

PRELIMINARY EVIDENCE FOR DIFFERENCES IN NEUROTRANSMITTER CONCENTRATIONS IN THE BRAINS OF AFRICANIZED AND EUROPEAN *Apis mellifera*

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ABSTRACT

Behavioral differences in defensive behavior are well-documented between Africanized and European honey bees. Since variation in neurotransmitter and neurotransmitter metabolite concentrations have been associated with behavioral differences in animals, we investigated brain neurotransmitter and neurotransmitter metabolite levels in honey bees of European and Africanized origin. The following neurotransmitters were examined: norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), gamma-amino butyric acid (GABA), and beta-alanine. Significant differences in the levels of beta-alanine were found in the brains of Africanized and European strains of *Apis mellifera*. While it would be premature to attribute the differences in behavior between the two strains to these differences in neurotransmitter levels, the differences reported here may be worthy of further attention. Confirmation of the neurotransmitter differences we detected using more extensive colony sampling and crossing experiments would provide evidence for a subspecific genetic basis for the variation.

INTRODUCTION

The honey bee, *Apis mellifera*, is an Old World species ranging from Scandinavia to the Cape of Africa, that has been classified into numerous subspecies or geographic races. Until the 1950's, honey bees of the Americas were primarily descendants of temperate-adapted European subspecies. However, in the 35 years following the introduction of a subsaharan subspecies into Brazil, descendent "Africanized" honey bees (AHB) spread to occupy most of the neotropics and now range north to Texas and south into Argentina.

Perhaps the most newsworthy difference between Africanized and European-derived honey bees is the degree of defensive behavior, with the AHB being much more prone to sting (Stort, 1974; Collins *et al.*, 1982). Differences in neurotransmitter and neurotransmitter metabolite concentrations have been associated with differences in behavior in various animals. We investigated the differences in brain neurotransmitter and neurotransmitter metabolite levels between European and Africanized honey bees.

MATERIAL AND METHODS

The following neurotransmitters were examined: norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), gamma-amino butyric acid (GABA), and beta-alanine. For this preliminary study, adult worker bees from one colony of Africanized and one colony of European origin were obtained from a university research apiary in Ribeirão Preto (Departamento de Genética,

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Universidade de São Paulo). The bees obtained for both strains were all five day old adult bees and had been allowed to emerge from sealed brood in an environmental chamber. The bees were preserved by freezing over dry ice from the time of collection to the time of analysis in the laboratory. The individual brain specimens were stored intact, in separate containers, and maintained at -70°C until the time of chromatographic analysis.

High pressure liquid chromatographic (HPLC) analysis of NE, DA, DOPAC, HVA, 5-HT and 5-HIAA were performed on brain material (Masters *et al.*, 1988) obtained from 10 Africanized and nine European honey bees. Bee brains were homogenized in 300 μl 2N perchloric acid using dihydroxybenzoic acid (DHBA) as the internal standard. The sample was centrifuged and then a straight injection of 50 μl was performed to determine the values for serotonin. Samples for catecholamine analysis were prepared using the method of Anton and Sayers (Masters *et al.*, 1988). To 200 μl of bee brain homogenate we added 1 ml of 1.5M Tris (pH 8.5) and 20 mg Al_2O_3 . The catecholamines were adsorbed to the Al_2O_3 by shaking for 10 minutes, the Al_2O_3 was then washed with deionized water two times and the catecholamines desorbed with 100 μl .2N perchloric acid. The column used was a 10 cm x 4.6 mm 3 μm Shandon ODS column packed in the laboratory. The ionic strength modifier was citric acid, the organic modifier was acetonitrile; triethyl amine was added to improve peak shape and octane sulfonic acid was the ion-pairing agent. The mobile phase was vacuum-filtered through 45 μm filters and degassed under vacuum prior to use. Solvent sample delivery was accomplished with a Spectrophysics Isochrome and detection was amperometric with a glassy electrode, using a silver chloride reference electrode and an amperometric detector (IBM) potential .6mV.

HPLC analysis of GABA and beta-alanine (Allison *et al.*, 1984) was performed on 12 Africanized and 10 European *Apis mellifera* brains. The GABA isoindole eluted at 10.7 min. and an unknown isoindole at 5.9 min. To assist in the identification of the unknown isoindole, 10 bee brains were extracted, reacted with OPA and T-BUSH, purified by HPLC for mass spectrometric analysis. The mass spectrum for this unknown was consistent with the isoindole of beta-alanine. Subsequently, the isoindole of beta-alanine (Sigma) was found to co-elute with the unknown isoindole on HPLC.

RESULTS AND DISCUSSION

The differences between neurotransmitter and neurotransmitter metabolite concentrations for the two strains are outlined in Table I. Significant differences for beta-alanine, DA, and the 5-HT metabolite 5-HIAA were found. However, after correction for multiple t-tests, only

the differences for beta-alanine remained significant. Our results suggest that differences may exist in certain neurotransmitter and neurotransmitter metabolite concentrations between the Africanized and European strains of *Apis mellifera*. While it would be premature to attribute the differences in behavior between the two strains to these differences in neurotransmitter levels, the differences reported here may be worthy of further attention. Confirmation of the neurotransmitter differences we detected using more extensive colony sampling and crossing experiments would provide evidence for a subspecific genetic basis for the variation. Future studies might examine the effect of interventions capable of changing the honey bee brain levels of these and perhaps other neurotransmitters, together with the effect that these interventions have on behavior. For instance, it would seem propitious to investigate the effect that beta-alanine dietary supplementation of the hive might have on AHB defensive behavior.

Table I - Honey bee strain.

	Africanized		European		t-value	P-value
	(Pmole/mg)	(SD)	(Pmole/mg)	(SD)		
Beta-alanine	5460	(960)	7256	(1205)	3.89	.0009
GABA	1226	(258)	1247	(302)	-0.17	.86
NE	0.26	(1.0)	0.29	(0.73)	-0.65	.52
DOPAC	1.26	(1.35)	0.97	(0.83)	0.57	.57
DA	0.53	(0.21)	0.37	(0.56)	2.22	.04
HVA	0.76	(1.52)	0.07	(0.14)	1.44	.16
5-HIAA	0.2	(0.19)	0.65	(0.52)	2.12	.05
5-HT	0.11	(0.04)	0.11	(0.03)	-0.20	.85

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RESUMO

Tem sido bem documentadas diferenças de comportamento de defesa entre abelhas Africanizadas e Européias. Desde que variação em concentrações de neurotransmissor de metabolitos de neurotransmissor foram associadas com diferenças de comportamento em animais, investigamos níveis de neurotransmissor no cérebro e de metabolitos do neurotransmissor em abelhas de origem Européia e Africanizadas. Os seguintes neurotransmissores foram examinados: norepinefrina (NE), dopamina (DA), ácido 3,4-dihydroxyphenyl-acético (DOPAC), ácido homovanílico (HVA), 5-hydroxytryptamina (5-HT), ácido 5-hydroxyindoleacético (5-HIAA), ácido gama-amino butírico (GABA) e beta-alanina. Foram encontradas diferenças significativas nos níveis de beta-alanina no cérebro de abelhas Africanizadas e Européias (*Apis*

mellifera). Embora seja prematuro atribuir as diferenças de comportamento entre os dois grupos a diferenças nos níveis de neurotransmissor, as diferenças mostradas aqui merecem atenção. Para a confirmação de diferenças no neurotransmissor precisa usar um número maior de colônias e experimentos de cruzamento para ter evidência para uma base genética subespecífica para esta variação.

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