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SEASONAL FLUCTUATION IN THE NEURONAL CLASSES OF PARAHIPPOCAMPAL AREA OF *P. krameri* (SCOPOLI, 1769) AND *E. scolopaceus* (LINNAEUS, 1758)

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Abstract

Hippocampus in birds is a relatively narrow, curved strip of tissue that lies on the dorsomedial surface of telencephalon. It is widest dorsally at the junction with parahippocampal area, and it tapers with septum. Parahippocampal area (APH), the most prominent field of hippocampus is a long structure that lies at the most rostral level and continues upto caudal extent. It has been indicated by behavioral studies that hippocampus in birds plays an important role in process of learning, memory formation, food storage and spatial navigation. The present study enlightens some interesting fluctuations occurring in the neuronal classes of parahippocampal area of two seasonally breeding birds viz. *P. krameri* and *E. scolopaceus* in terms of dendritic thickness, spine density and spine morphology during breeding and non-breeding time period of birds. The Golgi-impregnated sections were used to study these fluctuations and it was noticed that there was a significant increase in dendritic thickness, spine density, spine length and spine head diameter during breeding as compared to non-breeding period. The results obtained were comparable in two different seasonally breeding birds, supporting the view that avian parahippocampal area shows neuroanatomical plasticity associated with breeding and non-breeding period because of variations in endocrinology.

Key words:Seasonal plasticity, dendritic thickness, spine morphology, spine density, *Psittacula krameri* (order-Psittaciformes), *Eudynamys scolopaceus* (order- Cuculiformes).

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INTRODUCTION

In both mammals and birds, the most medial part of pallium is hippocampus formation, which is major pallial component of limbic system**.** In reference to birds, the term hippocampus is used to describe a strip of tissue lying close to the midline on the dorsal surface of telencephalon. This strip runs from posterior limit of telencephalon and extends to cover about two-thirds of anterior-posterior axis and is bounded ventrolaterally by lateral ventricle. The hippocampal complex is divided into five fields viz- Medial hippocampus (HCm), Lateral hippocampus (HCl), Parahippocampal area (APH), Central field of parahippocampus (PHc) and Crescent field (CF).The APH of zebra finch (36) and strawberry finch (34) seems to be a long structure that extends in a rostrocaudal direction and is located lateral to the hippocampus in the thin dorsal roof of the hemisphere at the caudal telencephalon. Hippocampus is involved in spatial memory and other learning functions (4,40) and plays role in the control of behavior related to food and appetite (37). The avian hippocampus changes seasonally as it is larger during hoarding season than during non-hoarding time in food-hoarding birds (33,19). The hippocampal volume has been found to be larger during breeding season in the sex(es) that actively looks for host nests in brood parasitic cowbirds (26). It has been noticed that navigational experiences affect hippocampal volume, the hippocampal size being larger in homing pigeons than to non-homing pigeons (28). The overall changes reported in all the above studies were related to increase in volume of hippocampus across the seasonal variations.

While studying seasonal plasticity in context to song control system during breeding season it has been noticed that HVC (robust nucleus of acropallium-RA) and Area X are more than double in volume in comparision to non-breeding season (38). Testosterone is believed to play supportive role in both singing behavior and seasonal changes in song control system (12,38). Synaptic morphology also varies seasonally in RA; the sizes of presynaptic and postsynaptic profiles were larger during breeding season in canaries (7). In captive red-winged blackbirds (*Agelaius phoeniceus*), density of dendritic spines on RA neurons were greater in males maintained on long spring-like day lengths than in males on short days (16).

A few investigations on the fluctuations in dendritic spine density of neurons of hippocampus are confined to mammals as it has been observed that apical dendritic spine density in CA1 hippocampal pyramidal cells undergo cyclic fluctuations with changing levels of estradiol and progesterone during estrous cycle in adult female rat (42). The sex steroid estradiol (E_2) in adult rodents increases spine density on hippocampal neurons (43) and affects hippocampal neurogenesis (25,35).

Barnea and Nottebohm (3) proposed that there is seasonal recruitment of hippocampal neurons in adult free ranging black-capped chickadees. According to them, relatively large neurons in a ventral layer of the hippocampal complex are constantly born in adult chickadee brain and is at peak during fall favoring their hypothesis that peak in neuronal recruitment occurred at a time when there was change in life-style and use of space generated an acute need for new spatial memories. This study presented an overview of seasonal plasticity in the terms of neuronal recruitment in hippocampal neurons but provided no information regarding changes occurring in neuronal characteristics. Therefore, the present study was undertaken in order to determine the seasonal plasticity occurring in the neuronal classes of hippocampus particularly APH region of birds which is the largest field of hippocampus with an

expectation to find if there is any significant difference in spine density between breeding and non-breeding period. Our investigation shows that there is an increase in spine turnover in birds during their breeding period and female *E. scolopaceus* exhibits greater seasonal fluctuations in neuronal characteristics in comparison to female *P. krameri*.

Golgi-impregnated tissues of two different birds- *Psittacula krameri* commonly known as Indian ring neck parrot (ability to learn and memorize) and *Eudynamys scolopaceus* i.e. Koel (brood parasite) were used to examine the plasticity in neurons of APH. The two different birds viz- *P. krameri* with ability to learn and memorize and *E. scolopaceus* showing brood parasitism used in the study were selected because their special features i.e learning and memory (8) and brood parasitic nature (27,32) are related to hippocampus.

MATERIALS AND METHODS

Ten adult female *Psittacula krameri* weighing 100-125 gms, measuring 32.3-40 cm in length (from tip of beak to end of tail) and Ten adult female *Eudynamys scolopaceus* weighing 80-197 gms, measuring 27.7-40.3 cm in length used in this study were collected from Allahabad(25° 28′ N, 81° 54′ E). The birds were brought and immediately sacrificed for experiment to avoid any stress condition. All the procedures were carried out according to institutional animal care guidelines.

The females of *P. krameri* and *E. scolopaceus* can be easily distinguished from their male partners as the female *P. krameri* posses' ring which is very pale in color while males bear a striking black ring with pink and pale blue outer rings attained at sexual maturity (2-3 years of age). In case of *E. scolopaceus,* females are brownish on the crown and have rufous streaks on the head. The back, rump and wing coverts are dark brown with white and buff spots. The underparts are whitish, but is heavily striped whereas their male partners are bluish-black with grey bill and red iris.

Cresyl-violet method

Two *P. krameri*, one during breeding (Oct-Feb) and other during non-breeding period (Mar-Sep), similarly two *E. scolopaceus,* one during breeding (Mar-Aug) and other during non-breeding period (Sep-Feb) were anaesthetized and perfused with 10 % formalin solution; the brain was removed, dehydrated and embedded in paraffin. 20 µm thick sections were cut and stained with Cresyl-violet staining solution (17) for the cytoarchitectonic study of hippocampal complex and especially the APH region (Fig 1).

Golgi-Colonnier method

Eight *P. krameri* and *E. scolopaceus* each were used for this study. Four brains (eight hemispheres) during breeding time (last week of December for *P. krameri* and last week of June for *E. scolopaceus*) and four brains (eight hemispheres) during non-breeding time (July for *P. krameri* and December for *E. scolopaceus*) were used for studying neuronal morphological features by applying Golgi-Colonnier method (5). The Birds were perfused transcardially with saline at 4°C for 15 minute and subsequently with the 2% Para formaldehyde in 0.1M phosphate buffer (4°C, pH 7.4) for 40 minute. The brains were removed from the skull and immersed in same fixative for 24 hours followed by prechromation (two times) in 2.5% potassium dichromate for 60 minute each treatment. Then they were transferred to the 5% glutarldehyde v/v and 2% potassium dichromate w/v solution at 4°C for 3 days for chroming. For the impregnation the brains were transferred to 0.60% w/v solution of the silver nitrate at 4°C for 2 days. Both of the chroming and impregnation steps were repeated twice and washed two to three times in distilled water between the solutions. After the completion of second and final impregnation, brains were dehydrated in series of alcohol, cleared in xylene and embedded in paraffin wax. 100 µm thick sections were cut, deparaffinised in xylene and dehydrated in downgrade and upgrade of alcohols and cleared in xylene before being coversliped using DPX mounting medium. Photomicrographs were taken with the help of computer aided Nikon eclipse 80i microscope. The camera lucida drawings were made by simple light microscope equipped with camera lucida. All the drawings were scanned and corrected with the help of Adobe Photoshop computer software.

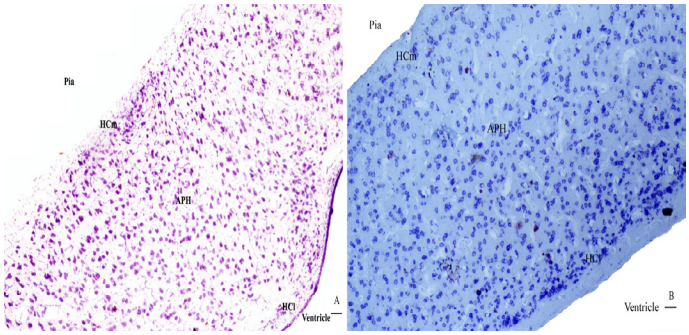

Figure 1. Photomicrograph showing Cresyl stained section indicating different regions of hippocampus of (A) female *P. krameri* and (B) female *E. scolopaceus*, where HCm= Medial hippocampus; APH= Parahippocampal region; HCl= Lateral hippocampus Scale bar = 10 µm.

Statistical Analyses

Dendritic thickness (both primary and secondary dendrites were used) was calculated with help of camera lucida drawings (with scales drawn on it) using Paint software whereas spine density, spine head diameter and spine length were calculated with help of photomicrographs taken with help of Nikon eclipse 80i microscope at 40x magnification.

For spine density, numbers of spines were calculated per 10µm of dendritic segment. All spines are not visible since some are obscured by opaque dendritic shaft therefore to predict 'true' spine density with reasonable accuracy, the formula given by Feldman and Peters (9) was applied.

$$N = \frac{n\pi\,[\,(Dr + Sl)^2 - (Dr + Sd)^2\,]}{[\,\theta/90 \,.\, \pi\,(Dr + Sl)^2\,] - 2\,[\,(Dr + Sl)\,\sin\theta .\,(Dr + Sd)\,]}.$$

Where, N = true spine density; n = number of visible spines; Dr = radius of dendrite; Sl = length of spine; Sd = diameter of spine head & θ = central angle.

All the parameters were assessed by Students t-Test. Differences between the seasons were tested for significance at $P<0.05$.

RESULTS

Animal 1- Female Psittacula krameri

Four types of neurons were identified in APH region of non-breeding and breeding female *Psittacula krameri* viz. Multipolar, Pyramidal, Bipolar and Unipolar neurons (Fig 2-5).

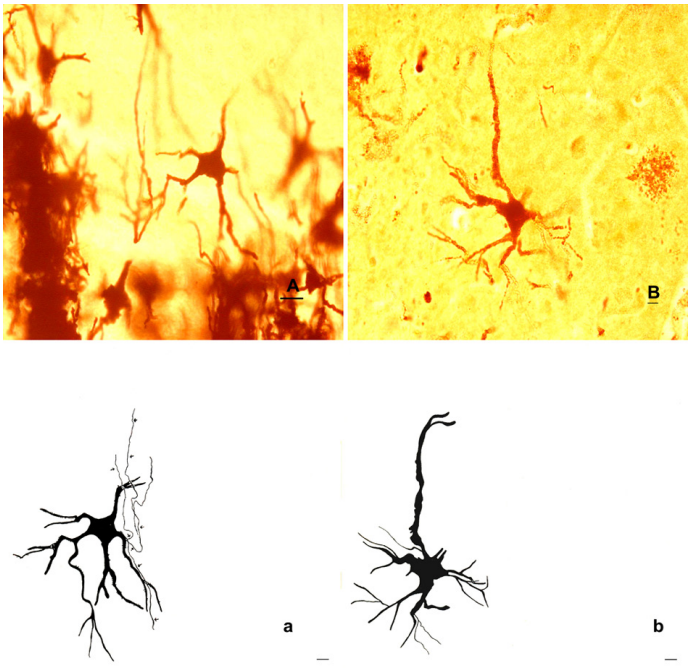

Figure 2. Photomicrographs and camera lucida drawings of multipolar neurons of APH region of female *P. krameri* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

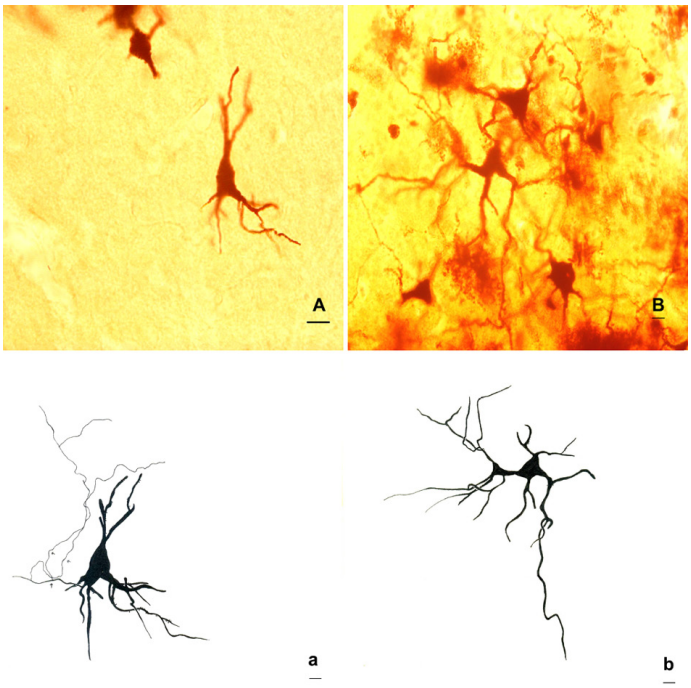

Figure 3. Photomicrographs and camera lucida drawings of pyramidal neurons of APH region of female *P. krameri* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

Multipolar neurons

These neurons gave rise to 4-6 thick dendritic branches towards all the possible directions originating from medium sized soma with diameter about 17-32 µm during non-breeding time and 14-26 µm during breeding time (Table 1). These neurons appeared to be main subtype as observed in chick and homing pigeon (36) and in APH region of strawberry finch (34) accounting for 56.41% and 57.64% during non-breeding and breeding period respectively of presently studied bird (Table 1). The soma shape varied from rectangular, oval to irregular, where a few multipolar neurons were of different type with irregular soma and apical dendrite elongated and thicker in comparison to basal dendrites acquiring nearly crab like appearance (Fig 2 B).

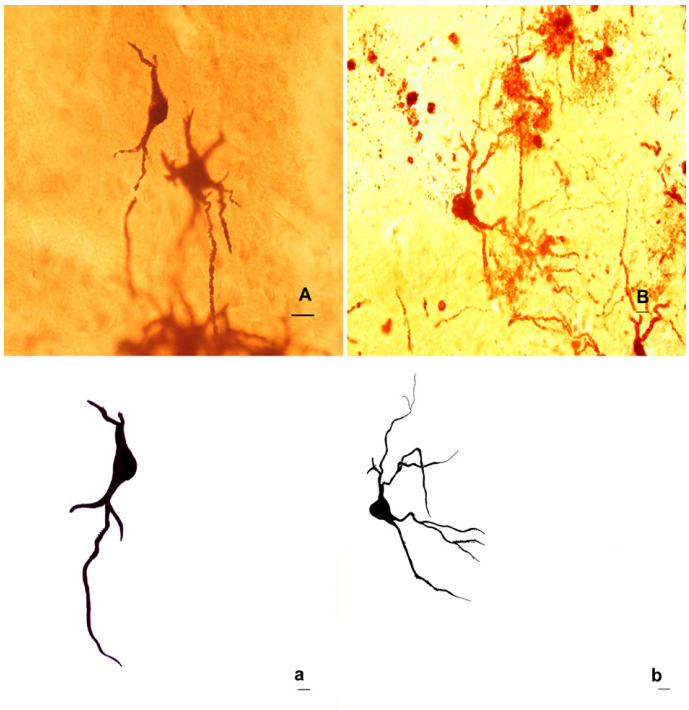

Figure 4. Photomicrographs and camera lucida drawings of bipolar neurons of APH region of female *P. krameri* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

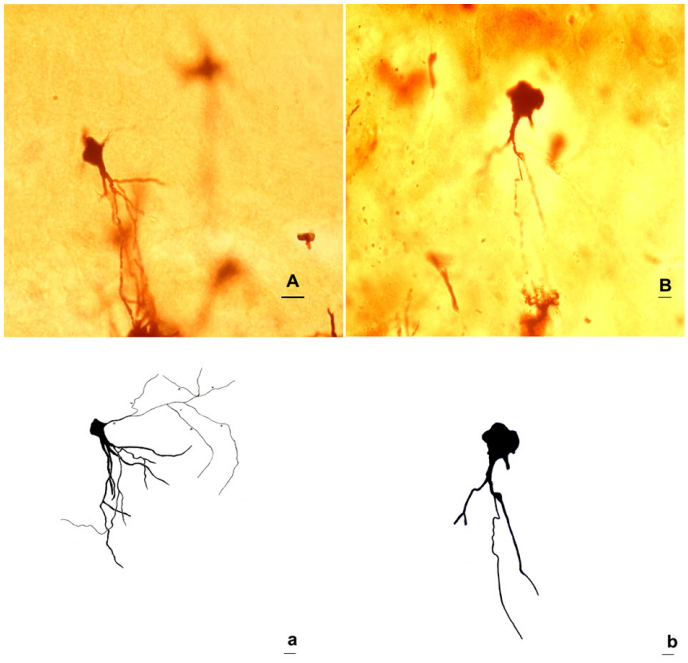

Figure 5. Photomicrographs and camera lucida drawings of unipolar neurons of APH region of female *P. krameri* during (A, a) non-breeding and (B, b) breeding seasons. Scale bar=10 µm.

Pyramidal neurons

The medium size somata of this type of neurons measuring 21-25 µm during non-breeding and 12-22 µm during

breeding time gave rise to thick apical dendrite with one or two side branches and finer basal dendrites (Table 1). The varied ranges of soma shape were observed like triangular, fusiform and pyramidal with stretched sides. It was observed that the apical dendrites were comparatively less developed than basal dendrites. i.e these neurons were different from mammalian pyramidal neurons in respect of highly developed apical dendrites.

Bipolar neurons

These neurons gave rise to dendritic branches in two directions one towards the pia and other towards ventricular with bean or oval shaped medium sized soma (20-22 and 14-22 µm). They were observed to be least in number among all 4 types of neurons during breeding period of bird (Table 1).

Table 1. Neuronal characteristics of the APH of female Indian ringneck parrot (*Psittacula krameri*) during non-breeding (N.B.) and breeding (B.) time period.

Type of Neuron	Soma diameter (in µm)		Dendritic field (in µm)		n (number of neurons)		Percentage %	
	N.B.	B.	N.B.	B.	N.B.	B.	N.B.	B.
Multipolar	17-32	14-26	63-316 x 19-213	145-228 x 130-227	110	117	56.41%	57.64%
Pyramidal	21-25	12-22	90-217 x 99-229	72-199 x 96-290	60	54	30.76%	26.60%
Bipolar	20-22	14-22	205-207 x 41-42	94-95 x 98-100	16	11	8.21%	5.42%
Unipolar	21-22	13-38	180-181 x 143-145	84-91 x 70-117	9	21	4.62%	10.34%

% is given as n.100/195 for N.B. and n.100/203 for B., 195 and 203 are the number of neurons examined in this study during non-breeding and breeding time of bird. Immature neurons were not used in this study.

Table 2. Thickness of dendrites (in µm) calculated for different types of neurons during non-breeding and breeding seasons in APH region of female *Psittacula krameri.*

Type of Neuron	Dendritic thickness (Non-breeding)	Dendritic thickness (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 1-4µm	Ad: 2-9µm	DF=6 t_{tab}=2.447	t_{cal}=3.41	Significant.
	Bd: 1-4 µm	Bd: 3-8 µm		t_{cal}=5.5	Significant.
Pyramidal	Ad: 2-5µm	Ad:3-8µm	DF=6 t_{tab}=2.447	t_{cal}=2.46	Significant.
	Bd: 2-4 µm	Bd: 2-8 µm		t_{cal}=3.24	Significant.
Bipolar	Ad: 0.74-3µm	Ad: 1-4µm	DF=6 t_{tab}=2.447	t_{cal}=3.21	Significant.
	Bd: 1-4 µm	Bd: 1-4 µm		t_{cal}=1.52	Insignificant.
Unipolar	Ad: 0.84-3µm	Ad: 1-6µm	DF=6 t_{tab}=2.447	t_{cal}=2.86	Significant.
	Bd: -	Bd: -			-

Ad= Apical dendrite; Bd= Basal dendrite.

Table 3. Length of Spines (in µm) calculated for different types of neurons during non-breeding and breeding seasons in APH region of female *Psittacula krameri*.

Type of Neuron	Spine length (Non-breeding)	Spine length (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 0.44-0.70µm	Ad: 0.7-1.82µm	DF=8 t_{tab}=2.306	t_{cal}=5.09	Significant.
	Bd: 0.53-1.23 µm	Bd: 0.56-1.47 µm		t_{cal}=3.18	Significant.
Pyramidal	Ad: 0.26-0.79µm	Ad: 0.56-1.96µm	DF=8 t_{tab}=2.306	t_{cal}=5.08	Significant.
	Bd: 0.44-1.23 µm	Bd: 0.7-1.47 µm		t_{cal}=2.55	Significant.
Bipolar	Ad: 0.79-1.23µm	Ad: 1.12-1.75µm	DF=6 t_{tab}=2.447	t_{cal}=4	Significant.
	Bd: 0.53-0.88 µm	Bd: 0.84-1.54 µm		t_{cal}=4.3	Significant.
Unipolar	Ad: 0.53-1.23µm	Ad: 0.84-1.54µm	DF=5 t_{tab}=2.571	t_{cal}=1.55	Insignificant.
	Bd: -	Bd: -			-

Ad= Length of spines present on apical dendrite; Bd= Length of spines present on basal dendrite.

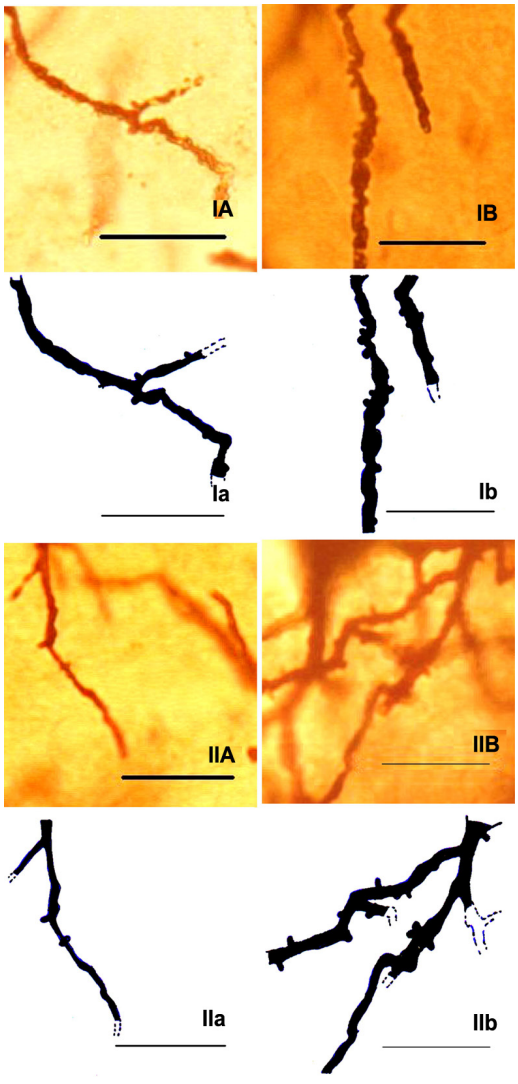

Figure 6. Segments of dendrite showing spines, where IA and IB are basal dendritic segments of multipolar neurons & IIA and IIB are the basal dendritic segments of pyramidal neurons of female *P. krameri* during non-breeding (A) and breeding (B) periods respectively Scale bar = 10 µm.

Unipolar neurons

These neurons gave rise to their dendrites towards single direction i.e. either towards the pole or towards the ventricle. They contributed very less during non-breeding period and only 4.62% of the total neurons belonged to this category (Table 1).

The Golgi impregnated brain sections revealed under mentioned facts about seasonal fluctuations among the neurons in terms of:

1) Dendritic thickness: Significant increase (P<0.05) in dendritic thickness (both primary and secondary dendrites included) of all four types of neurons except for the basal dendritic thickness of bipolar neuron was observed (Fig 6, Table 2).
2) Spine length: Significant increase (P<0.05) in length of spine in multipolar, pyramidal and bipolar neurons was noticed during breeding period of bird (Table 3).
3) Spine head/Spine head diameter: The differences in diameter of spine head present on basal dendrite of pyramidal and bipolar were significant at P<0.05 (Table 4).
4) Spine Density: Significant increase (P<0.05) in basal dendritic spine density of multipolar and pyramidal neurons, and significant difference in apical dendritic spine density of bipolar neuron were noticed (Table 5).

Animal 2- Female Eudynamys scolopaceus

Four types of neurons viz- Multipolar, Pyramidal and Bipolar neurons were observed in the APH of female *E. scolopaceus* during breeding time while during non-breeding time only three types of neurons could be observed (Multipolar, Pyramidal and Bipolar neurons; Fig. 7-9).

Multipolar neurons

These neurons gave rise to 4-6 thick dendritic branches towards all the possible direction originating from their medium/ large sized soma (26-35 µm during breeding and 23-29 µm during non-breeding time). They contributed to 74.07% and 75% of total neuronal population respectively

Table 4. Spine head diameter (in µm) for different types of neurons during non-breeding and breeding seasons in APH region of female *Psittacula krameri.*

Type of Neuron	Spine head diameter (Non-breeding)	Spine head diameter (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 0.26-0.70µm	Ad: 0.42-0.56µm	DF=8 t_{tab}=2.306	t_{cal}=1.25	Insignificant.
	Bd: 0.26-0.44µm	Bd: 0.49-0.77µm		t_{cal}=1	Insignificant.
Pyramidal	Ad: 0.18-0.35µm	Ad: 0.35-0.84µm	DF=8 t_{tab}=2.306	t_{cal}=1.93	Insignificant.
	Bd: 0.26-0.70µm	Bd: 0.42-1.19µm		t_{cal}=3.33	Significant.
Bipolar	Ad: 0.35-0.70µm	Ad: 0.42-0.70µm	DF=6 t_{tab}=2.447	t_{cal}=0.68	Insignificant.
	Bd: 0.18-0.53µm	Bd: 0.42-0.77µm		t_{cal}=3.57	Significant.
Unipolar	Ad: 0.44-0.53µm	Ad: 0.35-0.56µm	DF=5 t_{tab}=2.571	t_{cal}=1	Insignificant.
	Bd: -	Bd: -			-

Ad=Head diameter of spines present on apical dendrite; Bd= Head diameter of spines present on basal dendrite.

Table 5. Spine density (±SD) calculated for different types of neurons (during non-breeding and breeding time period) in APH region of female *Psittacula krameri.*

Type of Neuron	n (Non-breeding)	Spine density (Non-breeding) N	n (Breeding)	Spine density (Breeding) N	T test at P<0.05		
					Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Apical n= 1-3	Apical: 8.07±3.88	Apical n= 3-5	Apical: 10.66±4.08	DF=8 t_{tab}=2.306	t_{cal}=2.07	Insignificant.
	Basal n= 2-5	Basal: 10.08±4.25	Basal n= 3-6	Basal: 14.03±8.74		t_{cal}=2.56	Significant.
Pyramidal	Apical n= 2-4	Apical: 11.58±5.55	Apical n= 2-6	Apical: 10.79±6.53	DF=8 t_{tab}=2.306	t_{cal}=0.41	Insignificant.
	Basal n= 1-2	Basal: 4.33±3.23	Basal n= 1-5	Basal: 11.29±5.60		t_{cal}=4.83	Significant.
Bipolar	Apical n= 1-2	Apical: 3.13±1.29	Apical n= 3-5	Apical: 8.81±1.79	DF=6 t_{tab}=2.447	t_{cal}=3.28	Significant.
	Basal n= 1-2	Basal: 4.27±1.24	Basal n= 1-4	Basal: 5.91±2.54		t_{cal}=2	Insignificant.
Unipolar	Apical n= 1	Apical: 4.11±2.52	Apical n= 1-6	Apical: 6.26±5.29	DF=5 t_{tab}=2.571	t_{cal}=0.99	Insignificant.
	Basal n= -	Basal: -	Basal n= -	Basal: -		-	-

n= Number of visible spines per 10 µm of dendritic segment; N=True spine density.

during breeding and non-breeding period (Table 6).

Pyramidal neurons

The medium/ large sized somata (20-38 µm during breeding and 17-27µm during non-breeding time) with thick apical dendrite running towards the pia, and two to four finer basal dendrites (Table 6).

Bipolar neurons

These neurons gave rise to dendritic branches in two directions one towards the pia and other towards ventricular wall with bean or oval shaped medium sized soma (Table 6).

Unipolar neurons

These neurons gave rise to their dendrites towards single direction i.e. either towards the pole or towards the ventricle. These neuronal types were observed only during breeding time (Table 6).

Qualitative analysis of Golgi-stained slides provide the under mentioned results

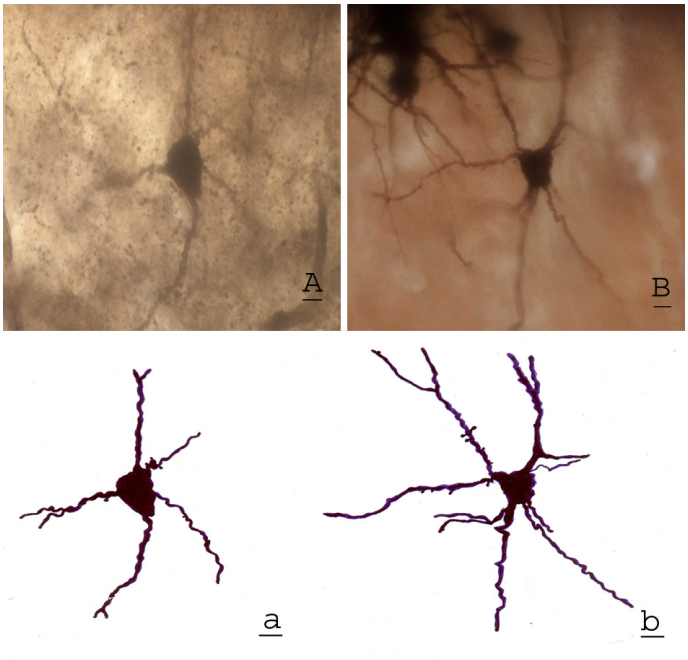

Figure 7. Photomicrographs and camera lucida drawings of multipolar neurons of APH region of female *E. scolopaceus* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

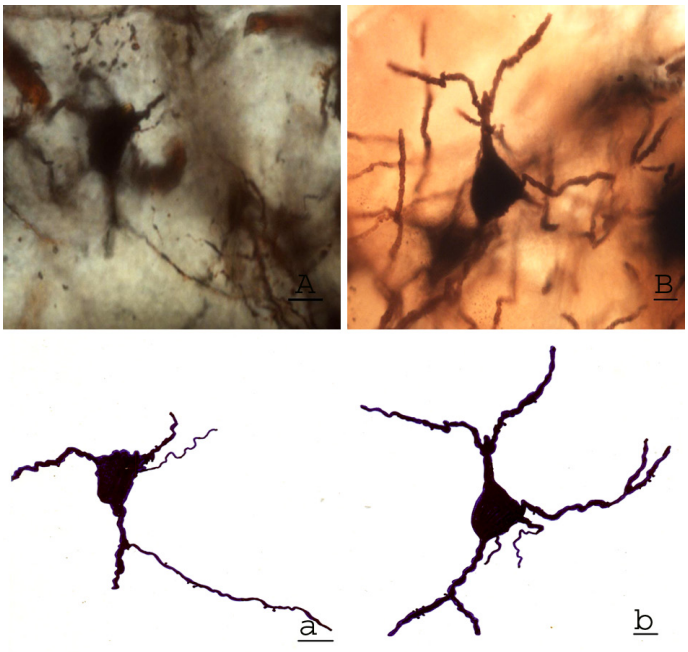

Figure 8. Photomicrographs and camera lucida drawings of pyramidal neurons of APH region of female *E. scolopaceus* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

1) Dendritic thickness: Significant increase ($P<0.05$) in dendritic thickness (both primary and secondary dendritic thickness included) was noticed during breeding time of female *E. scolopaceus* in all three types of neurons except for the basal dendritic thickness of multipolar neurons and apical dendritic thickness of bipolar cells (Table 7).
2) Spine length: In breeding bird significant increase ($P<0.05$) in spine length was recorded for spines present on apical and basal dendrites of multipolar and pyramidal neurons (Table 8).
3) Spine head/Spine head diameter: The spines present on the apical and basal dendrites of multipolar and pyramidal cells were more bulging i.e. with more diameters during breeding time of the bird (Table 9).
4) Spine Density: Significant increase ($P<0.05$) in apical and basal dendritic spine density of multipolar and pyramidal neurons during breeding time was observed (Table 10).

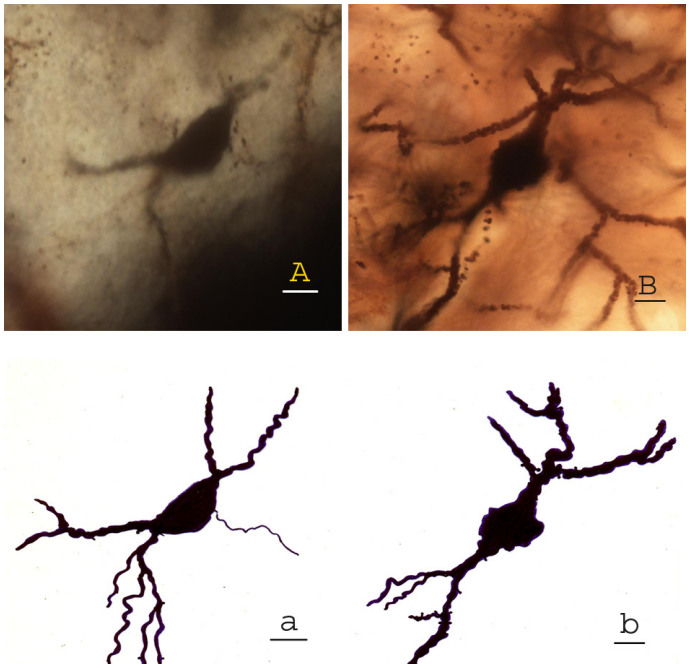

Figure 9. Photomicrographs and camera lucida drawings of bipolar neurons of APH region of female *E. scolopaceus* during (A, a) non-breeding and (B, b) breeding seasons Scale bar=10 µm.

DISCUSSION

The results obtained in this study demonstrate that multipolar neurons form the bulk of neurons followed by pyramidal cells and the former being major type of neuron found in APH of birds. This is in consonance with the findings of Tömböl et al. (36) in chick and homing pigeon and Srivastava et al. (34) in strawberry finch. Seasonal fluctuations in APH neurons of both the birds - *P. krameri* and *E. scolopaceus* were studied and it was found that there exists significant differences in dendritic thickness, spine morphology and spine density across the seasons. It was noticed that the APH neurons of *E. scolopaceus* were more sensitive to seasonal changes in comparison to *P. krameri* as marked significant increase was recorded in both apical and basal spine density of multipolar and pyramidal neurons of *E. scolopaceus*. However, only basal dendritic spine density of multipolar and pyramidal neurons and apical dendritic spine density of unipolar neurons of *P. krameri* showed significant increase. Similarly, there was a marked increase in spine head diameter of spines present on both apical and basal dendrites of multipolar and pyramidal neurons of *E. scolopaceus,* whereas in *P. krameri* only spine head diameter on basal dendrites of pyramidal and bipolar neurons showed significant increase. In the case of dendritic thickness and spine length, *P. krameri* overshadowed *E. scolopaceus* with significant increase during breeding time. Overall it can be summed up that APH neurons of *E. scolopaceus* are more prone to seasonal variations showing more significant results in spine density and spine head diameter during breeding period. The possible reason to this may be the brood parasitic nature of *E. scolopaceus* demanding for

Table 6. Neuronal characteristics of the APH of female koel (*Eudynamys scolopaceus*) during non-breeding (N.B.) and breeding (B.) time period.

Type of Neuron	Soma diameter (in µm)		Dendritic field (in µm)		n (number of neurons)		Percentage %	
	N.B.	B.	N.B.	B.	N.B.	B.	N.B.	B.
Multipolar	23-29	26-35	69-111 x 98-135	109-214 x 138-183	156	140	75.0%	74.07%
Pyramidal	17-27	20-38	80-153 x 36-140	78-216 x 90-183	44	39	21.15%	20.63%
Bipolar	21-22	31-32	50-145 x 59-60	160-109 x 62-110	8	5	3.85%	2.65%
Unipolar	Absent	15-30	-	25-30 x 30-31	0	5	-	2.65%

% is given as n.100/208 for N.B. and n.100/189 for B., 208 and 189 are the number of neurons examined in this study during non-breeding and breeding time of bird. Immature neurons were not used in this study.

Table 7. Thickness of dendrites (in µm) calculated for different types of neurons during non-breeding and breeding seasons in APH region of female *Eudynamys scolopaceus.*

Type of Neuron	Dendritic thickness (Non-breeding)	Dendritic thickness (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 2-4µm	Ad: 3-5µm	DF=6 t_{tab}=2.447	t_{cal}=3.91	Significant.
	Bd: 1-5 µm	Bd: 2-6 µm		t_{cal}=2.16	Significant.
Pyramidal	Ad: 1-4µm	Ad:3-6µm	DF=4 t_{tab}=2.776	t_{cal}=3.23	Significant.
	Bd: 1-4 µm	Bd: 3-6 µm	DF=6 t_{tab}=2.447	t_{cal}=6.68	Significant.
Bipolar	Ad: 1-4µm	Ad: 4-5µm	DF=4 t_{tab}=2.776	t_{cal}=2.58	Insignificant.
	Bd: 2-4 µm	Bd: 4-6 µm		t_{cal}=4.18	Significant.

Ad= Apical dendrite; Bd= Basal dendrite.

Table 8. Length of Spines (in µm) calculated for different types of neurons during non-breeding and breeding seasons in APH region of female *Eudynamys scolopaceus.*

Type of Neuron	Spine length (Non-breeding)	Spine length (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 0.52-0.64µm	Ad: 0.80-0.92µm	DF=8 t_{tab}=2.306	t_{cal}=14	Significant.
	Bd: 0.42-0.62µm	Bd: 0.80-0.97µm		t_{cal}=12	Significant.
Pyramidal	Ad: 0.44-0.54µm	Ad: 0.88-1.41µm	DF=8 t_{tab}=2.306	t_{cal}=10.4	Significant.
	Bd: 0.44-0.58µm	Bd: 0.53-1.32µm		t_{cal}=4.71	Significant.
Bipolar	Ad: 0.61-0.68µm	Ad: 0.70-0.79µm	DF=2 t_{tab}=4.303	t_{cal}=2	Insignificant.
	Bd: 0.46-0.58µm	Bd: 0.48-0.54µm		t_{cal}=0.125	Insignificant.

Ad= Length of spines present on apical dendrite; Bd= Length of spines present on basal dendrite.

Table 9. Spine head diameter (in µm) for different types of neurons during non-breeding and breeding seasons in APH region of female *Eudynamys scolopaceus.*

Type of Neuron	Spine head diameter (Non-breeding)	Spine head diameter (Breeding)	T test at P<0.05		
			Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Ad: 0.35-0.54µm	Ad: 0.53-0.88µm	DF=8 t_{tab}=2.306	t_{cal}=10	Significant.
	Bd: 0.26-0.35µm	Bd: 0.35-0.62µm		t_{cal}=2.875	Significant.
Pyramidal	Ad: 0.26-0.55µm	Ad: 0.44-0.70µm	DF=8 t_{tab}=2.306	t_{cal}=6.33	Significant.
	Bd: 0.26-0.40µm	Bd: 0.24-0.70µm		t_{cal}=3.5	Significant.
Bipolar	Ad: 0.28-0.36µm	Ad: 0.44-0.53µm	DF=2 t_{tab}=4.303	t_{cal}=1.976	Insignificant.
	Bd: 0.28-0.35µm	Bd: 0.26-0.44µm		t_{cal}=0.3	Insignificant.

Ad= Head diameter of spines present on apical dendrite; Bd= Head diameter of spines present on basal dendrite.

Table 10. Spine density (±SD) calculated for different types of neurons (during non-breeding and breeding time period) in APH region of female *Eudynamys scolopaceus.*

Type of Neuron	n (Non-breeding)	Spine density (Non-breeding) N	n (Breeding)	Spine density (Breeding) N	T test at P<0.05		
					Degree of freedom and t_{table} values	$t_{calculated}$	Significant/ Insignificant
Multipolar	Apical n= 4-5	Apical: 14.41±1.46	Apical n= 5-7	Apical: 21.58±1.90	DF=8 t_{tab}=2.306	t_{cal}=13.40	Significant.
	Basal n= 3-5	Basal: 14.39±1.86	Basal n= 5-8	Basal: 22.14±2.67		t_{cal}=11.85	Significant.
Pyramidal	Apical n= 3-4	Apical: 13.74±0.93	Apical n= 4-6	Apical: 16.27±3.22	DF=8 t_{tab}=2.306	t_{cal}=	Significant.
	Basal n= 3-5	Basal: 14.31±2.48	Basal n= 4-5	Basal: 19.30±8.19		t_{cal}=	Significant.
Bipolar	Apical n= 2-4	Apical: 11.43±6.89	Apical n= 2-4	Apical: 12.08±6.58	DF=2 t_{tab}=4.303	t_{cal}=2.6	Insignificant.
	Basal n= 2-3	Basal: 9.21±5.93	Basal n= 2-3	Basal: 11.80±9.90		t_{cal}=0.09	Insignificant.

n= Number of visible spines per 10 µm of dendritic segment; N=True spine density.

more active state of the bird for searching host's nest during breeding time.

The fluctuations in the spine density of dendritic spine of the APH neurons of both the birds could conceivably be due to variations in the levels of either ovarian steroids or pituitary hormones, as supported by the results of Gould et al. (13) according to which the spine density in the same neuronal population depends on estradiol and progesterone levels. Mac Lusky et al. (20) also proposed that estrogen stimulates hippocampal spine density. The seasonal changes in spine density of APH neurons appear to be similar to the seasonal changes in songbird because they are driven by seasonal changes in gonadal steroids (38).

The dendrites of multipolar, pyramidal and bipolar neurons in *P.krameri* and multipolar and pyramidal neurons in *E. scolopaceus* were found to be sensitive to variations in the season resulting in significant increase in dendritic spine density across the breeding time. These changes in dendritic spine density during breeding time are likely to have consequences for parahippocampal neuronal function. The spines are specialized postsynaptic structures that in part determine the electrical properties of their parent dendrite (6,18). Therefore the plasticity in the density of APH dendritic spines could have important consequences for the process of learning, memory and/ or other behavioral activities of hippocampus. During breeding time, the bird undergoes hormonal changes and becomes more active. This change in state and hormonal level is compensated by the increased spine density which indicates increased mental agility.

The significant increase in dendritic thickness in both the birds during breeding time may be probably to support more spines over the surface of dendrite. In contrast, a study by Adams et al. (1) reported that there was increase in mean dendritic length to almost three times during anestrus time in A15 DA neurons of Ewe and there was a corresponding increase in surface of the dendritic tree in anestrus. They opined that the increase in dendritic length in anestrus occurs in response to signals from afferent inputs from two different areas that contained cells projecting to A15 DA neurons.

In the presently studied birds i.e. *P. krameri* and *E. scolopaceus* there is significant increase in spine head size during breeding time indicating increase in synaptic surface area during this very time period thus enhancing larger synaptic transmission which is in line with the work of Fifková and Anderson (10) who reported about increase in spine head in distal spines after titanic stimulation of dentate gyrus. A direct relationship has been found between spine head size and postsynaptic density, number of postsynaptic receptors and releasable pool of neurotransmitter (23,29,44,45). Later Fifková (11) proposed that enlargement of spine head was in fact a result of increase in size of synapse and its stabilization might be mediated by local increase of Ca^{2+} mediated by mobilization of actin. On the contrary Trommald et al. (39) reported that these parameters remains unaffected. The repetitive stimulation produces an increment in size so that smaller spines are converted into spines with larger heads (22). Ballesteros et al. (2) reported significant differences in the head size and neck length of spines, being larger in M2 when compared to other cortical regions (S2 and V2L/TeA) in mice. These morphological differences may influence various functions. The larger spine heads have been correlated with more postsynaptic receptors (24). Hence during breeding time when the spine head diameter increases there is increase in post synaptic receptors allowing more and faster transmission of stimulus.

The neck length is one of the factors that control the rate of calcium decay as it diffuses from the synapse to the dendrite. The differences in the length of spine neck therefore alters calcium dynamics in spines which is supposed to be related to learning (31,44,45). It has been

proposed that small spines are preferential sites for long-term potentiation (learning spines), whereas larger spines (spines with larger spine length) might represent long term memory (memory spines) (21,22). Spines observed during breeding time in both the presently studied birds possessed significantly larger spine neck length indicating their relation with long term memory and might help the *P.krameri* to memorize any information and *E. scolopaceus* to remember the host nest. The spines present on apical and basal dendrites of multipolar, pyramidal and bipolar neurons of *P.krameri* and apical and basal spines of multipolar and pyramidal neurons of *E.scolopaceus* can be considered to be memory spines.

Fluctuation in length of spine neck appears to have important consequences with respect to ability of spine to transfer Ca^{2+} into parent dendrite (41). The dynamic changes in length and thickness in developing spines are supposed to have important implications for functional linkage of spine head to parent dendrite (30) thereby helping the birds studied in present case to functionally connect the spine head and parent dendrite to compartmentalize more Ca^{2+} during breeding period.

Spines are small structures which behave as individual postsynaptic compartments and can serve as integrative units in neuronal circuitry. They exhibit wide ranges of shapes and sizes and the differences in morphology between spines likely reflect their functional differences (15). During breeding time the animal becomes functionally active, there is change in hormonal level which may lead to changes in neuronal characteristics. As it is well known that hippocampus plays role in controlling emotions (14) therefore any change in emotional condition can cause plasticity in hippocampus. The physiological processes may be directly linked to spine changes, hence to acquire active state during breeding time there is increase in spine density, dendritic thickness, spine head diameter and spine length in neurons of hippocampus and particularly APH region of birds in present study.

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REFERENCES

1. Adams, V.L., Goodman, R.L., Salm, A.K., Coolen, L.M., Karsch, F.J., and Lehman, M.N., Morphological plasticity in the neuronal circuitry responsible for seasonal breeding in the Ewe. *Endocrinol.* 2006, **147**(10): 4843-4851.

2. Ballesteros-Yáñez, I., Benavides-Piccione, R., Elston, G.N., Yuste, R. and DeFelipe, J., Density and morphology of spines in mouse neocortex. *Neuroscience*. 2006, **138:** 403-409.

3. Barnea, A. and Nottebohm, F., Seasonal recruitment of hippocampal neurons in adult free-ranging black-capped chickadees. *Proc Natl Acad Sci USA*. 1994, **85:** 11217– 11221.

4. Bingman, V.P. and Able, K.P., Maps in birds: representational mechanisms and neural bases. *Curr. Opin. Neurobiol*. 2002, **12:** 745-750.

5. Blaesing, B., Nossoll, M., Teuchert Noodt., G. and Dawirs, R.R., Postnatal maturation of prefrontal pyramidal neurons is sensitive to single early dose of methamphetamine in gerbil (*Meriones unguiculatus*). *J. Neural. Trans.* 2001, **103:** 101-103.

6. Coss, R.G. and Perkel, D.H., The function of dendritic spines: a review of theoretical issues. *Behav Neural Biol.* 1985, **44:** 151-185.

7. DeVoogd, T.J., Nixdorf, B. and Nottebohm, F., Synaptogenesis and changes in synaptic morphology related to acquisition of a new behavior. *Brain Res.* 1985, **329:** 304-308.

8. El Falougy, H. and Benuska, J., History, anatomical nomenclature, comparative anatomy and functions of the hippocampal formation. *Bratisl. Lek. Listy.* 2006, **107:** 93- 108.

9. Feldman, M.L. and Peters, A., A technique for estimating total spine numbers on Golgi-impregnated dendrites. *J comp Neurol.* 1979, **188:** 527-542.

10. Fifková, E. and Anderson, C.L., Stimulation-induced changes in dimensions of stalks of dendritic spines in the dentate molecular layer. *Exp. Neurol.* 1981, **74:** 621-627.

11. Fifková, E., A possible mechanism of morphometric change in dendritic spines induced by stimulation, Cell. Mol. Neurobiol. 1985, **5:** 47-63.

12. Gentner, T.Q., Hulse, S.H. and Ball, G.F., Functional differences in forebrain auditory regions during learned vocal recognition in songbirds. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol*. 2004, **190:** 1001-1010.

13. Gould, E., Woolley, C.S., Frankfurt, M. and McEwen, B.S., Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci*. 1990, **10:** 1286–1291.

14. Hara, K. and Myers, R.E., Role of forebrain structures in emotional expression in opossum. *Brain Res.* 1973, **52:**131-144.

15. Harris, K.M. and Stevens, J.K., Dendritic spines of CA1 pyramidal cells in the rat hippocampus: serial electron microscopy with reference to their biophysical characteristics. *J. Neurosci.* 1989, **9:** 2982-2997.

16. Hill, K.M. and DeVoogd, T.J., Altered daylength affects dendritic structures in a song-related brain region in red-winged blackbirds. *Behav. Neural Biol.* 1991, **56:** 240-250.

17. Humason, G.L., Animal Tissue Techniques. San Francisco: WH Freeman and Co., 1972, pp. 641.

18. Kawato, M., Hamaguchi, T., Murakami, F. and Tsukahara, N., Quantative analysis of the electrical properties of dendritic spines. *Biol Cybern*. 1984, **50:** 447-454.

19. Lucas, J.R., Brodin, A., de Kort, S.R.. and Clayton, N.S., Does hippocampal size correlate with the degree of caching specialization? *Proc.R. Soc. B.* 2004, **271:** 2423–2429.

20. MacLusky, N.J., Luine, V.N., Hajszan, T. and Leranth, C., The 17α and 17β isomers of estradiol both induce rapid spine synapse formation in the CA1 hippocampal subfield of ovariecttomized female rats. *Endocrinol*. 2005, **146:** 287-293.

21. Matsuzaki, M., Ellis-Davies, G.C., Nemoto, T., Miyashita, Y., Iino, M., and Kasai, H., Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci.* 2001, **4:** 1086-1092.

22. Matsuzaki, M., Honkura, N., Ellis-Davies, G.C. and Kasai, H., Structural basis of long-term potential in single dendritic spines. *Nature*. 2004, **429:** 761-766.

23. Noguchi, J., Matsuzaki, M., Ellis-Davies, G.C. and Kasai, H., Spine-neck geometry determines NMDA receptor-dependent Ca^{2+} signaling in dendrites. *Neuron*. 2005, **46:** 609-622.

24. Nusser, Z., Lujan, R., Laube, G., Roberts, J.D., Molnar, E. and Somogyi, P., Cell type and pathway dependence of AMPA receptor number and variability in hippocampus. *Neuron.* 1998, **21:** 545-559.

25. Ormerod, B.K., Lee, T.T. and Galea, L.A., Estradiol initially enhances but subsequently suppresses (via adrenal steroids) granule cell proliferation in the dentate gyrus of adult female rats. *J Neurobiol*. 2003, **55:** 247-260.

26. Patel, S.N., Clayton, N.S. and Krebs, J.R., Spatial learning induces neurogenesis in the avian brain. *Behav Brain Res*. 1997, **89:** 115–128.

27. Reboreda, J.C., Clayton N.S. and Kacelnik, A., Species and sex differences in hippocampus size between parasitic and non-parasitic cowbirds. *Neuroreport*. 1996, **7:** 505- 508.

28. Rehkämper, G., Frahm, H.D. and Cnotka, J., Mosaic evolution and adaptive brain component alteration under domestication seen on the background of evolutionary theory. *Brain Behav Evol*. 2008, **71:** 115-126.

29. Sabatini, B.L, Maravall, M. and Svoboda, K., $Ca^{(2+)}$ signaling in dendritic spines. *Curr Opin Neurobiol*. 2001, **11:** 349-356.

30. Segal, M. and Anderson, P., Dendritic spine shaped by synaptic activity. *Curr Opin Neurobiol*. 2000, **10:** 582-586.
31. Segal, M., Changing views of Cajal's neuron: the case of the dendritic spine. *Prog Brain Res*. 2002, **136:** 101-107.
32. Sherry, D.F., Forbes, M.R.L., Khurgel, M. and Ivy, G.O., Females have a larger hippocampus than males in the broodparasitic brown-headed cowbird. *Proc Natl Acad Sci*. 1993, **90:** 7839-7843.
33. Smulders, T.V., Sasson, A.D. and Devoogd, T.J., Seasonal variation in hippocampal volume in a food-storing bird, the black-capped chickadee. *J Neurobiol*. 1995, **27:**15–25.
34. Srivastava, U.C., Chand, P. and Maurya, R.C., Cytoarchitectonic organization and morphology of the cells of hippocampal complex in strawberry finch, *Estrilda amandava*. *Cell Mol Biol*. 2007, **53:** 103–120.
35. Tanapat, P., Hastings, N.B., Reeves, A.J. and Gould, E., Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci*. 1999, **19:** 5792-5801.
36. Tömböl, T., Davies, D.C., Németh, A., Sebestény, T. and Alpár, A., A comparative Golgi study of chicken (*Gallus domesticus*) and homing pigeon (*Columba livia*) hippocampus. *Anat Embryol*. 2000a, **201:** 85–101.
37. Tracy, A.L., Jarrard, L.E. and Davidson, T.L., The hippocampus and motivation revisited: appetite and activity. *Behav Brain Res*. 2001, **127(1-2):** 13-23.
38. Tramontin, A.D. and. Brenowitz, E.A, Seasonal plasticity in the adult brain. *Trends in Neurosciences.* 2000, **23:** 251–258.
39. Trommald, M., Hulleberg, G. and Anderson, P., Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. *Learn. Mem*. 1996, **3:** 218-28.
40. Vargas, J.P., Petruso, E.J. and Bingman, V.P., Hippocampal formation is required for geometric navigation in pigeons. *Eur J Neurosci*. 2004, **20:** 1937-1944.
41. Volfovsky, N., Parnas, H., Segal, M. and Korkotian, E., Geometry of dendritic spines affects calcium dynamics in hippocampal neurons: theory and experiments. *J Neurophysiol*. 1999, **82:** 450-462.
42. Woolley, C.S., Gould, E., Frankfurt, M. and McEwen, B.S., Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons. *J. Neurosci*. 1990, **10:** 4035-39.
43. Woolley, C.S., Estrogen-mediated structural and functional synaptic plasticity in the female rat hippocampus. *Horm. Behav*. 1998, **34:** 140-148.
44. Yuste, R. and Bonhoeffer, T., Morphological changes in dendritic spines associated with long-term synaptic plasticity. *Annu Rev Neurosci*. 2001, **24:** 1071-1089.
45. Yuste, R. and Bonhoeffer, T., Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nat Rev Neurosci*. 2004, **5:** 24-34.