Quantitative LC-MS/MS analysis of dopamine in single ant brains

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Introduction

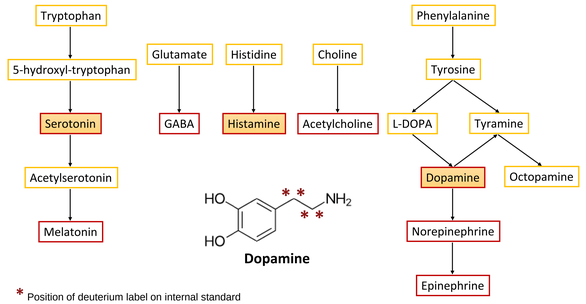
Ants are eusocial insects that operate collectively in colonies. Many ant species display persistent behavioral variation among individuals within a colony, as well as among colonies. The genetic, epigenetic, and neurophysiological mechanisms to differences among task groups within colonies has received some study, but little is known about variation among colonies in the neurophysiology of individual workers.

Differences in behavior have been linked to variation in biogenic amine signaling in many insect species. In ants, changes in dopamine levels (or ratios of dopamine to other biogenic amines) are associated with task.

To gain insight into variation among colonies in the dopaminergic neurophysiology of foragers of the harvester ant, Pogonomyrmex barbatus, we establish methodology enabling quantitation of dopamine levels in single ant brains.

FIGURE 1. *(A) Pogonomyrmex barbatus – red harvester ant, (B) – Forager ants at work, (C) – Field site near Rodeo, NM*

Analytes



SCHEME 1. Biogenic amines – major biosynthetic pathways. *Dopamine, serotonin and histamine – amines representing three different biosynthetic pathways – were monitored. Commercially available standards for dopamine, serotonin and histamine, and stable isotope labeled internal standard for dopamine, were purchased from Sigma-Aldrich and Cambridge Isotope Laboratories, respectively.*

Methods

Instrumentation

LC/MS: Quattro Premier triple quadrupole mass spectrometer (Waters) coupled with HP 1100 HPLC system (Agilent)

Column: Luna-PFP (Phenomenex)

150 mm x 2.1 mm, 3 µm particle size

Mobile phase A: 2 mM ammonium formate (AmF) pH 3.2

Mobile phase B: 2 mM ammonium formate (AmF) in methanol

Injection volume: 10 µl

Flow rate: 300 µl/min

Ionization mode: positive ESI

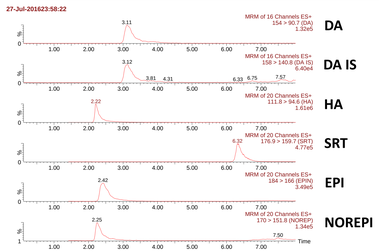
Scan mode: Selected Reaction Monitoring (SRM)

Data analysis: MassLynx/QuanLynx software (Waters)

TABLE 1. Analytes. *Five biogenic amines and one deuterium-labeled standard were monitored, using 2 to 3 SRM transitions for each.*

Analyte	Abbreviation	MW [g/mol]	SRM Transitions
Dopamine	DA	153.2	154.0 > 90.7, 118.7, 136.7
***d4*-Dopamine**	DA IS	157.2	158.0 > 121.7, 140.8
Histamine	HA	111.2	111.9 > 67.6, 94.6
Serotonin	SRT	176.2	176.9 > 114.6, 131.8, 159.7
Epinephrine	EPI	183.2	184.0 > 107.0, 134.9, 166.0
Norepinephrine	NOREPI	169.2	170.0 > 106.7, 134.7, 151.8

LC-MS/MS



Time [min]	%B
0	20
2	20
4	80
5	80
6	20
8	20

FIGURE 2. SRM chromatograms and LC gradient – *1 pmol on column of each analyte. Total run time – 8 min.*

Calibration curve and LLOD

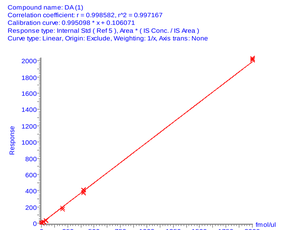
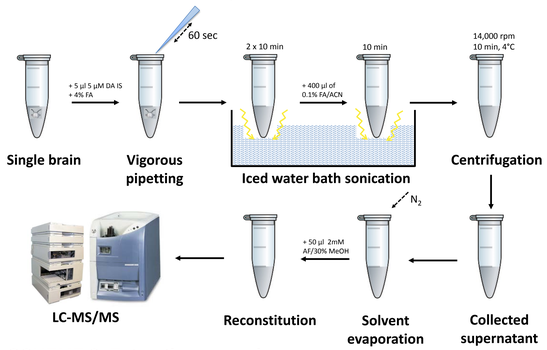


FIGURE 3. Dopamine calibration curve - *Range: 2 nM to 4000 nM prepared in surrogate matrix – 50x diluted homogenate of 3 ant brains pooled.*

Lower Limit of Detection (LLOD) *- in extracted brain homogenate – defined as signal to noise ratio (S/N) of 3 to 1 – was 50 fmol on column.*

Sample preparation

Assay setup: Brains dissected from 3 *Pogonomyrmex barbatus* were used for method development.



SCHEME 2. Extraction procedure.

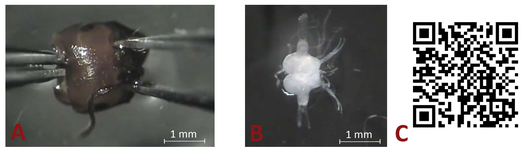


FIGURE 4. ***Pogonomyrmex barbatus*** **dissection:** *(A) head, (B) dissected brain, (C) brain dissection video (https://youtu.be/aS4C-AABaEI?t=1096)*

Results

Results

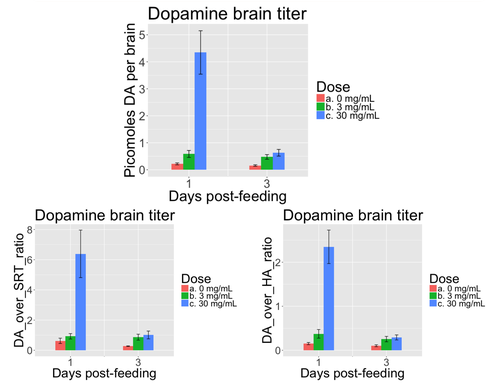


FIGURE 5. Dopamine dosing: 60 samples. *Collected foraging ants were anesthetized, divided in three groups and administered dopamine solution: 0 mg/mL (control), 3 mg/mL, or 30 mg/mL dopamine. After treatment, ants were collected after 1 or 3 days post-ingestion and dissected. Normalization of dopamine titers by either serotonin or histamine titers resulted in similar differences in dopamine levels among the treatment groups, suggesting that brain serotonin and histamine levels were qualitatively unaffected by the increases in dopamine.*

Conclusions

- Simultaneous measurements of serotonin and histamine – neurotransmiters not involved in dopamine biosynthetic pathway – were used for normalization of dopamine levels.
- This study establishes a sensitive LC-MS/MS method enabling efficient analysis of biogenic amines, particularly dopamine, in single ant brain samples.
- Transcriptomic analysis of brains from *P. barbatus* foragers revealed gene expression variation among colonies in several loci related to the metabolism and signaling of biogenic amines (Friedman et al., in review). Current work is focused on resolving the proximate mechanisms by which variation in forager brain biogenic amine signaling among colonies might contribute to ecological variation among colonies in foraging activity

Further reading

Deborah M. Gordon – "Ant Encounters: Interaction Networks and Colony Behavior" – Princeton University Press, 2010

Acknowledgments

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