

## Sexual differences in the brain catecholamine content in four species of tropical bats

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**Abstract:** The catecholamines dopamine, norepinephrine and epinephrine were studied in the brains of male and female tropical bats of four species, with different feeding habits (insectivorous, frugivorous, omnivorous and pollen eater). They were trapped in a refuge at 18°24'24"N, 99°02'08"W with a mean annual temperature of 25.8°C, in a tropical deciduous forest. The three catecholamines occur in both sexes of all four species, in levels which are statistically different among species as well as between sexes. Dopamine and norepinephrine levels were higher in males than females, but the opposite occurs with epinephrine. These findings suggest that changes in catecholamine levels are intimately involved in the reproductive pattern of the species studied.

**Key words:** catecholamines, tropical bats, sexual differences.

The maturation of the central nervous system follows specific patterns in the male and the female, as shown by morphological and structural, functional and behavioral differences. The monoamines (m.a.) -dopamine (DA), norepinephrine (NE) and serotonin (5HT)- are present in the central nervous system of animals from all major phyla and they may act as neurotransmitters or neuromodulators in brain function (Agrawal *et al.* 1968).

Accumulated evidence indicates the importance of the m.a. in the control of gonadotropin hormone secretion and other sexual functions in

the adult rat, and there has been speculation about the role of m.a. in the process of sexual differentiation (Ladosky and Gaziri 1970). Since gonadotropic hormone secretion shows a clear sexual difference in rats during the first three weeks of age (Döhler and Wuttke 1975), it is possible that analogous to the adult rat, m.a. may play an important role in the control of the gonadotropin secretion and hence in the brain's sexual differentiation. Vogt (1954) showed that m.a. were unequally distributed in mammalian brain tissue. Catecholamines (CA) are very important for

the neuroendocrine processes related to the bats' reproductive biology (Richardson 1979).

Here we compare the CA brain levels in both sexes of four species of tropical bats which live under similar environmental conditions, but differ in feeding habits.

## MATERIAL AND METHODS

The bats were trapped near Túnel del Arco, 8 km south of San Juan Chinameca, Morelos,

México (18°34'24"N, 99°02'08"W), 1,025 m asl. The surrounding vegetation is a tropical deciduous forest, with a mean annual temperature of 25.8°C.

Four different species, *Pteronotus parnellii mexicanus*, *Artibeus jamaicensis triomylus*, *Glossophaga leachii* and *Leptonycteris sanborni* were studied. Vouchers are deposited at the Colección de Mamíferos, Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Reproductive patterns and feeding habits are summarized in Table 1.

TABLE 1

*Reproductive patterns and feeding habits of bats used in this study*

SPECIES	REPRODUCTIVE PATTERN	AUTHOR	FEEDING HABIT	AUTHOR
<i>P. parnellii mexicanus</i>	Seasonally monoestrous cycle, dextral asymmetry of the bihorned uterus	Garrido <i>et al.</i>	Insectivorous 1984	Villa 1967 Garrido <i>et al.</i> 1984
<i>A. jamaicensis triomylus</i>	Seasonally polyoestrous cycle. Bimodal polyestry in Panama. Reliable information about this species in Mexico not available	Wilson 1973	Frugivorous	Villa 1967
<i>G. leachii</i>	No information available about the cycle. Bimodal polyestry including specimens from Mexico.		Omnivorous with preference for nectar	Villa 1967
<i>L. sanborni</i>	Monoestrous or bimodal polyoestrous because of a birth peak in early November in Mexico. Apparently another peak on May or June. Maybe migratory.	Hayward & Cockrum 1971	Polen eater	Villa 1967

In order to avoid possible seasonal and circadian variations (Philo *et al.* 1977), animals were studied only during last winter months, February and March. The specimens were trapped in mist nets with plant camouflage during a twelve hour period, from 18:00 to 6:00hs. Only *P. parnellii* were trapped in the mist nets at the entrance of the tunnel, which serves them as a refuge. The bats were then put in a previously prepared cage inside the tunnel to reduce stress.

All the animals were young adults, as shown by the gonadal tissues and epiphyseal ossification of the wings. Due to the difficulty of keeping animals alive for a long time, they were sacrificed by cervical dislocation between 9:00 and 11:00hs on the morning after they were trapped. Each bat was weighed and measured and a smear was taken from the females for the

observation of their cycle phase. Smears were fixed in 70% alcohol and were stained by the Papanicolaou technique. The brain was quickly removed and frozen at -70°C in dry ice-acetone and kept in a special box with dry ice. Then, all tissues were stored in the laboratory at -70°C until the assay was performed.

The extraction and assay of the CA-DA, NE and E-were carried out according to the method described by Ben-Jonathan and Porter (1976), with slight modifications. The tissues were homogenized in a Potter-Elvehjem homogenizer in 0.4M perchloric acid (1/5, w/v) and then the homogenates were centrifuged at 15,000 g for 10 minutes. The supernatant was decanted and the CA determination in a 50 µl was used for a 75 minute incubation, with <sup>3</sup>H-S-adenosyl-methionine, previous to CA extraction. The

catecholamines were separated by thin layer chromatography on plastic plates with silica gel without gypsum (Polygram Sil G, Brinkmann Instruments, Inc.).

The plates were developed as described elsewhere (Ben-Jonathan and Porter 1976) and then were dried and exposed briefly to iodine vapor, revealing three spots corresponding to the CA metabolites. Each spot, with the underlying plastic support, was cut out and placed in a scintillation vial with 0.5 ml of 0.1M acetic acid. After vigorous shaking, 10 ml of Instagel (Packard) were added and radioactivity was counted by a Packard Tri-Carb liquid scintillation spectrometer (Mod. 544). The counting conditions allowed for 40%  $^3\text{H}$ -counting efficiency and 80%  $^{14}\text{C}$  efficiency.

The results are expressed as the mean  $\pm$  standard error of the mean along with the number of individual observations (n). Experimental values obtained within the same range of (n) were compared by use of Student's t-test. Males were also compared with females.

Comparisons among *P. parnellii* and the other species were carried out by the Newman-Keuls test, which permits a comparison

among multiple means obtained from samples of very different size (Steel and Torrie 1985).

## RESULTS

Vaginal smears obtained from 40 females revealed that 22 of them were in the metaoestrous phase and therefore, only the animals in this phase were used. Results of CA-DA, NE and E- brain content are presented in Tables 2 to 4, respectively. Statistically significant differences among species and between sexes ( $p < 0.001$ ) were found.

The highest concentration of DA (Table 2) was found in male *L. sanborni* (915 ng/g of wet tissue) and the lowest in female *G. leachii* (61 ng/g of wet tissue). In *P. parnellii*, no significant differences were observed between male and female. Data obtained from this species were statistically different ( $p < 0.001$ ) from the other studied. For instance, we got 638 vs 368 ng/g of wet tissue for males of *P. parnellii* and *A. jamaicensis* respectively (Newman-Keuls test,  $p < 0.01$ ).

TABLE 2

Sex differences in brain dopamine levels in four tropical bat species; neurotransmitter is expressed as ng/g of wet tissue

	<i>P. parnellii</i>	<i>A. jamaicensis</i>	<i>G. leachii</i>	<i>L. sanborni</i>
Male	638 $\pm$ 6.90 $\sqrt$ (12)	468 $\pm$ 30.0 $\pm$ (4)	483 $\pm$ 19.08 $\pm$ (8)	915 $\pm$ 28.32 $\pm$ (12)
Female	568 $\pm$ 23.00 (4)	65 $\pm$ 2.04 $\alpha$ , $\pm$ (6)	61 $\pm$ 5.20 $\alpha$ , $\pm$ (3)	236 $\pm$ 8.00 $\alpha$ , $\pm$ (9)

Data represent mean  $\pm$  standard error of the mean; the number in parenthesis is the sample size. The analysis of the samples was done by triplicate. Female bats were in metaoestrous phase.

$\alpha$  =  $p < 0.001$  male vs female data, Student "t" test.

$\pm$  =  $p < 0.001$  *P. parnellii* vs the other species, Student "t" test.

$\sqrt$  =  $p < 0.01$  Newman Keuls test.

The NE levels (Table 3) are different between sexes ( $p < 0.001$ ). The highest value was found in male *A. jamaicensis* (556 ng/g of wet tissue) and the lowest in *G. leachii* (104 ng/g of wet tissue). However, in *P. parnellii* significant differences between sexes were not observed. Statistical differences ( $p < 0.001$ ) were noted when the data of *P.*

*parnellii* were compared with those of the other species.

Sex differences in E brain levels appear in Table 4. In most cases the levels of E were higher in the females ( $p < 0.001$ ). However, no differences were found between the sexes of *L. sanborni*. The highest concentration was observed in female *P. parnellii* (117 ng/g of wet

TABLE 3

Sex differences in brain norepinephrine levels in four tropical bat species; neurotransmitter is expressed as ng/g of wet tissue

	<i>P. parnellii</i>	<i>A. jamaicensis</i>	<i>G. leachii</i>	<i>L. sanborni</i>
Male	446 ± 12.40 $\phi$ (12)	556 ± 32.0 ± (4)	245 ± 9.89 ±,√ (8)	482 ± 3.47 ±,√ (12)
Female	443 ± 21.00√ (4)	130 ± 2.86 $\alpha$ ,±,√ (6)	104 ± 2.89 $\alpha$ ,±,√ (3)	526 ± 4.00 $\alpha$ ,± (9)

Data represent mean ± standard error of the mean; the number in parenthesis is the sample size. The analysis of the samples was done by triplicate. Female bats were in metaoestrous phase.

$\alpha$  =  $p < 0.001$  male vs female data, Student "t" test.

± =  $p < 0.001$  *P. parnellii* vs the other species, Student "t" test.

√ =  $p < 0.01$  Newman Keuls test.

$\phi$  =  $p < 0.05$  Newman Keuls test.

TABLE 4

Sex differences in brain epinephrine levels in four tropical bat species; neurotransmitter is expressed as ng/g of wet tissue

	<i>P. parnellii</i>	<i>A. jamaicensis</i>	<i>G. leachii</i>	<i>L. sanborni</i>
Male	49 ± 1.73 √ (12)	33 ± 2.00 ± (4)	39 ± 1.06 (8)	73 ± 2.02 ± (12)
Female	177 ± 9.5 $\alpha$ (4)	125 ± 4.49 $\alpha$ ,± (6)	118 ± 7.51 $\alpha$ ,± (3)	80 ± 3.67 ± (9)

Data represent mean ± standard error of the mean; the number in parenthesis is the sample size. The analysis of the samples was done by triplicate. Female bats were in metaoestrous phase.

$\alpha$  =  $p < 0.001$  male vs female data, Student "t" test.

± =  $p < 0.001$  *P. parnellii* vs the other species, Student "t" test.

√ =  $p < 0.01$  Newman Keuls test.

tissue) and the lowest in male *A. jamaicensis* (33 ng/g of wet tissue). When the data of *P. parnellii* were compared with the other species, significant differences were observed ( $p < 0.001$ ). For instance, we obtained 49 vs 33 ng/g of wet tissue from male *P. parnellii* and *A. jamaicensis* respectively.

## DISCUSSION

To assess the role of neurotransmitters in the neural circuitry, their concentration levels and turnover rates need to be studied. Although the latter provide a more accurate evaluation, data obtained by studying physiological levels are also useful. The neurotransmitters that we have studied -DA, NE and E- are closely related to

the vital functions of neuroendocrine control and adaptive and behavioral mechanisms (Ortega-Corona *et al.* 1982).

These results confirm that three CA are present in the brain of bats and also show that there are important differences between sexes and species. Some authors (Ladosky and Gaziri 1970, Gordon and Shellenberger 1974, Gorski *et al.* 1978, Ortega-Corona *et al.* 1979 and Vaccari 1980), have reported sexual differences in rats as well as in other species. Vaccari (1980) found that male brains contained more NE than female brains. We accordingly found that the NE levels in two bat species are higher in the male.

The overall lower content of NE in female brains may be a reflection of a greater and faster rate of NE utilization. This is confirmed by the fact that DA levels are lower in females. An

indirect proof of a faster "functioning" of the CA-system in female rats is that the brain slices of pro-oestral females take up more NE. This difference could be explained by either a higher rate of synthesis or a less efficient catabolism in the male.

Finally, given the classical wisdom that animals often synchronize their reproduction with food peaks, as has been demonstrated for *P. parnellii* in México (Garrido *et al.* 1984) and *A. jamaicensis* in Panama (Wilson 1973), we surmised that differences in CA levels among these four species could be explained by seasonal changes in food quality and content. The differences may be due also to feeding habits or composition of the diet; if this is so, the neurotransmitters may be intimately involved in the adaptation process of each species to its environment.

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#### RESUMEN

Se estudiaron las catecolaminas -dopamina, norepinefrina y epinefrina- en el cerebro de machos y hembras de cuatro especies de murciélagos tropicales, con diferentes hábitos de alimentación (insectívoro, frugívoro, omnívoro y polínívoro). Estos fueron capturados cerca del Túnel del Arco, estructura de un acueducto que corre en un bosque tropical caducifolio cuya temperatura anual media es 25.8°C. Las tres catecolaminas se encuentran presentes en el cerebro de todas las especies estudiadas y muestran diferencias estadísticamente significativas tanto entre especies, como entre sexos. Mientras la dopamina y la norepinefrina son consistentemente más elevadas en los machos, la epinefrina lo es en las hembras. Estos hallazgos sugieren que los cambios en los niveles de las catecolaminas se encuentran íntimamente ligados al patrón reproductivo de cada especie e involucrados en funciones cerebrales importantes, tales como la conducta, la integración endocrina y la diferenciación sexual del cerebro. El ambiente, la

dieta y el sexo posiblemente participan en los mecanismos de adaptación de estas especies a su entorno.

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