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Measurement of natural variation of neurotransmitter tissue content in red harvester ant brains among different colonies

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Abstract

Colonies of the red harvester ant, *Pogonomyrmex barbatus*, regulate foraging activity based on food availability and local conditions. Colony variation in foraging behavior is thought to be linked to biogenic amine signaling and metabolism. Measurements of differences in neurotransmitters have not been made among ant colonies in a natural environment. Here, for the first time, we quantified tissue content of 4 biogenic amines (dopamine, serotonin, octopamine, and tyramine) in a single forager brain from 9 red harvester ant colonies collected in the field. Capillary electrophoresis coupled with fast-scan cyclic voltammetry (CE-FSCV) was used to separate and detect the amines in individual ant brains. Low levels of biogenic amines were detected using field-amplified sample stacking by preparing a single brain tissue sample in acetonitrile and perchloric acid. The method provides low detection limits: 1 nM for dopamine, 2 nM for serotonin, 5 nM for octopamine, and 4 nM for tyramine. Overall, the content of dopamine (47 ± 9 pg/brain) in ant brains was highest, followed by octopamine (36 ± 10 pg/brain), serotonin (20 ± 4 pg/brain), and tyramine (14 ± 3 pg/brain). Relative standard deviations were high, but there was less variation within a colony than among colonies, so the neurotransmitter content of each colony might change with environmental conditions. This study demonstrates that CE-FSCV is a useful method for investigating natural variation in neurotransmitter content in single ant brains and could be useful for future studies correlating tissue content with colony behavior such as foraging.

Graphical Abstract

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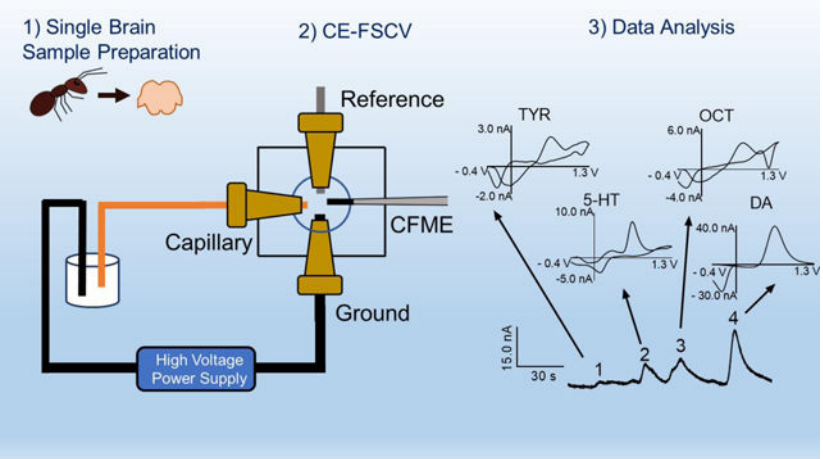
Conflict of Interest: The authors declare that they have no conflict of interest.



Keywords

Neurotransmitters tissue content; Pogonomyrmex Barbatus / red harvester ants; Dopamine; Serotonin; Octopamine; Tyramine; Capillary electrophoresis / Electrophoresis; Fast-Scan Cyclic Voltammetry; Carbon-Fiber microelectrode

Introduction

Colonies of red harvester ants (*Pogonomyrmex barbatus*) forage for seeds in the desert as their food and water source [1,2]. Differences in collective regulation of foraging activity are associated with forager brain gene expression differences, specifically highlighting biogenic amine neurophysiology [3–5]. The ant nervous system consists of a large central brain, which mediates the use of olfaction in ant social behavior, with smaller optic lobes attached to each end [6]. Ants utilize neurotransmitters, such as dopamine, serotonin, octopamine, and tyramine, to modulate complex behavior related to aggression, learning, and memory [5,7,8]. In ants, exterior and foraging workers tend to have higher levels of brain dopamine than interior or nursing workers, and manipulation of brain dopamine signaling can influence foraging activity [9]. A recent field study demonstrated that dopamine plays a central role regulating foraging activity in harvester ants [4]. Colonies treated with exogenous dopamine increased their foraging activities but colonies treated with 3-iodotyrosine, a dopamine biosynthesis inhibitor, decreased their foraging activity. Starvation and social context lead to changes in biogenic amine levels in working ants [10]. Therefore, methods to study neurotransmitter content in the ant brain could offer valuable information about which neurotransmitters are upregulated or downregulated, and how the variation in neurotransmitters contributes to behavioral phenotypes.

Separating and detecting neurotransmitters in the ant brain is difficult, because the absolute amount of neurotransmitter is so small in each tiny brain. High-performance liquid chromatography (HPLC) has been used for the separation and quantification of neurotransmitters in ant brains, typically from pooled samples of multiple brains, in colonies that were housed in the laboratory, after pharmacological treatment [4,10–12]. An alternative method for tissue content analysis in limited sample volumes is capillary electrophoresis

(CE) with electrochemical detection,[13] which has been used to determine neurotransmitter tissue content in individual *Drosophila* brains [14–18]. Micellar electrokinetic chromatography (MEKC) was initially coupled with amperometric detection to measure neurotransmitters and their metabolites in individual *Drosophila* brains [17–20]. Additionally, dopamine and octopamine were further detected in the subregions of fly brains [18]. However, amperometry lacks chemical selectivity thus peaks identification relies on the analyte retention time and requires the use of internal or external standards. Fast-scan cyclic voltammetry (FSCV) uses a cyclic voltammogram (CV) to identify the analyte, and has been extensively used to measure neurotransmitters in animal models [21–24]. The detector is a carbon-fiber microelectrode (CFME), which is excellent for neurochemical detection because of its small size, excellent electrochemical properties, and sensitivity [25–27]. In previous work, CE-FSCV was used to analyze neurotransmitters, including dopamine, serotonin, tyramine, octopamine, and histamine, in single larval or adult *Drosophila* central nervous systems [14–16]. The red foraging ant brain is small, approximately 950 μm × 600 μm × 550 μm (length, width, thickness), a volume of 314 nL, so CE should be a good method to quantify biogenic amines in a single ant brain.

Here, we developed CE-FSCV to quantify four major insect neurotransmitters: dopamine, serotonin, octopamine, and tyramine, in a single red harvester ant brain from colonies collected in the field. These are the first measurements of tissue content of biogenic amines in single forager brains of ants collected in a natural population. Dopamine was the most abundant biogenic amine, followed by octopamine, serotonin, and tyramine. Tissue content of biogenic amines in ant brains showed significant differences across colonies. The relative standard deviation within a colony was lower than when data from all colonies were pooled, showing that variance is greater among colonies than within a single colony. Levels of dopamine did not correlate with the other neurotransmitters, but levels of octopamine did correlate with levels of tyramine and serotonin. There was a trend towards correlation of behavioral response to exogenous dopamine with dopamine content; thus, future studies can investigate the relationship between biogenic amine contents and colony behavior to understand the neurophysiological basis of the evolution of collective behavior.

Experimental methods

Chemicals

All chemicals were purchased from Sigma unless stated otherwise. 10 mM of Tyramine, octopamine, serotonin, and dopamine were prepared as standard stock solution in 0.1 M perchloric acid. Ant brains were dissected in cold citric acid. 200 mM NaH_2PO_4 with 1mM tetraborate (pH 4.5) was used for the separation buffer and 100 mM NaH_2PO_4 (pH 6.5) for the detection cell buffer. Any solutions injected into CE system were filtered with 0.2 μm nylon filter (Fisher, Suwanee, GA, USA).

Ant Brain Homogenate Preparation

Detailed methods for collecting foragers from 9 focal colonies in natural populations are described in Friedman. *et.al.* [4]. Foraging ants were collected in the morning of 9/4/2017 at a long-term study site near Rodeo, New Mexico [28]. Ants were directly collected into

liquid nitrogen and stored in a – 80 °C freezer until dissection. Ant brains were dissected in 50 mM chilled citric acid using fine tweezers and stored in individual tubes at – 80 °C freezer until the day of the experiment. The protocol for homogenizing ant brains was adapted from Fang *et. al.* [14]. For the brain homogenate, a sample vial was prepared using a gel-loading pipette by trimming the tip and then sealing it with a flame. An isolated frozen ant brain was thawed and transferred to the sample vial with minimal buffer which was then replaced with 5 μl of 70% 5 mM perchloric acid and 30% acetonitrile. The sample vial containing the buffer and brain was centrifuged at 9000 rpm for 1 min (Brinkman Instruments, Westbury, NY) at room temperature. A silver metal wire (28 gauge, 0.325 mm o.d.) was used as a pestle to break up the brain. The sample was centrifuged again at 9,000 rpm for 2 min and the homogenized sample was sonicated in a water bath for 10 min (Fisher, Suwanee, GA). The sonicated sample was centrifuged through a 40 μl aerosol filter pipette (VWR, PA) to an empty sample vial at 13,000 rpm for 3 min. Electrokinetic injections were made by placing the end of the separation capillary into this vial. The standard and brain homogenate samples were kept on ice in the dark until analysis to minimize degradation of the samples.

Capillary Electrophoresis with Fast-Scan Cyclic Voltammetry

The CE with FSCV detection system was built in-house, and the procedure was performed as described in Fang *et. al.* [14]. Briefly, a separation capillary (11 μm i.d, 148 μm o.d., Polymicro Technologies, Phoenix, AZ) was cut to a length of 40 – 42 cm using a capillary column cutter (Scientific Instrument Services, Ringoes). The end of the capillary used for the detection was flame etched, removing about 1 cm of the polyimide coating to expose the fused silica. The etched end was glued into a 2 cm long larger capillary (250 μm i.d,, 359 μm o.d., Polymicro Technologies, Phoenix, AZ), leaving the etched region exposed, and the capillary was then inserted into the CE detection cell (Figure 1).

To fabricate a carbon-fiber microelectrode, a 30 μm carbon fiber (World Precision Instruments, Sarasota, FL) was aspirated into a capillary which was pulled into two electrodes, and the carbon fiber was trimmed as close as possible to the end of the capillary glass sheath. The electrode was placed approximately 10 μm from the end of the separation capillary. Stainless-steel and chloridized silver wires were attached to the cell to serve as the CE ground and reference electrode, respectively. End column detection was employed to mitigate the impact of high separation voltage on FSCV response. 15 KV was applied for 15 s for the sample injection, and 9 KV was applied for the separation, using a DC power supply (Spellman, Plainview, NY). The cell buffer was slowly flowed through the cell to prevent analytes from accumulating. Standard solutions of 50 nM dopamine and octopamine and 25 nM serotonin and tyramine were first injected for a calibration run. Then a tissue sample was injected.

For the FSCV detection, we used a Waveneuro potentiostat to collect data (5 MΩ headstage, Pine Research Instrument, Durham, NC). A triangular waveform of – 0.4 V to 1.3 V and back at – 0.4 V at 400 V/s was applied to the electrode every 100 ms. HDCV software (provided by R.M. Wightman, University of North Carolina, Chapel Hill, NC) was used for

data collection and analysis. PCIe-6363 (National Instruments, Austin, TX) was used to apply FSCV waveform and to collect data.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 7.02 (GraphPad Software, Inc., La Jolla, CA). Error bars represent mean ± standard error of the mean.

Results and Discussion

Capillary electrophoresis and fast-scan cyclic voltammetry

The objective of this study was to analyze and compare variation in 4 neurotransmitters in single brains across 9 colonies of the red harvester ant (P. barbatus). This is the first study reporting the quantitative measurements of all major biogenic amines from foraging ants collected from a natural environment. CE-FSCV separation had not been used for ant brains before, but procedures were adapted from *Drosophila* studies [14]. The amount of neurotransmitter in each ant brain is small, so field-amplified sample stacking was employed as a preconcentration step to inject much of the analyte onto the capillary in a narrow zone. Field-amplified sample stacking works by using a lower conductivity sample buffer; here, acetonitrile/water with perchloric acid to stabilize the amines. This method is convenient because it allows one to dilute the ant brain samples in slightly larger volumes (5 μl). For FSCV detection, a standard waveform was applied to the CFME scanning from – 0.4 V to 1.3 V and back to – 0.4 V at 400 V/s every 100 ms, which can detect all the compounds of interest [14]. Serotonin, octopamine, and tyramine form side products that can foul electrodes, and thus analyte specific waveforms have been developed [29,30]. However, fouling from side products is negligible in this study because the amount of these neurotransmitters available in a fly brain is small and thus byproduct formation is minimal. CE experiments with standards were run before and after the tissue samples, and sensitivity for the standards was the same for pre- and post-experiment calibration runs. This standard waveform has been previously used for detection of these biogenic amines [14,16].

Analysis of tissue content of biogenic amines in a single ant brain

Figure 2 shows representative examples of CE-FSCV separations of biogenic amines in a standard solution and from a single ant brain. The standard solution contains 50 nM dopamine and octopamine and 25 nM serotonin and tyramine. Each biogenic amine has a distinguishable cyclic voltammogram (CV) which helps identify the analytes (Figure 2A, top). Dopamine and serotonin have one oxidation and one reduction peak in their CVs at approximately 0.6 V and – 0.2 V for dopamine, and 0.5 V and 0.1 V for serotonin. However, serotonin also has an additional peak both on the forward and backward scan due to the formation of a side product [31]. Tyramine exhibits a primary oxidation peak at 1.1 V, a secondary oxidation peak at 0.6 V, and reduction peak at – 0.2 V [29]. While these peak potentials are similar to octopamine, due to their similar chemical structures, they are clearly separated using CE (Figure 2A, center). Because all the neurotransmitters have oxidation peaks around 0.6 V, a current vs time trace at this potential is used to visualize all the peaks in one trace, as in a traditional electropherogram. However, it is better to visualize all the data in a color plot (Figure 2A, bottom) which represents potential on the y-axis, time on the

x-axis, and current in false color. The color plot, CVs, and electropherogram are used to identify the separated analytes from potential interferents. We estimate the limit of detection (LOD, S/N=3) was 1 nM for dopamine, 2 nM for serotonin, 5 nM for octopamine, and 4 nM for tyramine, similar to previously published values [14].

Figure 2B shows an example separation from a single ant brain. As shown in the electropherogram (Fig. 2B, center), all four neurotransmitters in the tissue sample were detected and well resolved. The brain tissue sample is more complex, containing other electroactive species that are not found in the standard solution. For example, the color plot shows an extra peak below the serotonin oxidation peaks in the potential window between 0.4 V and 0.5 V. This extra peak for serotonin is less intense but also appears in the standard solution and elutes after the primary oxidation peak, indicating it is likely the byproduct of serotonin oxidation. Similarly, for octopamine, extra peaks are visible in the potential window between 0.4 V and 0.5 V (on the forward scan) and 1.2 V and 1.1 V (on the back scan), below and above the octopamine oxidation peak, respectively. These extra peaks could be due to the formation of side products or another electroactive compound that coeluted. Previously, HPLC with amperometry detection was used for *Drosophila* brain tissue analysis and an unidentified analyte coeluted with octopamine, resulting in the overestimation of octopamine signal [32]. Here, FSCV provides a better analysis of co-eluting interferent due to the color plot that shows the response at all voltages and the electropherogram extracted at the specific potential (0.6 V) bypassing the interferent signals. Other than these possible interferent signals, the migration order and time of each neurotransmitter are the same as those from the standard sample and the color plot shows no other signals.

To quantify the concentration of neurotransmitters in the brain sample, the oxidation current of each analyte was converted to concentration using the calibration factor from the standard sample analysis. The example sample (Fig. 3B), from colony D30, had 17 nM tyramine, 31 nM serotonin, 34 nM octopamine, and 59 nM dopamine. Since each sample was prepared with one brain in 5 μl solution, the concentration can be back calculated to the amount of each neurotransmitter in pg per brain. For this ant brain, the amount (pg/brain) was 15 for tyramine, 33 for serotonin, 32 for octopamine, and 56 for dopamine.

Comparisons of biogenic amines in different red harvester ant colonies

Red harvester ants from 9 colonies were collected in the field as described in Friedman *et. al.* [4]. Figure 3 shows the data from each individual ant graphed by neurotransmitter and colony. Data are organized for dopamine in order of the most dopamine content per colony to the least, and then the same colony order is used for the other biogenic amines. Overall, the most abundant biogenic amine content in forager brains (n=53 from 9 colonies) was dopamine (47 ± 9 pg), followed by octopamine (36 ± 10 pg), serotonin (20 ± 4 pg), and tyramine (14 ± 3 pg). One-way ANOVA shows the significant main effect of colony on tissue content of each neurotransmitter: for dopamine, $F_{(8, 45)} = 7.05$, $p<0.0001$; for serotonin, $F_{(8, 45)} = 3.199$, $p=0.0058$; for octopamine, $F_{(8, 44)} = 4.676$, $p=0.0003$; for tyramine, $F_{(8, 44)} = 3.003$, $p=0.0089$. One interesting observation from this data is that colony D24 has the significantly higher tissue content of every neurotransmitter than most

other colonies. For instance, D24 has significantly higher level of tissue content compared to other colonies except D29 and D33 for both dopamine and serotonin and D27 only for serotonin. Similarly, D24 has greater octopamine and tyramine tissue content than all other colonies except D33, D25, and D19 for tyramine.

Additionally, we tested for correlations between average values of neurotransmitters within each colony. Interestingly, dopamine content was not correlated with tissue content of serotonin (Pearson, r^2=0.02, p=0.71), octopamine (r^2=0.16, p=0.29), or tyramine (r^2=0.004, p=0.86). However, octopamine content was strongly correlated with serotonin (r^2=0.80, p=0.0011) and tyramine (r^2=0.69 p=0.0057), and tyramine was correlated with serotonin (r^2=0.66, p=0.0076).

Figure 4 shows the same data as Fig. 3, but the four neurotransmitters are grouped for each colony to observe the variation of tissue content within each colony. A two-way ANOVA shows a significant variation of neurotransmitter tissue content within a colony ($F_{(3,177)}$ = 19.62, p < 0.0001), especially in D24, D29, D33, and D26. In D24, the amount of dopamine was significantly higher than both serotonin (p=0.0002, n=7) and tyramine (p<0.0001, n=7), and octopamine was significantly higher than serotonin (p<0.0001, n=7) and tyramine (p<0.0001, n=7). Similarly, colonies D29 and D33 had higher dopamine content than serotonin and tyramine (D29: serotonin p<0.05 and tyramine p<0.0001, n=6; D33: serotonin and tyramine p<0.05, n=6). In D26, dopamine was significantly more than tyramine (p<0.05, n=6). In general, dopamine was the most abundant biogenic amine in every colony except D24 and D30, which had slightly higher octopamine levels. Tyramine was generally lowest except for colony D19, which had lower serotonin content. In each colony, tyramine content was lower than octopamine content. Tyramine is the synthetic precursor to octopamine, thus higher octopamine could lead to lower tyramine levels. This inverse relationship was also clearly shown in different life stages of *Drosophila* [16].

In previous studies of other ant species, the dopamine tissue content was significantly higher than other biogenic amines, similar to red foraging ants here [33,34]. Similar patterns were observed in other insects, including *Drosophila* larvae, where dopamine concentrations were highest and tyramine concentrations the lowest, while tyramine was higher than octopamine in adult brains [16]. In honeybees, as in the ants reported here, dopamine tissue content in the brain was found to be highest followed by octopamine, serotonin, and tyramine. [35]. In higher order species, mammals also have higher dopamine tissue content than serotonin content [13,36]. Thus, the patterns observed here for ant colonies are similar to those observed in other species.

The relative standard deviations (RSD) within and among colonies were examined to understand variance. Table 1 shows the RSD for each colony for each neurotransmitter, the average of those values, as well as the overall RSD when the individual values of all the colonies are pooled together. Variances within a colony in level of each neurotransmitter was lower than the variance for all samples pooled, indicating that differences among colonies are larger than variation within colonies. Colony variance for each neurotransmitter (53 % for dopamine, 72 % for serotonin, 63 % for octopamine, and 65 % for tyramine) was lower than overall RSDs (71 % for dopamine, 90 % for serotonin, 125 % for octopamine, and 126

% for tyramine). Although D24 has significantly larger tissue content of each neurotransmitter than other colonies, the overall dopamine RSD when D24 is removed (68 %) is still larger than the mean RSD. This analysis shows that there is more variance in neurotransmitter content among colonies than within a single colony. Therefore, there may be environmental factors like food availability, heat, or humidity promoting the variation in neurotransmitters between colonies that contribute to the larger variance.

Variation of neurotransmitter levels among colonies could be correlated with differences in expression or activity of synthesis or metabolic enzymes. Dopamine is biologically derived from L-tyrosine, which is converted to L-DOPA, by the enzyme tyrosine hydroxylase [37]. L-tyrosine is also the precursor for tyramine and octopamine, as they are synthesized through tyrosine decarboxylase to tyramine and then through tyramine-β-hydroxylase to octopamine [38]. Therefore, if there is upregulation of L-tyrosine, levels of dopamine, tyramine, and octopamine are expected to rise. For example, here we found that the colony with the highest content for dopamine, also had high content for octopamine and tyramine, suggesting that L-tyrosine might have been high. However, dopamine tissue content is not significantly correlated to that of octopamine and tyramine. In comparison, octopamine and tyramine contents were highly correlated, likely due to their shared synthesis pathway. Serotonin, on the other hand, has a completely separate synthesis pathway with tryptophan as the synthetic precursor. However, serotonin is significantly correlated with octopamine and tyramine content across colonies, suggesting that colonies may vary in amino acids intake, like L-tyrosine and tryptophan, or in the expression of key enzymes that cause the broad upregulation of synthesis pathways.

Differences in neurotransmitter content across colonies may also be due to differences in the brain metabolism of biogenic amines. The main difference in metabolism between insects and mammals is that monoamine oxidase, the main metabolic enzyme in mammals, is not present in insects [39]. The primary dopamine metabolic pathway in insects occurs through N-acetylation by the enzyme, arylalkylamine N-acetyltransferase (aaNAT), which converts dopamine to N-acetyl dopamine (NADA). NADA is taken up either by glial cells in the brain or cuticle in sclerotization [40]. Dopamine can also be metabolized by Ebony to β-alanyl-dopamine (BADA), which is directly converted back to dopamine by Tan, BADA hydrolase [23]. The aaNAT enzyme is involved in the metabolic pathway of all the biogenic amines, thus their tissue contents would be expected to change similarly if aaNAT activity was affected.

A previous study demonstrated that dopamine plays a central role regulating foraging activity in the harvester ants [4]. Ants treated with dopamine at a dose that increased brain dopamine titer increased their foraging activity compared to control ants treated with buffer. Here, there was a trend toward a significant correlation between dopamine tissue content and the changes of foraging trips after exogenous dopamine treatment (Figure 5). Colonies with higher dopamine levels have less increase in foraging activity after dopamine treatment, which implies exogenous dopamine treatment could be ineffective at promoting foraging activity if endogenous dopamine level are already elevated (Figure 5, Pearson r^2=0.55, $p<0.05$). However, this effect is largely driven by the one colony with large amounts of dopamine (colony D24) and so future studies are needed to understand the relationships

between ant behavior and biogenic amine content. For example, biogenic amines could be correlated with colony behaviors such as foraging or as they change with environmental conditions such as humidity. A future study might investigate metabolites along with biogenic amines to determine if metabolism changes are correlated with foraging behavior among colonies in the field. F

Conclusions

Capillary electrophoresis with fast-scan cyclic voltammetry (CE-FSCV) was used to separate and quantify dopamine, serotonin, octopamine, and tyramine in single harvester ant brains. This is first time quantifying biogenic amines in 9 different ant colonies that were obtained in the field. Levels of neurotransmitters are about 10–100 pg of each neurotransmitter per brain, with more dopamine than serotonin. There was significant variation among colonies in each biogenic amine were observed across colonies, with larger differences between than within colonies. Colonies that had higher dopamine content were less responsive to foraging activity in response to dopamine treatment. Future studies could investigate the effect of environmental condition, like humidity, on each neurotransmitter within and among colonies. Furthermore, CE-FSCV could be used to examine natural variations of neurotransmission among colonies by analyzing metabolites.

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Data Availability

The data supports the finding of this study are available in figshare. [DOI: 10.6084/m9.figshare.10023080]

Biographies

Mimi Shin is a postdoctoral researcher in the Department of Chemistry at the University of Virginia. She received her PhD at University of Kansas in 2016. Her research interest is in developing various analytical techniques, using electrochemistry and imaging, to characterize neurotransmitter release in the brain of various animal models from insects to rodents.

Daniel Ari Friedman is currently a postdoctoral researcher in Entomology at University of California, Davis. He received his PhD student at Stanford University in 2019, advised by Professor Deborah Gordon, in the Department of Biology. He is interested in the evolution of genetics, physiology, and colony traits in eusocial insects.

Deborah M. Gordon is a Professor in the Department of Biology at Stanford University. Prof. Gordon's lab group studies the collective regulation of behavior in ants and how it functions ecologically

B. Jill Venton is Professor and Chair of the Department of Chemistry at University of Virginia. She is also affiliated with the Neuroscience Graduate Program and Brain Institute. Her research interests are in developing new electrochemical and separations techniques for measurements of neurotransmitters in tissue.

References

1. Barron AB, Søvik E, Cornish JL. The Roles of Dopamine and Related Compounds in Reward-Seeking Behavior Across Animal Phyla. Front Behav Neurosci. 2010;4:163. [PubMed: 21048897]
2. Gordon DM. The rewards of restraint in the collective regulation of foraging by harvester ant colonies. Nature. 2013;498(7452):91–3. [PubMed: 23676676]
3. Jandt J, Gordon D. The behavioral ecology of variation in social insects. Curr Opin Insect Sci. 2016;15:40–4. [PubMed: 27436730]
4. Friedman DA, Pilko A, Skowronska-Krawczyk D, Krasinska K, Parker JW, Hirsh J, Gordon DM. The Role of Dopamine in the Collective Regulation of Foraging in Harvester Ants. iScience. 2018;8:283–94. [PubMed: 30270022]
5. Friedman DA, Gordon DM. Ant Genetics: Reproductive Physiology, Worker Morphology, and Behavior. Annu Rev Neurosci. 2016;39(1):41–56. [PubMed: 27050321]
6. Kamhi JF, Traniello JFA. Biogenic Amines and Collective Organization in a Superorganism: Neuromodulation of Social Behavior in Ants. Brain Behav Evol. 2013;82(4):220–36. [PubMed: 24281765]
7. Kamhi JF, Arganda S, Moreau CS, Traniello JFA. Origins of Aminergic Regulation of Behavior in Complex Insect Social Systems. Front Syst Neurosci. 2017;11:74. [PubMed: 29066958]
8. Szczuka A, Korczyńska J, Wnuk A, Symonowicz B, Gonzalez Szwacka A, Mazurkiewicz P, Kostowski W, Godzinska. The effects of serotonin, dopamine, octopamine and tyramine on behavior of workers of the ant Formica polyctena during dyadic aggression tests. Acta Neurobiol Exp 2013;73(4):495–520.
9. Entler BV, Cannon JT, Seid MA. Morphine addiction in ants: a new model for self-administration and neurochemical analysis. J Exp Biol. 2016;219(Pt 18):2865–9. [PubMed: 27655824]

10. Wada-Katsumata A, Yamaoka R, Aonuma H. Social interactions influence dopamine and octopamine homeostasis in the brain of the ant Formica japonica. J Exp Biol. 2011;214(10):1707–13. [PubMed: 21525317]
11. Cuvillier-Hot Alain Lenoir V Biogenic amine levels, reproduction and social dominance in the queenless ant Streblognathus peetersi. Naturwissenschaften. 2006;93:149–53. [PubMed: 16514515]
12. Okada Y, Sasaki K, Miyazaki S, Shimoji H, Tsuji K, Miura T. Social dominance and reproductive differentiation mediated by dopaminergic signaling in a queenless ant. J Exp Biol. 2015;218(Pt 7):1091–8. [PubMed: 25687437]
13. Fang H, Pajski ML, Ross AE, Venton BJ. Quantitation of dopamine, serotonin and adenosine content in a tissue punch from a brain slice using capillary electrophoresis with fast-scan cyclic voltammetry detection. Anal Methods. 2013;5(11):2704–11. [PubMed: 23795210]
14. Fang H, Vickrey TL, Venton BJ. Analysis of biogenic amines in a single Drosophila larva brain by capillary electrophoresis with fast-scan cyclic voltammetry detection. Anal Chem. 2011;83(6).
15. Denno ME, Privman E, Borman RP, Wolin DC, Venton BJ. Quantification of Histamine and Carcinine in Drosophila melanogaster Tissues. ACS Chem Neurosci. 2016;7(3):407–14. [PubMed: 26765065]
16. Denno ME, Privman E, Venton BJ. Analysis of neurotransmitter tissue content of drosophila melanogaster in different life stages. ACS Chem Neurosci. 2015;6(1):117–23. [PubMed: 25437353]
17. Berglund EC, Kuklinski NJ, Karagunduz E, Ucar K, Hanrieder J, Ewing AG. Freeze-Drying as Sample Preparation for Micellar Electrokinetic Capillary Chromatography-Electrochemical Separations of Neurochemicals in Drosophila Brains. Anal Chem. 2013;85(5):2841–6. [PubMed: 23387977]
18. Kuklinski NJ, Berglund EC, Engelbrektsson J, Ewing AG. Biogenic Amines in Microdissected Brain Regions of Drosophila melanogaster Measured with Micellar Electrokinetic Capillary Chromatography-Electrochemical Detection. Anal Chem. 2010;82(18):7729–35. [PubMed: 20738098]
19. Omiatek DM, Santillo MF, Heien ML, Ewing AG. Hybrid capillary-microfluidic device for the separation, lysis, and electrochemical detection of vesicles. Anal Chem. 2009;81(6):2294–302. [PubMed: 19228035]
20. Ream PJ, Suljak SW, Ewing AG, Han K-A. Micellar Electrokinetic Capillary Chromatography-Electrochemical Detection for Analysis of Biogenic Amines in Drosophila m elanogaster. Anal Chem. 2003;75(16):3972–8. [PubMed: 14632107]
21. Pyakurel P, Shin M, Venton BJ. Nicotinic acetylcholine receptor (nAChR) mediated dopamine release in larval Drosophila melanogaster. Neurochem Int. 2018;114:33–41. [PubMed: 29305920]
22. Shin M, Field TM, Stucky CS, Furgurson MN, Johnson MA. Ex vivo measurement of electrically evoked dopamine release in zebrafish whole brain. ACS Chem Neurosci. 2017;8(9):1880–8. [PubMed: 28617576]
23. Shin M, Copeland JM, Venton BJ. Drosophila as a Model System for Neurotransmitter Measurements. ACS Chem Neurosci. 2018;9(8):1872–83. [PubMed: 29411967]
24. Shin M, Venton BJ. Electrochemical Measurements of Acetylcholine-Stimulated Dopamine Release in Adult Drosophila melanogaster Brains. Anal Chem. 2018;90(17):10318–25. [PubMed: 30073836]
25. Cao Q, Puthongkham P, Venton BJ. Review: New insights into optimizing chemical and 3D surface structures of carbon electrodes for neurotransmitter detection. Anal Methods. 2019;11(3):247–61. [PubMed: 30740148]
26. Ganesana M, Lee ST, Wang Y, Venton BJ. Analytical Techniques in Neuroscience: Recent Advances in Imaging, Separation, and Electrochemical Methods. Anal Chem. 2017;89(1):314–41. [PubMed: 28105819]
27. Shin M, Wang Y, Borgus JR, Venton BJ. Electrochemistry at the Synapse. Annual Review of Analytical Chemistry. 2019 12;12(1):297–321.

28. Ingram KK, Pilko A, Heer J, Gordon DM. Colony life history and lifetime reproductive success of red harvester ant colonies Coulson T, editor J Anim Ecol. 2013;82(3):540–50. [PubMed: 23368713]
29. Cooper SE, Venton BJ. Fast-scan cyclic voltammetry for the detection of tyramine and octopamine. Anal Bioanal Chem. 2009;394(1):329–36. [PubMed: 19189084]
30. Jackson BP, Dietz SM, Wightman RM. Fast-scan cyclic voltammetry of 5-hydroxytryptamine. Anal Chem. 1995;67(6):1115–20. [PubMed: 7717525]
31. Hashemi P, Dankoski EC, Petrovic J, Keithley RB, Wightman RM. Voltammetric Detection of 5-Hydroxytryptamine Release in the Rat Brain. Anal Chem. 2009;81(22):9462–71. [PubMed: 19827792]
32. Hardie SL, Hirsh J. An improved method for the separation and detection of biogenic amines in adult Drosophila brain extracts by high performance liquid chromatography. J Neurosci Methods. 2006;153(2):243–9. [PubMed: 16337274]
33. Aonuma H, Watanabe T. Changes in the Content of Brain Biogenic Amine Associated with Early Colony Establishment in the Queen of the Ant, Formica japonica Gronenberg W, editor PLoS One. 2012 8;7(8):e43377. [PubMed: 22905265]
34. Mannino G, Abdi G, Emilio Maffei M, Barbero F. Origanum vulgare terpenoids modulate Myrmica scabrinodis brain biogenic amines and ant behaviour. PLoS One. 2018;13(12):e0211749.
35. Cook CN, Brent CS, Breed MD. Octopamine and tyramine modulate the thermoregulatory fanning response in honey bees (Apis mellifera). J Exp Biol. 2017;220(10):1925–30. [PubMed: 28314750]
36. Brenes JC, Fornaguera J. The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum. Behav Brain Res. 2009;198(1):199–205. [PubMed: 19027796]
37. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. Cell Commun Signal. 2013;11(1):34. [PubMed: 23683503]
38. Bauknecht P, Jékely G. Ancient coexistence of norepinephrine, tyramine, and octopamine signaling in bilaterians. BMC Biol. 2017;15(1):6. [PubMed: 28137258]
39. Sloley B Metabolism of Monoamines in Invertebrates: The Relative Importance of Monoamine Oxidase in Different Phyla. Neurotoxicology. 2004;25(1–2):175–83. [PubMed: 14697892]
40. Yamamoto S, Seto ES. Dopamine dynamics and signaling in Drosophila: an overview of genes, drugs and behavioral paradigms. Exp Anim. 2014;63(2):107–19. [PubMed: 24770636]

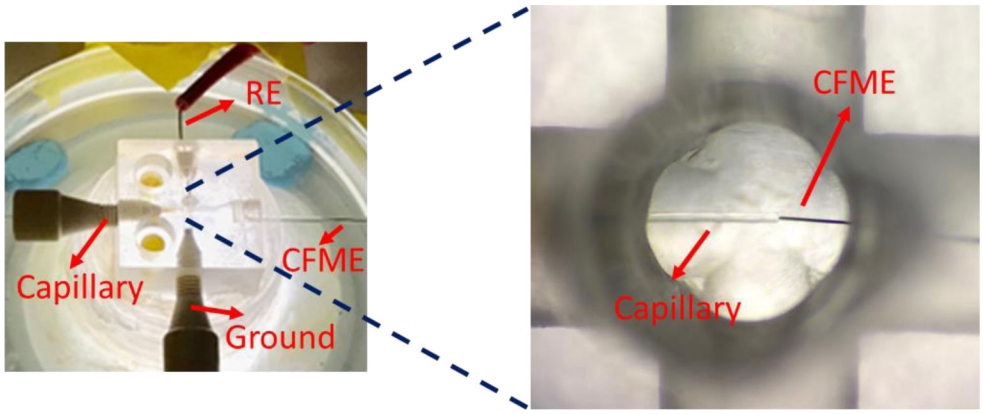

Figure 1.

Image of capillary electrophoresis detection cell. A reference electrode (RE) and ground wire are positioned inside of a detection cell. A carbon-fiber microelectrode (CFME), used for detection, is placed opposite of separation capillary, approximately 10 μm away.

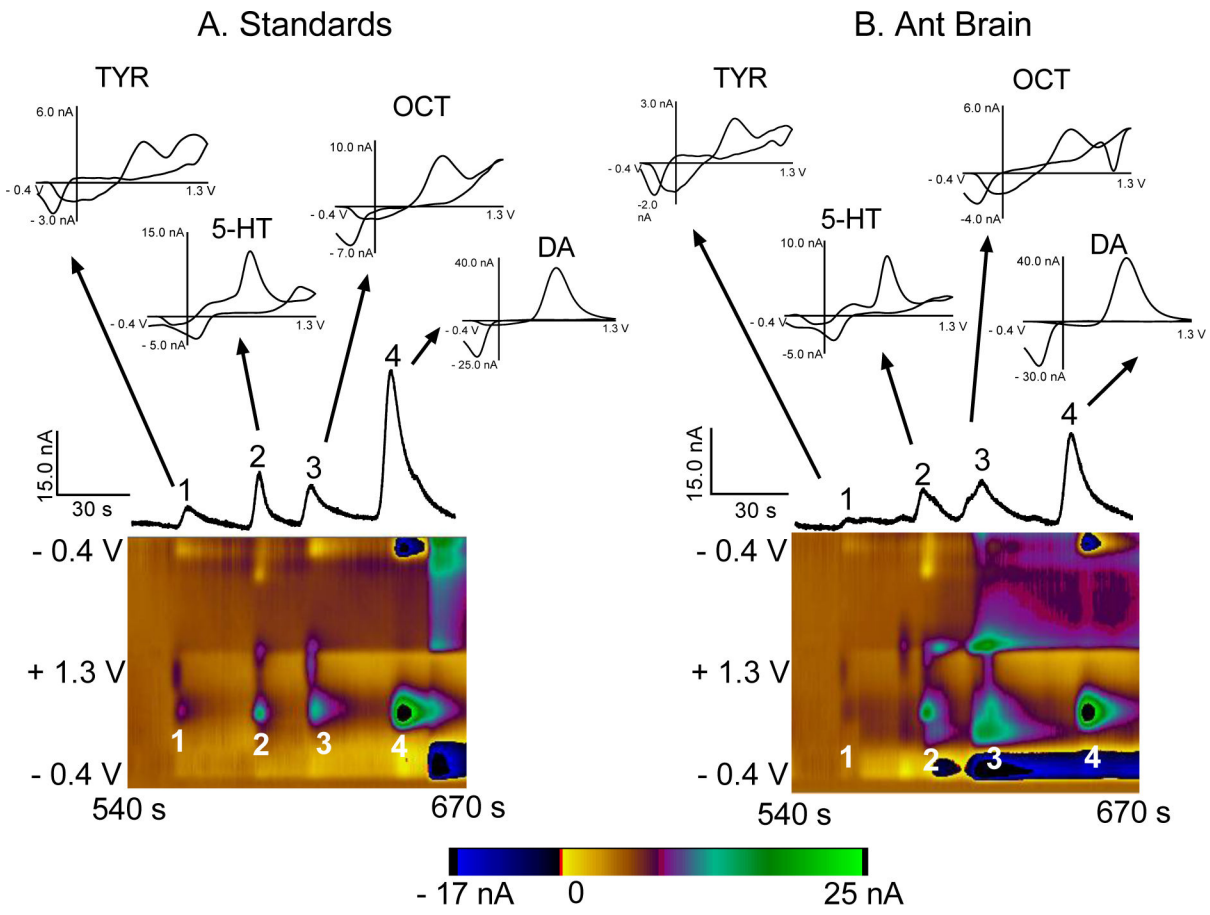

Figure 2.

Representative CE-FSCV data for (A) standard (50 nM dopamine and octopamine and 25 nM serotonin and tyramine) and (B) single red foraging ant brain sample from colony D30. Brain was prepared in 5 μl of 70% 5 mM $HClO_4$:30% acetonitrile. Standard solution was injected prior to the tissue sample on the same capillary. As shown in the electropherogram, current versus time plot extracted at 0.6 V, neurotransmitters were well separated and eluted in order of (1) tyramine (TYR), (2) serotonin (5-HT), (3) octopamine (OCT), and (4) dopamine (DA). Each eluted neurotransmitter is identified by its cyclic voltammogram (inset). The color plot has time on x-axis, potential on y-axis, and current in false color enabling to visualize each separated analyte. The carbon-fiber microelectrode was calibrated with a standard solution and a calibration factor was used to convert current response in the tissue sample into concentrations (and the amounts per brain); 17 nM (15 pg/brain) tyramine, 31 nM (33 pg/brain) serotonin, 34 nM (32 pg/brain) octopamine, 59 nM (56 pg/brain) dopamine.

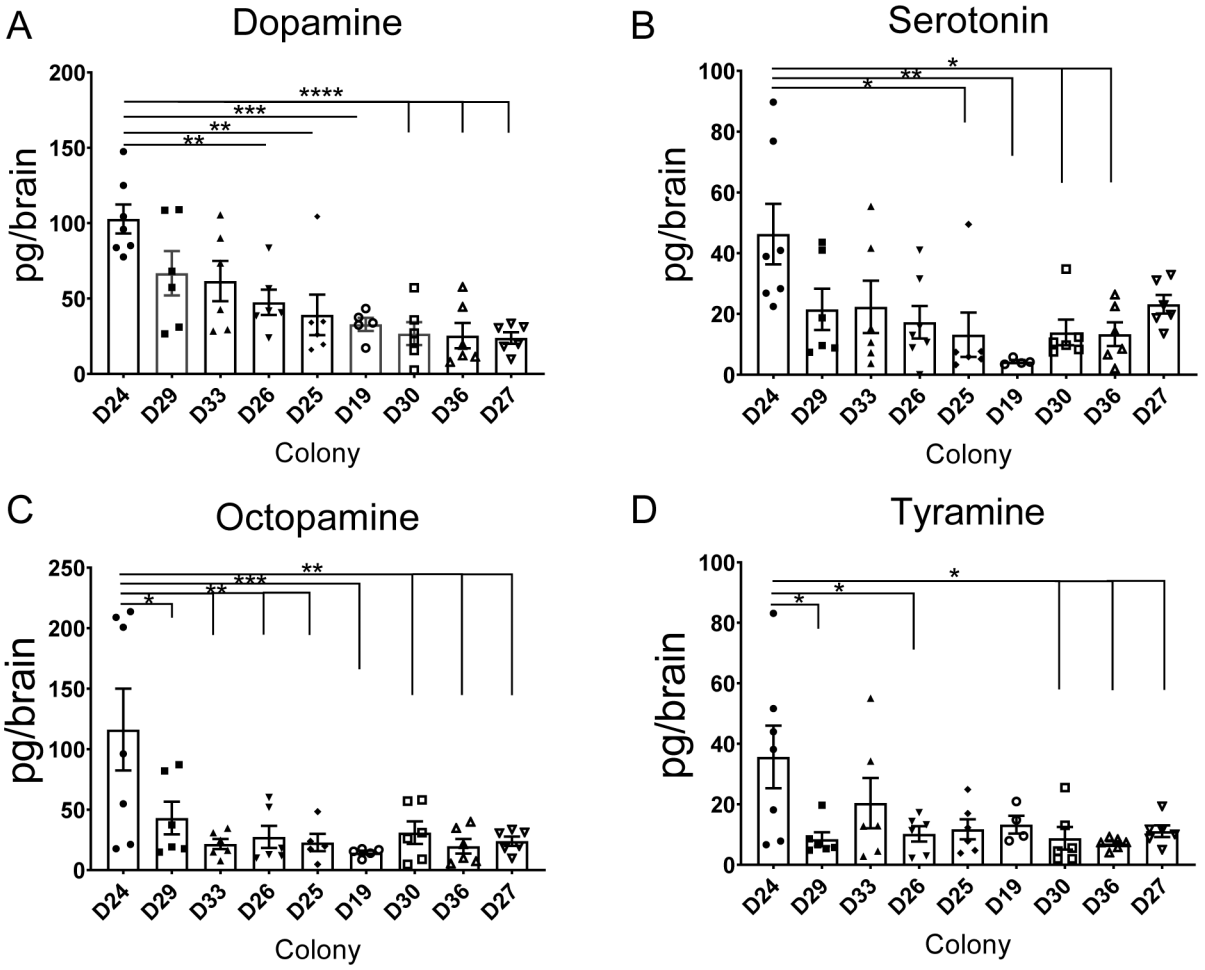

Figure 3.

Comparison of natural variations of (A) dopamine, (B) serotonin, (C) octopamine, and (D) tyramine levels (pg/brain) in different red harvester ant colonies. X-axis gives the different colony ID in order from the highest dopamine content to the lowest. Y-axis shows the tissue content of biogenic amines in the individual ant brain (pg/brain). Points are individual ants, with the bar being the average and error bar the SEM. The data were analyzed using one-way ANOVA. There is a main effect of colony on biogenic amine tissue content; dopamine ($F_{(8, 45)}$ = 7.073, p<0.0001), serotonin ($F_{(8, 45)}$ = 3.199, p=0.0058), octopamine ($F_{(8, 44)}$ = 4.676, p=0.0003), and tyramine ($F_{(8, 44)}$ = 3.003, p=0.0089). Post-test differences between colonies are marked. * p<0.05, ** p< 0.01, *** p<0.0001, Turkey's multiple comparisons test.

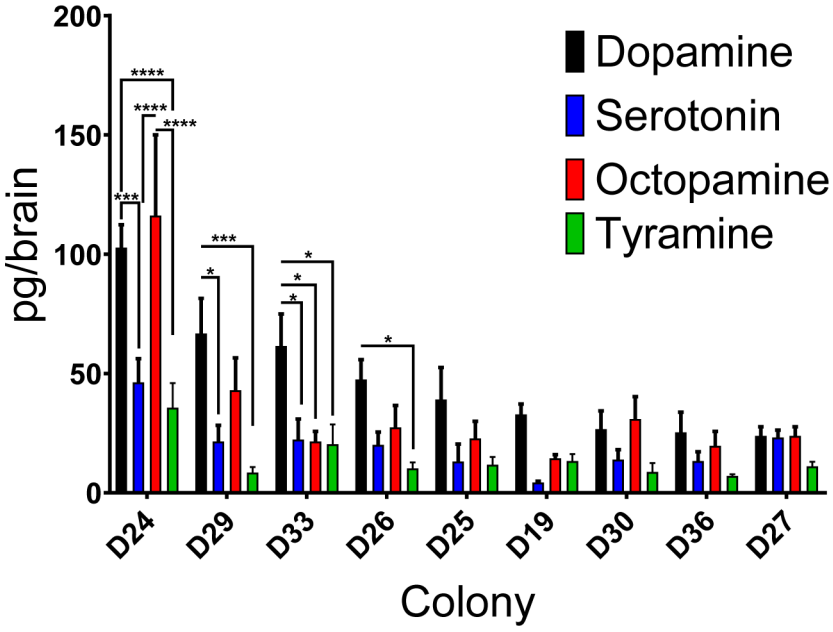

Figure 4.

Tissue content of neurotransmitters within each colony. There was a significant variation of tissue content within a colony ($F_{(3,177)}$ = 19.62, p < 0.0001, two-way ANOVA) and across the colonies ($F_{(8, 177)}$ = 14.6, p < 0.0001). Significant differences in neurotransmitters were shown in D24, D29, D33, and D26. In D24, dopamine was higher than serotonin (p = 0.0002, Tukey's multiple comparison test) and tyramine (p<0.0001) and octopamine was higher than serotonin (p<0.0001) and tyramine (p<0.0001). In D29, dopamine was higher than serotonin (p<0.05) and tyramine (p<0.001). D33 had higher dopamine content compared to serotonin, octopamine, and tyramine (p<0.05) whereas dopamine was only higher than tyramine in D26 (p<0.05)

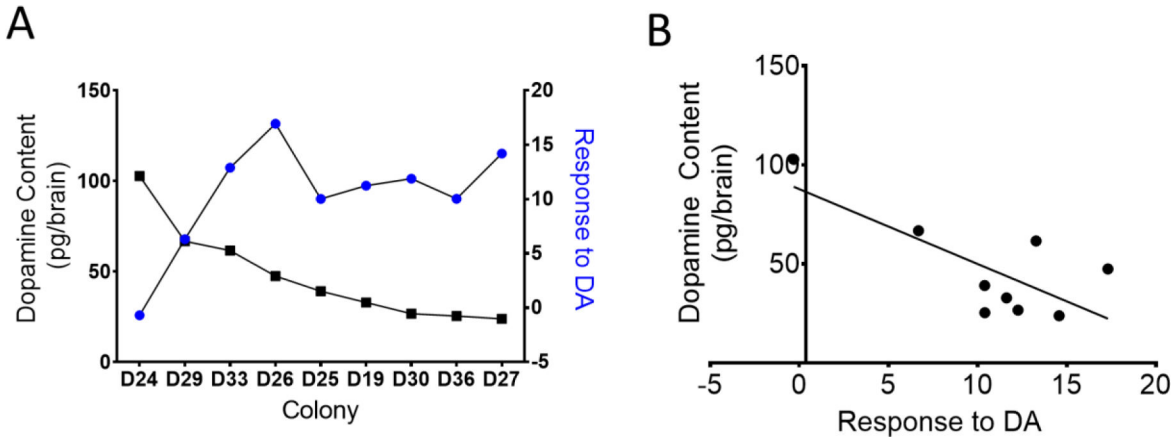

Figure 5.

Comparison of dopamine content in each colony to exogenous dopamine treatment response. (A) Left and right y-axis represent DA content (pg/brain, n= 6 or 7 in each colony, black squares) in a single forager brain and foraging activity in response to orally administrated dopamine (3mg/ml, blue dots) from 9 different colonies, respectively. The foraging activity behavior data is reprinted from Friedman *et. al.* [4]. Colonies with less dopamine content available in the brain were more responsive to exogenous dopamine treatment. (B) Correlation of dopamine tissue content and response to exogenous dopamine. There was a significant correlation (Pearson r^2=0.55, $p<0.05$).

Table 1.

Relative standard deviations (RSDs) of measured neurotransmitter in each colony.

RSD (%)	D24	D29	D33	D26	D25	D19	D30	D36	D27	Average colony RSD	Overall RSD (not by colony)
Dopamine	25	54	53	43	84	30	70	81	39	53	71
Serotonin	57	77	95	82	136	24	75	71	32	72	90
Octopamine	77	77	48	82	67	25	74	75	39	63	125
Tyramine	77	67	100	61	69	44	105	27	43	66	106