Direct-zol RNA extraction kit (Zymo Research). RNA concentration was assessed using Qubit 2.0 RNA HS reagents (Thermo Fisher Scientific) and purity using a NanoDrop (ND 2000, Thermo Scientific). Total RNA was assessed for quality using a BioAnalyzer tapestation (Agilent Technologies), and samples with RNA Integrity Number (RIN) > 8.0 were used to make RNA libraries. 3 libraries were made for each of 6 colonies. Each library consisted of poly-AAA+ mRNA extracted from the pooled dissected brains of 3 foragers. Libraries were generated using Illumina's TruSeq Stranded mRNA Sample Prep Kit. Reads are available in the Short Read Archive (BioProject: PRJNA277638).

For the kallisto/sleuth differential gene expression analysis pipeline, the reference transcriptome was indexed by kallisto v.0.42.5 (Bray et al., 2016). RNA-seq reads were pseudoaligned to the indexed reference transcriptome with kallisto, a method with good consistency to other RNA-seq and rt-qPCR quantifications of differential expression (Costa-Silva et al., 2017). The k-mer bias correction option was implemented and 100 bootstrapped transcriptomes were generated for each library to estimate the variation of expression for each transcript. The kallisto output was analyzed using the sleuth v0.28.0 package (Pimentel et al., 2016) in R v.3.3.0 (R Development Core Team, 2014). Across the 18 libraries from 6 colonies, there were a total of ~355 million 75 basepair paired-end RNA-seq reads. All workers sampled in this study are part of the same interbreeding J1/J2 population of *P. barbatus* at a long-term study site (Ingram et al., 2013) and no colonies or libraries displayed a mapping bias to the reference transcriptome used. A post-correction q-value threshold of 0.01 was used to call a transcript as differentially-expressed. To generate a transcript co-expression network, the “ExpressionCorrelation” plugin was used within Cytoscape (Su et al., 2014) with a cutoff of transcript-transcript Pearson correlation $r^2 > 0.93$ across all 18 libraries.

To generate functional annotations of the reference transcriptome, InterProScan (Jones et al., 2014) was used to query each transcript’s predicted protein translation against 11 protein databases (Profile HMM models: CATH-Gene3D, Superfamily, PIRSF, TIGRFAMs, Panther, Pfam, and Smart. Profile models: HAMAP, Prosite, ProDom. Pattern models: PRINTS, Prosite). InterProScan protein domain-level GO terms were merged with the GO terms inferred by blastx homology in Blast2GO. Annotation augmentation (ANNEX) was performed in Blast2GO. Lists of transcripts identified from differential expression co-expression analyses were tested for GO term enrichment using Fisher’s Exact Test in Blast2GO. Multiple test correction was implemented according to the False Discovery Rate (Benjamini and Hochberg, 1995). Enriched or depleted GO terms with three or fewer annotated representatives in a given gene set were not considered.

B. Pharmacology Methods

All solutions were administered to ants as follows: an ant was collected with an aspirator and placed in a 50 mL tube. The 50 mL tube was immersed in ice until the ant stopped moving. The ant was tapped out onto a paper towel, and gently grasped by a rear leg. To mark the ant, a small dab of oil-based paint (Uni-Paint PX-20) was placed on the back of the ant’s head using a small toothpick, using a unique color for each of the treatment groups. To feed the solution to the ant, 0.2 μ L of aqueous solution was placed on the mandibles of the anesthetized ant. The droplet is captured between the mandibles via surface tension. The contents of the solution used in each experiment are described for each experiment specifically. Where applicable, all drug solutions were prepared fresh from powder and measured with a Mettler Toledo AT261 Delta Range FACT to 0.1 mg accuracy. After administering a solution to an ant, the ant was placed on its lateral side, and it eventually began to move around

C. Laboratory experiments on the effect of dopamine administration.

Foraging *Pogonomyrmex barbatus* were collected from adult colonies near Phoenix, AZ in 5/2016, and driven to Stanford, CA. Laboratory ants were kept on a 14:10 LD cycle in a temperature controlled room (74 degrees F). Ants were given ad libitum access to wet cotton balls inside of glass tubes for water, and provided apple slices and millet seed every 3 days. Ant nests consisted of a foraging arena (2' x 3') with Fluon-coated sides (BioQuip) that had an open top and was exposed to light, connected to a series of smaller plastic boxes (4" x 4" ranging to 8" x 12") that were kept in the dark. Ants for brain dopamine quantification were collected from the foraging arena of the laboratory colony. All collected ants were placed into the same container, then randomly sorted into three treatment groups.

For treatment, ants were individually slowed on ice then orally administered either pure water (control), water with 3 mg/mL dopamine (3.714 mg/mL dopamine hydrochloride salt) (Sigma-Aldrich, PubChem 24277897), or water with 30 mg/mL dopamine. The solubility of dopamine in water is 600 g/L (PubChem CID: 681) and all dopamine solutions were made immediately before administration. After treatment, ants were kept in laboratory conditions in a fluon-coated plastic box. Ants were collected the following morning at 9am (day 1 time point) or 3 days after ingestion at 9am (day 3 time point). At the time of collection, ants were flash frozen in liquid nitrogen and stored at -80C until dissection. The brain was refrozen in 50 μ L PBS on dry ice, then stored at -80C until dopamine quantification was performed at the Stanford University Mass Spectrometry facility with an internal radiolabeled dopamine standard as follows.

D. Mass spectrometry methods:

Brain samples containing one ant brain and 50 μ L PBS were placed on ice in 1.7 mL tubes and 5 μ L of 5 μ M *d4*-dopamine (dopamine internal standard, Cambridge Isotope Laboratories) solution was added followed by 60 μ L of 4% formic acid. Samples were vigorously pipetted for 60 sec to start the homogenization. After that samples were placed in ice water bath and sonicated for 10 minutes twice. Next 400 μ L of 0.1 % formic acid in cold acetonitrile was added to the sample and sonicated for 10 more minutes. Samples were then centrifuge for 10 min @ 4°C @ 14000 rpm, dried under nitrogen and reconstituted in 50 μ L of HPLC sample buffer (2 mM ammonium formate pH 3.2 / 20% Methanol). The separation of derivatized amino acids was performed on an HP1100 HPLC system (Agilent, Santa Clara, CA) using a 150 x 2.10 mm Luna-PFP column (Phenomenex, Torrance, CA) after injection of 5 μ L at a flow rate of 0.3 mL/min. Mobile phase A was 2 mM ammonium formate pH 3 in water. Mobile phase B was 2 mM ammonium formate in methanol. The gradient program was as follows: 0.00-2.00 min – 20%B, 4.00 min – 80% B, 5 min – 80% B, 6-8 min – 20% B. All samples were injected in triplicates. A Quattro Premier triple quadrupole mass spectrometer (Waters, Milford MA) was operated in positive electrospray ionization mode. Capillary voltage was set to 3.00 kV, and cone voltage was set to 21 V. The detection of the analytes was performed in selected reaction monitoring mode (SRM). Two or three precursor ion - fragment ion pairs were selected for each analyte and compound specific cone voltage and collision energy values were used (see Table S1). Stable isotope labeled *d4*-dopamine was utilized as an Internal Standard (IS). The most intense transition for each analyte was selected for quantitation and the subsequent ones for confirmation.

An 8-point dopamine calibration curve was used with all analytes ranging from 2nM to 4000nM, and *d4*-dopamine at fixed 5 nmol/mL concentration as an internal standard. The LLOD for dopamine was 50 fmol on column and LLOQ was 150 fmol.

E. Field pharmacology experiments

Behavioral pharmacological experiments were performed with a set of 10 colonies in 7-8/2016 (D19, D24, D25, D26, D27, D29, D30, D33, D34, D36), and in 9 of the same 10 colonies in 8/2017 (all the previous colonies except D34). The colonies were near but not on the long-term study site (Ingram et al., 2013). Foragers were collected 1-2 meters from the nest entrance and identified as foragers because they were not carrying anything and walked in a straight line off the nest mound towards a foraging trail or fan (Gordon, 1986). The ants were brought back to the laboratory at the Southwestern Research Station and randomly sorted into treatment groups, defined as follows. In 2016, there were two treatment groups: 1x phosphate-buffered saline (PBS, Electron Microscopy Sciences), and 3 mg/mL dopamine in 1x PBS group. In 2017, there were three treatment groups: 1x PBS, 3 mg/mL dopamine in 1x, and 3 mg/mL 3-iodo-tyrosine (3IY, Sigma-Aldrich). In both years, each treatment group consisted of 100-150 foragers per colony, the same number of foragers was used between groups for each colony replicate. Ants were returned to their nest mound later the same day and most returned marked ants immediately descend into their nest. Foragers of *P. barbatus* tend to be the oldest ants in the colony, and workers marked while foraging do not later switch to perform other tasks (Gordon, 1989).

Observations began early the following day before foraging began. Counts of foraging trips by marked ants began when the first marked ant was observed to leave the nest. For colonies with a single foraging trail, a foraging trip was recorded when a marked ant crossed a line ~1-2 meters from the nest entrance on the trail. For colonies with more than one foraging trail, a foraging trip was recorded when a marked ant was observed leaving the nest entrance, carrying nothing, and walking in a straight line off of the nest mound (Gordon, 1986). In 2016, two colonies were observed each morning in alternating observation periods of 15-20 minutes. During each 15-20 minute observation period, all foraging trips made by marked ants were marked and counts were recorded in 30-second intervals. Foraging counts ended when the colony had stopped foraging for the morning and no ants had left the nest for 3 minutes. In 2017, one colony was observed per day, and outgoing foraging trips of marked ants were counted in 30-second intervals as before.

For each colony we calculated the response to drug treatment as the increase in foraging trips made by dopamine- or 3IY-treated ants divided by the total number of foraging trips made by control-treated foragers. This design minimizes the effects of day, as all comparisons are being made between two groups of foragers within the same colony on the same day. In each interval the numbers of foraging trips made by workers from each treatment were not very different and the results in each interval did not always reflect the totals: in only about one-third of the 30-second intervals were there were more dopamine-treated foragers than PBS-treated nestmates.

F. Behavioral ecological methods

Observation of undisturbed colony foraging behavior occurred during August 2017. Observation was performed over 12 consecutive days (8/3/17 - 8/14/17) on the set of 9 colonies later used in pharmacological experiments. Each colony was surrounded with 5 field flags, each placed 1.5 meters from the nest entrance, with the first flag pointing due North. On observation days, colonies were observed in a circuit by the same observer, in an order that was altered every day. Observation began before any colony had begun foraging for the day, at around 5am. If colonies were not foraging at all at the time of observation, then the observer proceeded to the next colony immediately. An outgoing foraging trip was defined as occurring when an unladen ant walked in a straight line off the nest mound and crossed an invisible line between two of the field flags surrounding the colony. An incoming foraging trip was defined as occurring when an ant crossed an invisible line between two field flags, heading towards the nest entrance. If more than 1 incoming and/or outgoing foragers were observed in the first 30 second scan per each side of the colony, rates of incoming and outgoing foragers were counted in 3 sequential 30-second intervals for that side. Observation of focal colonies continued until all colonies had finished foraging for the day. Average incoming and outgoing foraging rates per colony per observation were calculated as follows. First, the average incoming and outgoing foraging rate per minute per side was calculated by doubling the average of the 3 sequential 30-second counts per side. Then, the colony overall foraging rate per minute was calculated for each observation by summing foraging rates across all 5 sides of the colony.

The estimated overall number of foraging trips made by each colony each day was calculated as the area under the curve of colony overall outgoing foraging rate through time, as the integral of outgoing foraging rate through time is the overall number of foraging trips. The “weatherData” package was used to obtain weather data for all foraging days from a climate station near the fieldsite in Rodeo, NM. For each day where undisturbed colony behavior was observed, the average relative humidity measurement between 07:00 and 12:00 was calculated. Parametric ANOVA modeling was used to test for differences among colonies in how their total number of trips per day was associated with the daily humidity. To quantify the sensitivity of each colony to humidity, the total number of foraging trips made by the colony per day was regressed using a Theil-Sen non-parametric estimator (R “mblm” package) against the average relative humidity that day. The slope of this regression represents the estimated number fewer total foraging trips made by the colony per percent decrease in relative humidity, where higher values reflect higher colony sensitivity to humidity.

To test for a relationship between colony sensitivity to humidity and response to pharmacology, the value of the regression slope estimated above was correlated with the percent increase in foraging trips made by dopamine-treated ants relative to control-treated ants from that colony. Both parametric Pearson and non-parametric Kendall correlation tests were performed and correlation estimates are reported in the main text.

G. Brain biogenic amine content methods.

Natural variation in biogenic amine titer among foraging ants from colonies of *P. barbatus* was measured with High-Performance Liquid Chromatography (HPLC).

Foragers from the 9 focal colonies were collected on the morning of 9/4/2017 between 7am and 9am. Collected ants were frozen directly into liquid nitrogen, and kept in liquid nitrogen or a -80C freezer until dissection in cold citric acid. For HPLC we measured 5 samples from each of the 9 colonies with collected ants. Each sample consisted of 2 pooled brains and was measured for dopamine and serotonin content as per Hardie and Hirsh (Hardie and Hirsh, 2006).

Statistical analysis of natural variation among colonies in forager brain neurotransmitter titer was performed in R v3.4.0 with an ANOVA test from the library “heplots”. The library “granovaGG” was used to visualize the ANOVA results.

2. Table S1. Mass Spectrometry information for analyte detection, Related to Figure 2

Analyte	Abbreviation	SRM transitions	Cone Voltage [V]	Collision Energy [eV]
Dopamine	DA	154.0 > 90.7 154.0 > 118.7 154.0 > 136.7	17 17 17	21 17 11
Dopamine IS	DA IS	158.0 > 121.7 158.0 > 140.8	17 17	21 11
Histamine	HA	111.8 > 67.6 111.8 > 94.6	22 22	19 13
Tyrosine	TYR	138.0 > 76.7 138.0 > 94.6 138.0 > 102.7	15 15 15	25 16 20
Norepinephrine	NOREPI	170.0 > 106.7 170.0 > 134.7 170.0 > 151.8	11 11 11	22 16 7
Serotonin	SRT	176.9 > 114.6 176.9 > 131.8 176.9 > 159.7	18 18 18	28 22 12
Epinephrine	EPI	184.0 > 107.0 184.0 > 134.9 184.0 > 166.0	15 15 15	22 15 9

3. Data S1. Pharmacology Raw Data, Related to Figure 3.

File attached as supplemental dataset.

4. Supplemental References.

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