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Quantitative analysis of the trigeminal ganglion neurons in the human, monkey and baboon

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Summary

The present study provides comparative quantitative data on trigeminal ganglion neurons in order to enable appropriate use of experimental animals. Quantitative analysis of 11 trigeminal ganglia of humans, 4 of vervet monkey *(Cercopithecus aethiops)* and 4 ganglia of 2 baboons *(Papio anubis)* was carried out using the model based method of stereology and total cell counts in serial sections. Mean total numbers of neurons in trigeminal ganglion were found to be in humans 123,010 (stereology); in the vervet monkey 98,073 (counts) or 101,178 (stereology) and in the olive baboon 137,250 (counts) or 153,555 (stereology). The mean total number of neurons reflects intra- and interspecies differences, as well as differences in the method of estimation. The different values of the volume density (V_v) (for humans 0.67, for the monkey 0.35 and for the baboon 0.4) may be related to structural differences in the trigeminal ganglia. In humans, the mean diameter of neurons in the trigeminal ganglion was 39.6 μm; in the monkey it was 48 μm, and in the baboon 54 μm, respectively. These specific differences found between the neurons of the human, monkey and baboon trigeminal ganglion may be related to differences in body (head) size and can help in the evaluation of data obtained from experimental animals to human studies.

Key Words: Trigeminal ganglion - Neurons - Quantification - Primates.

Introduction

The trigeminal (semilunar) ganglion (TG) consists mainly of large and medium size pseudounipolar and bipolar neurons surrounded by satellite cells. These neurons innervate skin, the oral and nasal mucosa, cornea, teeth pulps and periodontal ligaments and have some peculiar features (Lieberman, 1976; Dubner et al., 1978; Kruger and Young, 1981). There is extensive collateral innervation of adjacent oral and dental regions from the single ganglion neuron (Atkinson and Kenyon, 1990). The somatotopic organization of the TG has been reported in the monkey and several other species (Kruger and Young, 1981; Marfurt and Echtenkamp, 1988; Shigenga et al., 1989).

Human extraocular muscles are richly supplied by proprioceptive receptors (like muscle spindles). These proprioceptors, having innervation through the trigeminal nerve (ophthalmic division), play a role in binocular vision (Lukas et al., 1997). The primary afferent perikarya of the same function in rats, sheep, pigs, cat and monkeys have been reported (see Aigner et al., 1997).

The TG is suitable for quantification by the method of stereology using serial sections (Vujaskovic and Malobabic, 1991). Quantification data are relevant for changes related to aging, denervation and vascular headaches (Truex, 1940; Mayenberg et al., 1981). Studies on the size and number of TG neurons have been carried out in the rat (Aldskogius and Arvidsson, 1978; Gregg and Dixon, 1973; Sugimoto et al., 1986) and the cat (Marfurt, 1981; Shigenga et al., 1989; Holland and Robinson, 1990). There are no conclusive data on the quantification of TG neurons in the cat, rat or humans (Blinkov and Glezer, 1976). Inter- and intraspecies variability in the estimated

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number of TG neurons may be due to the difference in methodology (section thickness, reference area, correction factors, counting split cells, over or under counting), the age of the animal and whether unilateral or bilateral counting was done (Abercrombie, 1946; Konigsmark, 1970).

Use of experimental animals often requires comparisons with related data from humans. The aim of present study was to carry out a comparative quantitative analysis of the TG neurons in humans, the monkey and the baboon using stereological methods (Weibel, 1979, 1980; West and Gundersen, 1990) as well as total cell counts in serial sections. Our findings may be valuable for researchers of the TG and trigeminal system, addressing specific morphological and functional interspecies differences.

Materials and Methods

The trigeminal ganglia (TG) of 11 humans (6 males, 5 females; age range 20-70 years) were analyzed using stereological methods by the second and third author. The subjects investigated were without known disease of the nervous system and postmortem delay was between 10 and 24 hours. Four trigeminal ganglia from 3 monkeys *(Cercopithecus aethiops)* (age 2-4 years; weight 2-4 kg) and 4 ganglia from 2 baboons *(Papio anubis)*, one adult (8 years, weight 16 kg) and one young (2 years, weight 6 kg) were analyzed by the first author. A total of 8 TG from these two animal species was investigated using stereological as well as total cell count methods in serial sections.

The human TG were removed with the dural sheath and fixed for four weeks by immersion in 10% formalin. After dehydration, serial paraffin wax sections 10 µm thick were cut and stained with Kluver-Barrera method (Luxol fast blue-cresyl violet).

The monkeys and baboons were deeply anaesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and perfused transcardially with phosphate-buffered saline followed by 10% formalin for 30 minutes. The trigeminal ganglia were dissected, dehydrated and embedded in wax. Serial sections 7-10 µm thick (see Table 2) were cut and stained with haematoxylin and eosin. Prior to dehydration, the volumes of the ganglia were measured using the water displacement method of Scherle (1970). The linear shrinkage constant of the ganglia was determined by measuring the block of fixed and processed ganglia to be about 10-15%.

Humans

Stereological analysis. For the human TG, every 10th section was analyzed. The surface of each section (Ps) was measured by the MOP-3 (Zeiss) image analyzer system. For stereology, Weibel's M42 multipurpose test system was used. The surface of the test system (Pp) on the related magnification (x100) was determined and was 0.92 mm^2. The ratio (Ps/Pp) between the surface of the each TG section and of the test system was calculated. Two independent observers (GV and SM) performed counting and the interobserver difference was 3%. By a random selection procedure on each section investigated, 6 fields of the test system were used and only neurons in the field (test system area) containing nucleoli or fragments of nucleoli were counted. The neurons on the right and lower border of the test system were not counted. Thereafter, the mean value of the neuron number for each section was calculated and was then multiplied by the Ps/Pp ratio. The resulting number was multiplied by 10 (since every 10th section was used) and so the final number of neurons was obtained. The Floderus correction (0.91) was applied, according to the formula $t_s = t + 2R - 2h$ (t_s = thickness of supersection, t = thickness of section, R = maximal observed radius and h = the thickness of smallest detected cap). Using the formula $N = N' \cdot (t/t_s)$, (where N' is the number of neurons obtained by counting, t_s is thickness of supersection, and t is thickness of investigated section), we obtained corrected number of neurons (N) (Kalisnik, 1985). In spite of suggestions that the Floderus correction may be ignored (Aherne and Dunnill, 1987), we used it (0.91) because it could obviously only underestimate the final number of neurons very slightly.

The volume density (V_v) was calculated using the formula $Vv = P_f/P_t$, where P_f is number of neurons lying in the test system and Pt is the number of all test points in the test system (Pt).

Monkey and baboon

1. Stereological analysis. About 10-20 equidistantly spaced sections (every 5th or 10th) of the monkey and baboon TG ganglia were analyzed using an integrating grid with 100 points at a magnification of x100. The region of the ganglion with a concentration of the neurons -"ganglionic region"- was used as a reference area (Fig. 1).

The sections were analyzed field by field to obtain mean values of the volume density (V_v), the number of neurons per unit area (N_A) for each section and subsequently for the total reference area of the ganglion. The total volume density was analyzed by counting the number of points falling on the neurons while the number per unit area was determined from neuron profiles within the test area. The area of the grid with 100 points was calibrated using a slide-etched scale. The measured volume of the whole ganglion was corrected for the "ganglionic region" for the estimation of total neuron counts.

The mean values of volume density (V_V), the number per unit area (N_A), the mean diameter of neurons and the section thickness were used to calculate the numerical density (N_V), using the formulae computed by Weibel and Gomez (1962):

$N_V(a) = NA^{3/2} / N_V^{1/2} \times \beta$ where $\beta = 1$ for spheroid; and by Weibel (1980).

$N_{V(b)} = N_A/D+T$ where D= mean caliper diameter of neurons and t= section thickness.

The total estimated number of neurons (N_{Test}) was calculated by the formula $N_{Test}=N_{va}$ x the volume of the "ganglionic region" (reference area).

2. Total cell counts of the TG neurons were obtained by counting the number of neurons with a clear nucleus in five equidistantly spaced sections of the ganglion. The mean value obtained for the sections counted was multiplied by the number of sections to obtain the total cell counts (Tcount).

Size of Trigeminal Ganglion Neurons

In the human. The sizes of the neurons were measured in 6 TG. An ocular grid was used for measurements of the longest axis of the neurons, which were generally ovoid or spherical in shape. The same sections as those used for stereology were employed and the random selection procedure was implemented; 150 neurons (with nucleolus) in each ganglion were measured (magnification x 100). Neurons were divided into 5 size classes for detailed analysis and mean values were calculated.

In the monkey and baboon. For each species the longest diameter of the 100 TG neurons randomly selected, with a clearly visible nucleus, was measured using a calibrated ocular grid (magnification x 400) and mean values were obtained. Student's t-test was used to calculate the statistical significance between the total number and the size of neurons in the monkey and baboon.

Results

Trigeminal Ganglion Neuron Counts

Humans. Table 1 shows the sex and age distribution of the number of trigeminal neurons found in humans.

A variation can be seen in the number of total human TG neurons in the different ganglia, with a mean number of 128,009.09 (minimal- 78,000; maximal-153,000).

Table 1.– The sex, age and numbers of trigeminal neurons in the humans

Sex	age (yrs)	Number of neurons
Male	58	120,300
Male	57	147,000
Male	70	137,000
Male	54	153,000
Male	61	78,000
Male	60	78,000
Female	54	122,000
Female	20	141,000
Female	22	145,800
Female	20	152,000
Female	24	134,000

MEAN 128,009 SD 27,000
RANGE 78,000 - 153,000

The mean volume density (Vv) of the TG was 0.6724 (SD: 0.0205).

Monkey and Baboon

1. Stereology. Table 2 shows the mean values and SD of the volume proportion (V_V), number per unit area (N_A) and the numerical density(Nv) of the TG neurons in (a) monkey and (b) baboon.

Table 2.– The mean values and SD of volume density (V_V), number per unit area (N_A) and numerical density (N_V), estimated total number of neurons (Ntest) and total cell counts (Tcount) of the trigeminal ganglion neurons in (a) Vertet monkey and (b) Olive baboon

$$N_{Va} = \frac{N_A^{3/2}}{V_V^{1/2}} \qquad N_{Vb} = \frac{N_A}{D+t}$$

(D= mean diameter of neurons, monkey 48 µm and baboon 54 µm, t= section thickness)

a) Vervet Monkey

TG	t(µm)	V_V	NA/mm²	Nva/mm³	NVb/mm³	Ntest	Tcount
a	7	0.38	116	2028	2109	101490	108290
b	7	0.34	133	2622	2418	104880	96000
c (rt)	10	0.30	128	2640	2207	105600	90000
d (lt)	10	0.36	130	2470	2241	92740	98700
Mean		0.35	127	2440	2244	101178	98073
SD		0.034	7	285	117	6903	7613

rt-right ganglion, lt- left ganglion

b) Olive baboon

TG	t(µm)	V_V	NA/mm²	Nva/mm³	NVb/mm³	Ntest	Tcount
Young (rt)	10	0.45	137	2390	2141	136980	153000
Young (lt)	10	0.40	124	2185	1938	124020	127750
Adult (rt)	10	0.35	71	1179	1109	141960	162750
Adult (lt)	10	0.30	73	1140	1141	146040	160720
Mean		0.40	101	1723	1582	137250	153555
SD		0.034	34	657	571	10274	11343

There was very little variation in the volume density between the dorso-ventral sections from one ganglion to the other, suggesting that the neurons were in general evenly distributed amongst the intraganglionic fibers and blood vessels. In the analysis of a section, the volume density of the neurons was lower at the periphery of the ganglionic band in the anterior region where the three divisions branched from the ganglion. The mean V_v shows that about 35-40% of the "ganglionic region" is composed of neurons with the satellite cells and the rest is made up of intraganglionic fibers and blood vessels (Fig. 1).

Fig. 1.– Photomicrograph of the trigeminal ganglion of the monkey (*Cercopitebcus aethiops*) showing the ganglionic region and the three divisions of the trigeminal nerve: A) ophthalmic B) maxillary C) mandibular D) centralroot (H&E; x10).

The number per unit area was lower in the monkey than in the adult baboon, but greater than that of the younger baboon. The distribution of the neurons in the dorso-ventral extent of the ganglion varied, the range being 102-158/mm^2 in the monkey and 60-94/mm^2 in the adult baboon.

The calculated numerical densities (N_{Va}) and (N_{Vb}) for monkeys and baboons using the respective formulae shown in Table 2 are fairly close.

The measured volume of the whole ganglion was corrected for the non-ganglionic region, which comprised about 30% of the ganglion. The measured volume of the monkey ganglion ranged from 0.06-0.08 cc, and of the baboon 0.08-0.17cc. The corrected volume of the "ganglionic region" ranged from 0.037-0.05cc in the monkey, 0.057cc in the young baboon and 0.12-0.13cc for the adult baboon.

The corrected volume of the ganglionic region x N_{Va} was used to estimate the total number of neurons by stereology (N_{test}), see Table 2. Some variations in the number of total TG neurons in the monkey and baboon were observed.

2. The total neuron counts (Tcounts) showed that the mean of five sections in the monkey (n=4) ranged from 1300-1500, and the mean of total neuron count was 98,073±7613 (90,000-108,290). In the baboon (n=4), the range of the mean of 5 sections was 1460-1700; the total cell counts was 153,555±11,343 (range 137,750-162,650).

The difference between the total mean neuron counts in the monkey and baboon was significant ($p<0.01$)

Size of Neurons

Humans. Table 3 shows age, sex and the percentage size distributions of TG neurons in the humans. The greatest number of neurons (72%) in humans was in the 20-40 μm size range with a mean 39.6 μm. The whole size range was between 13 μm (minimal) and 76 μm (maximal).

Table 3.– The sex, age and percentage size distribution of the trigeminal ganglion neurons in the humans

Size of Neurons			**sex/age (yrs)**			
(μm)	**F/20**	**F/20**	**M/58**	**M/54**	**M/65**	**M/70**
<20	514.9	3.33	0.93	5.43	6.25	3.92
20-29.99	23.9	42.5	34.57	41.08	28.57	25.49
30-39.99	47.8	32.5	44.86	34.11	43.75	37.25
40-40.99	21.6	14.16	14.95	17.05	18.75	25.49
≥50	5.20	7.51	4.69	2.33	2.68	7.85

Monkey and Baboon. The mean diameter of the TG neurons in the monkey (n=100) was 48±12 μm (range 25-65 μm). In the baboon, the mean diameter (n=100) was 54±13 μm (range 30-80 μm). Student's t- test showed that the difference between the size of the mean diameter in monkeys and baboons was significant ($P<0.001$). The corrected mean diameter of the neurons for 10% shrinkage would be 53 μm in the monkey and 60 μm in the baboon.

Discussion

Quantification of the TG neurons in humans, the monkey and baboon was carried out independently at our two laboratories using the methods of stereology and total cell counts with comparable findings. Differences were found in the total number of neurons between the individual ganglia as well as among the three species. Apart from the differences in the total number of TG neurons within the species due to the method of counting and histological processing, other factors that may account for the observed variability may be age, nutrition and environment, dental status, and the size of peripheral innervation (Gregg and Dixon, 1973; Cavanaugh, 1951). In the present study, the individual differences in the number of TG neurons of each species may be explained by age differences and the status of dentition in the monkey and baboon (deciduous mixed, and permanent dentition). The total cell counts obtained from the mean number of neurons per section may involve errors from the cell counts and total number of sections, although this method has been suggested by Blinkov and Gleezer (1976).

The total number of neurons of about 123,010 in the human compares well with the 140,000 fibers estimated in the trigeminal sensory root of humans (Sjogquist, 1938). In man, according to Belyaev (1963), the number of motor root fibers varies from 5348 to 14,601 and sensory root fibers from 76,842 to 150,079. In humans we did not find any apparent pattern related to sex and age in the number of neurons. The available literature does not contain quantitative data for TG neurons in primates.

In the rat, counting neurons in every 10^{th} section 10 μm thick, Gregg and Dixon (1973) found between 40,910–62,030 cells with a mean of 49,350 in 12 TG and Aldskogius and Arvidson (1978), counting neurons with nuclei in every 5^{th} section 15 μm thick, found 23,279-46,713 neurons in 4 rat TG.

The difference in the estimated total number of TG neurons between the rat, human, monkey and baboon may well be due to the morphological differences in the size of the peripheral innervation of the head region. However, compared to humans, the number of TG neurons is higher in the baboon. The trigeminal nerve in the baboon has similarities to its human counterpart, although the maxillary and mandibular divisions are large due to marked prognathism in the baboon (Gasser and Wise, 1972). The vervet monkey is comparably smaller than the baboon.

In the young baboon, the total number of neurons is similar to that seen in the older one, although the numerical density is almost twice that found in the older animals. There is twofold increase in the volume of the TG from young to the old and it is likely that the axons and blood vessels contribute to this increase in volume. V_v shows the greatest values in humans. The study methods may also influence the results obtained for humans, monkey and baboon, and these values may be further evaluated using other methods, such as design-based methods of stereology (West and Gundersen, 1990; Braendgaard and Gundersen, 1986).

The measurement of TG neurons with the nucleolus has been shown to afford a satisfactory representation of cell size (Palmer and Holland, 1988). In the guinea pig TG cells of different size were found to be primary afferent perykarya from extraocular muscles (Aigner et al., 1997). In this species, 55.71% of TG neurons are between 30 and 50 μm (Aigner et al., 1997). The size of the human ganglion neuron (mean 39.6 μm) compares well with mean of 46 μm reported for the cat (Marfurt and Echtenkamp, 1988; Shigenga et al., 1989). The size of the neurons was significantly greater in the baboon as compared to the monkey. The large cell size in the baboon may provide for wider arborization of the peripheral fibers to innervate a larger area. Cell size analysis in the cat and rat has revealed 3 distinct groups of TG neurons: small cells (20-30 μm) in cutaneous branches; medium-size cells (30-50 μm) in corneal afferents, and large-size cells (30-80 μm) in oral and perioral branches, including tooth pulps (Sugimoto et al., 1986; Marfurt, 1981).

There is a positive correlation between cell size and axon diameter (Sjogquist, 1938) and between cell size and the size of the peripheral area innervated (Lieberman, 1976). It has been suggested that the difference in neuron size would be related to the various sensory mechanisms transmitting the information from the different regions of the head. Despite the differences in head size, the relative size of the TG neurons in the rat, cat, monkey and baboon may reflect similar basic organization of investigated trigeminal ganglia.

In conclusion, quantification of TG neurons in humans, the monkey and the baboon provides a necessary comparative data for estimation of the number of neurons and volume densities. Bearing in mind the differences in methodology (used in our two laboratories, as well as by other authors) or interspecies differences related to morphology and the size of the head, our quantitative study on TG neurons in the species investigated would be useful in further studies on the trigeminal system, including its role in ocular motility.

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References

Abercrombie M (1946). Estimation of nuclear population from microtome sections. *Anat Rec*, 94: 239-247.

Aherne WA and Dunnill MS (1987). *Morphometry*. Edward Arnold, London, p 63.

Aigner M, Lukas JR, Denk M and Mayr R (1997). Sensory innervation of the guinea pig extraocular muscles: A 1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate tracing and calcitonin gene-related peptide immunohistochemical study. *J Comp Neurol*, 380: 16-22.

Aldskogius H and Arvidson J (1978). Nerve cell degeneration and death in the trigeminal ganglion of the adult rat following peripheral nerve transection. *J Neurocytol*, 7: 229-250.

Atkinson M and Kenyon C (1990). Collateral branching innervation of rat molar teeth from trigeminal ganglion cells shown by double labeling with fluorescent retrograde tracers. *Brain Res*, 508: 289-292.

Blinkov SM and Gleezer I (1976). *The human brain in figures and tables*. Plenum Press and Basic Books Inc., New York. pp 11-14, 279, 322.

Braendgaard H and Gundersen HJ (1986). The impact of recent stereological advances on quantitative studies of nervous system. *J Neurosci Methods*, 8: 39-78.

Cavanaugh MW (1951). Quantitative effects of the peripheral innervation area on nerves and spinal ganglion cells. *J Comp Neurol*, 94: 181-219.

Dubner R, Sessle B and Storey A (1978). *The neural basis of oral and facial function*. Plenum Press, London. pp. 1-56.

Gasser RF and Wise DM (1972). The trigeminal nerve in the baboon. *Anat Rec*, 172: 511-522.

Gregg JM and Dixon AD (1973). Somatotopic organization of the trigeminal ganglion in the rat. *Arch Oral Biol*, 18: 487-498.

Holland GR and Robinson PP (1990). Cell counts in the trigeminal ganglion of the cat after inferior alveolar nerve injuries. *J Anat (Lond)*, 171: 179-186.

Kalisnik M (1985). Temelji stereologije. *Acta Stereologica (Ljubljana)*, 4 (Suppl. 1): 66-69.

Konigsmark BW (1970). Methods for counting neurones. In: Nauta WH (ed). *Contemporary research methods in Neuroanatomy*. Springer, New York, pp 315-340.

Kruger L and Young RF (1981). Specialised features of the trigeminal nerve and its central connections. In: Samii M and Janeta PJ (eds.). *The Cranial Nerves*. Springer Verlag, Berlin, pp 273-301.

Lieberman AR (1976). Sensory Ganglia. In: Landon DN (ed). *The peripheral nerve*. Chapman and Hall, London, pp 188-263.

Lukas JR, Blumer R, Aigner M, Denk M, Baumgartner I and Mayr R (1997). Propriozeption aus auseren Augenmuskeln des Menschen: Zur Morphologie ihrer Muskelpsindeln. *Klin Monatsblatt Augenheilknd*, 3: 183-187.

Marfurt CF (1981). The somatotopic organization of the cat trigeminal ganglion as determined by the horseradish peroxidase technique. *Anat Rec*, 201: 105-118.

Marfurt CF and Echtenkamp SF (1988). Central projection and trigeminal ganglion location of corneal afferent neurones in the monkey Macaca fascicularis. *J Comp Neurol*, 272: 370-382.

Mayberg M, Langer SR, Zervas NT and Moskowitz MA (1981). Perivascular meningeal projections from cat trigeminal ganglion: possible pathway for vascular headache. *Science*, 213: 28-230.

Palmer J and Holland GR (1988). Nucleolus eccentricity in trigeminal ganglion neurones. *J Anat (Lond)*, 157: 163-168.

Scherle W (1970). A simple method of volumetry of organs in quantitative stereology. *Mikroskopie*, 26: 57.

Shigenaga Y, Nishimura M, Suemune S, Nishimora T, Doe K and Tsuru H (1989). Somatotopic organisation of tooth pulp primary afferent neurones in the cat. *Brain Res*, 477: 66-89.

Sjogquist O (1938). Studies of pain conduction in the trigeminal nerve. A contribution to surgical treatment of facial pain. *Acta Physiol Scand (Suppl.)*, 17: 1-39.

Sugimoto T, Takemura M, Sakai A, Ishimura M (1986). Cell size analysis of trigeminal primary afferent neurones comprising individual peripheral branches of the rat mandibular nerve. *Exp Neurol*, 93: 565-573.

Truex CR (1940). Morphological alterations in the gasserian ganglion cells and their association with the senescence in man. *Am J Pathol*, 16: 255-268.

Vujaskovic G and Malobabic S (1991). The absolute number of neurons in human trigeminal ganglion: The estimation of the necessary number of sections. *Med Istr*, 24: 1-2, 135-138.

Weibel ER (1979). Stereological methods. Vol 1. Academic Press, London, pp 9-196.

Weibel ER (1980). Stereological methods. Vol 2. Academic Press, London, pp 140-151.

Weibel ER and Gómez DM (1962). A principal for counting tissue structures on random sections. *J Appl Physiol*, 17: 343-348.

West JM and Gundersen JG (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *J Comp Neurol*, 296: 1-22.