Disector counts reveal changes of synaptic bodies in cultured chicken pineal glands

Holger Jastrow, Michael Hainz and Lutz Vollrath, Anatomisches Institut, Histologie, J. Gutenberg-Universität, Becherweg 13, D-55128 Mainz, Email: jastrow@goofy.zdv.uni-mainz.de, http://www.uni-mainz.de/~jastrow/

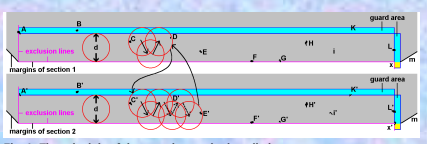
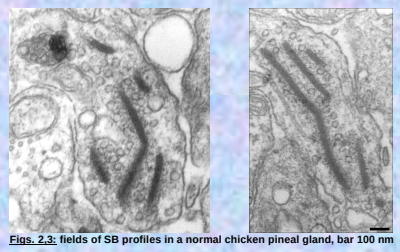


Fig. 1: ***The principle of the counting method applied:***
a. **Undamaged straight margins of 2 adjacent sections are measured at x 400.**
b. **The counting frames of sections 1 and 2 are defined by the measured lengths of their lower margins minus the guard area, which is wider than the largest dimension of a particle (here particle H's diameter), and the screen diameter at x 20.000 (d). The circles corresponding to an electron microscope screen demonstrate the procedure: supposed, particle C's profile was the last one checked on both sections, section 1 is scrutinised moving it up and down along the margin from the left to the right until the profile of particle D is seen. Then section 2 is investigated correspondingly starting from the profile of C'. As here no profile of D' is present, scanning proceeds till profile E' is reached. Investigation now restarts at profile D on section 1 proceeding towards profile E (not shown).**
Illustration of the structures counted by SSPC* and DC: Profile A on section 1 extends from the counting frame through the guard area and hits the exclusion line on the left, thus it is not counted. Profile A' extends from the counting frame into the guard area but does not hit the exclusion line thus it is counted in the SSPC of section 2. Profiles B and B' are both not counted as they are not inside the counting frame but lie exclusively in the guard area. Profile C is counted in SSPCon both sections. Profile D is counted in DC and SSPCin section 1 as profile D' is missing. Profile E and E' are subject to SSPCin their sections. Profiles F, F' and G are not counted as they hit the exclusion line at the margin of the section. Profile G' is a candidate for SSPCon section 2. Profile H (the profile with the maximal diameter) and H' are in the SSPCof their sections. A profile i is missing, thus i' is counted in the disector and the SSPCfor section 2. Profile K is missing consequently profile K' is due to DC and SSPCof section 2. Profiles L and L' are both in the SSPCof their sections. Profile X is absent; a profile X' extending from the counting frame through the guard area reaching the section's margin may be counted erroneously by DC and SSPC, as it extends into a missing part of the guard area. However this error is of theoretical interest only as its probability would be < maximal particle diameter² / counting area x percentage of particles that would have the necessary c-,γ- or u- shape and the orientation necessary to extend from the counting frame through the guard area and down and back to touch the exclusion line (bend of >180°). In this study this possibility was insignificant. In reality the scanned strip would be much wider and SBs much smaller than shown in this figure. Counts are summarised in Table 1:

*SSPC = single section profile count, DC = disector count

		Profiles :												
Count	Section	AA'	BB'	CC'	DD'	EE'	FF'	GG'	HH'	ii'	KK'	LL'	XX'	S
SSPC	1	-	-	+	+	+	-	-	+	-	-	+	-	5
	2	+	-	+	-	+	-	+	+	+	+	+	*	9
DC	1	-	-	-	+	-	-	-	-	-	-	-	-	1
	2	-	-	-	-	-	-	-	-	+	+	-	*	3



Figs. 2,3: **fields of SB profiles in a normal chicken pineal gland, bar 100 nm**

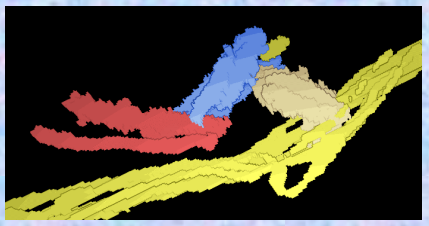


Fig. 4: **Three dimensional reconstruction of a field of synaptic bodies of a normal chicken pineal gland seen from above. SBs lie close to the cell membrane (yellow). The red SB has a considerable torsion in itself, therefore it seemingly consist of two parts (synaptic vesicles not reconstructed).**

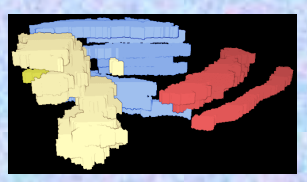


Fig. 5: **Electron-dense bodies of 5 plate-like SBs seen from of the cell membrane.**

Fig. 6: **Front and topside of the same SBs with the cell membrane shown in yellow.**

Abstract:

Synaptic bodies (SBs) are small, rare organelles involved in signal transduction in the pineal gland. In vivo they undergo changes in number and size. The disector, a counting method independent of size and shape of investigated particles, was used to clarify numerical changes in vivo and in vitro. Counts of SBs were performed on electron microscopic sections of chicken pineal glands removed at 12:00 or 24:00 and cultured in an incubator for 12 hours. In vivo significant increases of SB number, SB profile number and SB profile length were seen from noon to midnight. Pineal glands removed at 12:00 and incubated for 12 hrs showed significant increases of SB number, SB profile number and length. The increase in length was less pronounced than in vivo at 24:00, whereas the SB and SB profile increase was more evident. In glands removed at 24:00 and incubated for 12 hrs significant decreases of SB profile number and length and an insignificant decrease of SB number were encountered. In vitro significantly more SBs and SB profiles were present, the latter were insignificantly shorter (p = 0.3) than in vivo. Our data suggest that SB number and length in the chicken pineal gland are subject to endogenous control, retaining their day/night rhythm in organ culture. As the changes in number were less pronounced in vitro than in vivo, it is assumed that in vivo the day/night rhythm is accentuated either by the innervation of the gland or by light or by both.

Introduction

Synaptic bodies (SBs; **Figs.2,3**) are electron-dense structures surrounded by synaptic vesicles involved in signal transduction processes. They are present in pinealocytes, hair-cells, retinal photoreceptor and -bipolar cells. The mostly plate-like SBs (**Figs.4-6**) undergo changes in number and size (Jastrow et al. 1997a). Using the disector (**Fig.1**), which, in contrast to counts in a standard area, is independent of shape and size of particles, it was checked whether under in vitro conditions the day-night rhythm of SB number and size persisted in chicken pineal glands.

Material and Method

Three-week-old male chicken (n = 22) were kept under standard laboratory conditions (12/12 h LD cycle). In deep anaesthesia 12 chicken were killed at 24:00. Pineal glands were quickly removed under dim-red light, six of them were transferred into organ culture dishes and kept in a dark incubator (37°C, 95% O_2, 5% CO_2) for 12 hrs before fixation. 10 chicken were sacrificed at 12:00. Five of their pineal glands were cultured as described, the others fixed immediately. Series of up to 21 sections (thickness: 50 nm) were examined on Formvar® coated one-hole grids. On a Zeiss TEM 10, disector counts (DC) and single section profile counts (SSPC) were performed simultaneously on two adjacent sections taken from the centre of a series [examined areas: ~25,000 µm²; magnification: x 20,000; **Fig.1**]. A modification of the disector method (Jastrow et al. 1997b) was applied to cope with problems due to size and rareness of SBs. Differences in day/night normal and culture groups were tested using Student`s t-test. Further, the lengths of SB profiles were measured by means of a morphomat at x 20,000.

Results (Data shown in **Table 2** and **Diagrams 1 - 4**)

In vivo significant increases of SB number, SB profile number and SB profile length were seen from noon to midnight. Pineal glands removed at 12:00 and incubated for 12 hrs showed significant increases of SB number, SB profile number and length. The increase in length was less pronounced than in vivo at 24:00, whereas the SB and SB profile increase was more evident. In glands removed at 24:00 and incubated for 12 hrs significant decreases of SB profile number and length and an insignificant decrease of SB number were encountered. In vitro significantly more SBs and SB profiles were present, the latter were insignificantly shorter (p = 0.3) than in vivo.

Discussion

The results obtained in the present study show that the established day/night rhythm of SB profile number (Vollrath and Spiwoks-Becker 1996) is confirmed by disector and single section profile counts in chicken in vivo and in vitro. Our data further suggest that quantity and length of SBs in the chicken pineal gland are subject to endogenous control since changes in number occur also in vivo albeit to a lesser extend than in vivo. The fact that in organ culture SBs retain their day/night rhythm of number and size is in agreement with the intrinsic rhythmicity of individual pinealocytes. It is assumed that in vivo this day/night rhythm is accentuated either by the innervation of the gland or by light or by both. Compared to the in vivo values there was a greater difference of mean profile lengths of both groups of pineal glands kept in culture pointing to more pronounced changes in mean size of SBs. However the standard deviation from the mean profile length is much smaller in vitro than in vivo pointing to a more uniform size of SBs. Due to the larger size of individual SBs at night, the latter may bind more synaptic vesicles (SVs) at night than during the day. Due to this there are less SVs free to participate in signal transduction at night provided that total SV number is not altered. Thus it is not astonishing that electric recordings of chicken pineal glands on average showed a lower activity at night (Schenda et al. 1998). In contrast to this, melatonin synthesis is higher during night than day pointing to the fact that hormone secretion is not - or inversely correlated to electric or synaptic activity of the cells. As pinealocytes in cell dispersion culture under light produce less melatonin, it remains to be elucidated whether organ culture in an incubator with lighting will result in smaller and less SBs in the cells.

Acknowledgements
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References:

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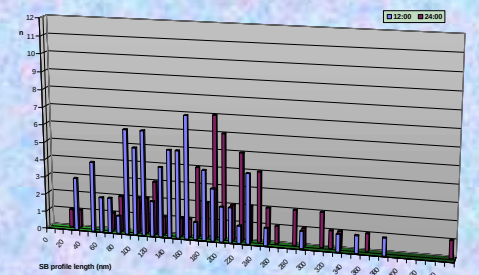
Jastrow H, von Mach MA and Vollrath L (1997b): Adaptation of the disector method to rare small organelles in TEM sections exemplified by counting synaptic ribbons of the rat pineal gland. Journal of Anatomy 191: 399-405.
Schenda J, Holtmann B and Vollrath L (unpublished results)
Vollrath L and Spiwoks-Becker I (1996): Plasticity of retinal ribbon synapses. Microscopy Research and Technique 35: 472-487.

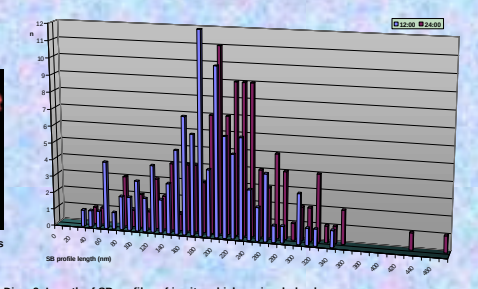
Table 2: **Results**

	n/20.000µm²	n/1000µm³	length* nm
in vivo			
12:00 #1	24,56	7,67	n.m.
12:00 #2	25,30	9,43	n.m.
12:00 #3	17,06	5,09	198,30
12:00 #4	23,96	7,36	178,58
12:00 #5	18,33	5,66	169,27
mean	**21,84**	**7,04**	**182,05**
increase (%)	**70,97**	**39,07**	**12,41**
24:00 #1	56,07	18,09	n.m.
24:00 #2	51,38	16,00	n.m.
24:00 #3	50,08	14,39	210,57
24:00 #4	54,00	17,03	204,83
24:00 #5	46,68	12,71	206,31
24:00 #6	53,71	14,32	199,14
mean	***51,99***	***15,42***	***205,21***
in vitro			
12:00 #1	30,30	9,45	n.m.
12:00 #2	38,35	19,19	161,85
12:00 #3	31,64	10,93	n.m.
12:00 #4	34,93	11,73	184,73
12:00 #5	40,00	12,80	185,55
12:00 #6	36,70	13,34	182,00
mean	**35,32**	**12,91**	**178,53**
24:00 #1	51,42	18,60	239,40
24:00 #2	32,72	12,73	171,09
24:00 #3	43,56	14,12	226,40
24:00 #4	31,00	9,85	213,29
24:00 #5	36,45	12,31	208,13
mean	**39,03**	**13,52**	**211,66**

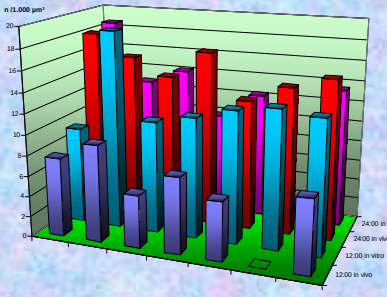
* length = mean SB profile length; n.m. = not measured
12:00 = killed at 24:00 ->12h in vitro -> fixed at 12:00



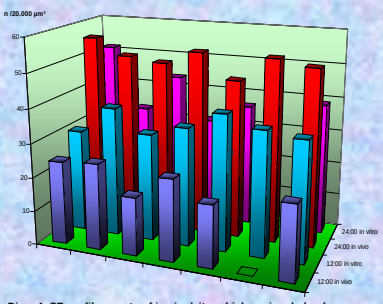
Diag. 1: **Length of SB profiles of in vivo chicken pineal glands**



Diag. 2: **Length of SB profiles of in vitro chicken pineal glands**



Diag. 3: **SB disector counts of in vivo/vitro chicken pineal glands**



Diag. 4: **SB profile counts of in vivo/vitro chicken pineal glands**