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A Study on Head Measurements in Different Laboratory Rat Strains

Farklı laboratuvar sıçanı soylarında baş ölçümleri üzerine bir araştırma

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ABSTRACT

This study is conducted to determine whether there was a difference between the lineages of Brown-Norway, Lewis, Sprague-Dawley, and Wistar Albino rats which are used as laboratory animals and have over 300 sub-lineages. For this purpose, a total of 24 male rats composed of 6 each from every lineage possessing similar weight (average 400 g) and age. The rats used in the individual studies in the Experimental Animal Unit, Faculty of Medicine at Dokuz Eylül University were included in the study after the researchers completed the required samples. After removing the skin of rats, the cadavers were boiled, and the muscles were separated from the bones. After clearing the skeletal bones from the muscles, the skull measures were taken with the aid of a caliper. The skull and facial measurements were taken, and craniofacial indices (nasal index, facial index, skull index, basal index, and neurocranial index) were calculated. As a result, 27 measurements were made on the skull of four different rats and craniofacial indices were calculated and, high–level significant differences were found in terms of craniofacial indices among the lineages.

Keywords: *Craniometry, morphometry, rat strain, skull index.*

ÖZET

Bu çalışma, laboratuvar hayvanları olarak kullanılan ve 300'ün üzerinde alt soya sahip Brown-Norveç, Lewis, Sprague-Dawley ve Wistar Albino sıçanlarının soyları arasında farklılık olup olmadığını belirlemek amacıyla yapılmıştır. Bu amaçla her soydan benzer ağırlıkta (ortalama 400 g) ve yaşta 6'şar adet olmak üzere toplam 24 erkek sıçan oluşturuldu. Dokuz Eylül Üniversitesi Tıp Fakültesi Deney Hayvanları Birimi'nde yapılan bireysel çalışmalarda kullanılan sıçanlar, araştırmacıların gerekli örnekleri tamamladıktan sonra çalışmaya dahil edildi. Sıçanların derisi çıkarıldıktan sonra kadavralar kaynatıldı ve kaslar kemiklerden ayrıldı. İskelet kemikleri kaslardan temizlendikten sonra kumpas yardımı ile kafatası ölçüleri alındı. Kafatası ve yüz ölçümleri alınarak kraniyofasiyal indeksler (nazal indeks, fasiyal indeks, kafatası indeksi, bazal indeks ve nörokraniyal indeks) hesaplandı. Sonuç olarak, dört farklı sıçanın kafatası üzerinde 27 ölçüm yapılmış ve kraniyofasiyal indeksler hesaplanmış ve soylar arasında kraniyofasiyal indeksler açısından yüksek düzeyde anlamlı farklılıklar bulunmuştur.

Anahtar Kelimeler: *Kafatası indeksi, kraniometri, morfometri, sıçan soyları*

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INTRODUCTION

The laboratory rat originated from the Norwegian rats or Brown rat (Rattus norvegicus), a member of the Rodentia group and the Muridae family, whose genus Rattus contains more than 130 species and entered the laboratory a century ago.[4] The Rodents class constitutes the largest class of mammals and covers 1,800 species.[6] Rodents are the most preferred animals in biomedical experimental animal studies, and rodents constitute 80-90% of all animals used for this purpose.[17] They are the second most commonly used laboratory animal species after rats. These individuals have many significant advantages: they are rodents, which are active at night, and easy to adapt to the environment. In addition to that, they are easy to transport, and they can make many litter. Also, they can take care of more animals in one unit area.

One of the most specialized structures of the skeletal system is the cranium. The skull is a morphologically specialized shape and structure in animal species. The cranial bones are located in the flat bones group and are divided into two groups: ossa faciei - facial bones and ossa crani-cranial bones. In rats, the skull bones, the cavum cranii, the cavum oris, and the cavum nasii encircle three essential cavities. The most important of these is the cranium-cranial cavity. It consists of 7 bones, including the brain and cerebellum, cavum cranii, os temporale and os parietale (double), os frontale, os ethmoidale, os occipitale, os sphenoidale and os interparietale (single). The shape and structure of these bones show significant differences among the species. It does show differences even in the sub-lineages of the same species. For example, when externally looking at the dog breeds, it is possible to see that the bone structures have remarkably differentiated in short and long-headed dogs. Yildiz et al., have shown that there is a proportional difference in head and face measurements of German wolf and Turkish shepherd dogs.[24] Moving from this point, it is aimed to compare head and skull measurements among the laboratory rat lineages in this study.

The morphological and morphometric studies in the skull reflect the contribution of both genetic and environmental factors to individual development and also make up the basis of clinical and surgical practices.[23] The difference in skull measurements does not only emanate from the difference between species and genus.[16] but also the age and gender differences between different individuals of the same genus.[11] There are morphological and morphometric studies applied to the skulls of various animal species. [3,7,10,14,15,19] In rats, the amount of protein available in the diet was found to be effective on skeletal development and craniofacial skeletal measurements.[9] Aminabadi et al. have stated that the anterior cranial base length and viscerocranium measures have significantly increased in the rats with prenatal stress in comparison to the control group.[1] Several differences have been found when the head skeletons of South American rodent (Proechimys trinitatus) and Rattus norvegicus were compared.[8] Due to the studies on skull measurements of different rat strains and sexes, the effects of various pathologies on the bone development of rats have been investigated in different studies above. However, the skull measurements of different rat lineages and their comparison have not been done before, and no detailed information is available on this subject. This study aimed to investigate whether or not there was a difference in the morphometry of the skull between Brown-Norway, Lewis, Sprague-Dawley, and Wistar Albino lineages commonly used in laboratories and biomedical researches.

MATERIAL AND METHODS

In this study, the rats that were used in the individual studies at the Experimental Animal Unit, Faculty of Medicine at Dokuz Eylül University (DEU), where the tissues and samples were taken as biological wastes after taking samples were included in the study. The study was started after the ethical approval by DEU Multidisciplinary Laboratories Local Ethics Committee for Animal Experiments. The adult male rats (about 400 g), which were not affected by their skeletal system, were taken to study. A total of 24 rats composed of 6 each from every lineage; Brown-Norway, Lewis, Sprague-Dawley, and Wistar Albino have been utilized. The rats that were taken to the study were first removed from their skins, then the muscles and soft tissues were separated from the bones by a maceration technique (Figure 1 and Figure 2).[18] All bone measurements were made with calipers.

Then, according to the metric determined by Driesch,[21] measurements were taken from 27 different skull zones using calipers (Figure 3 and Figure 4). Indexes were calculated using the method defined by Onar et al..[13]

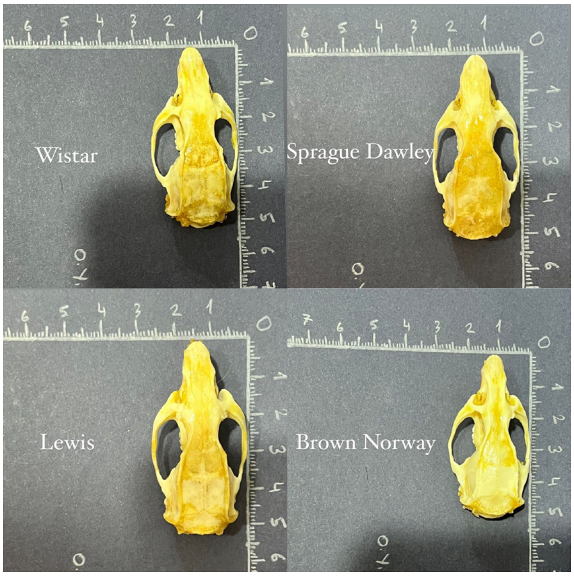

Figure 1. Dorsal view of skulls

Figure 2. Ventral view of skulls

Measurement points defined on the skull:

Acrocranion (A). The most aboral point on the vertex of the cranium in the median plane, Basion (B). Orobasal border of foremen magnum in median plane, Bregma (Br). Median point of parietofrontal suture Lambda (L). Median point of parietooccipital suture, Nasion (N). Median point of nasofrontal suture, Prosthion (P). Most oral points of premaxillae on the median plane (Figure 1-2).

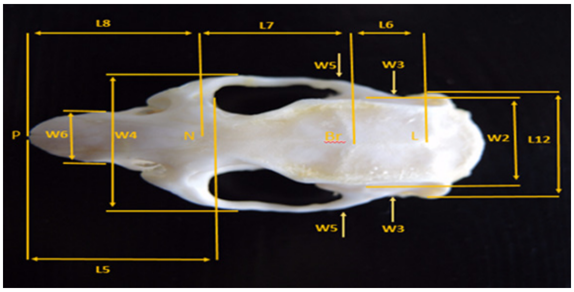

Figure 3. Measurement points on skull (dorsal view)

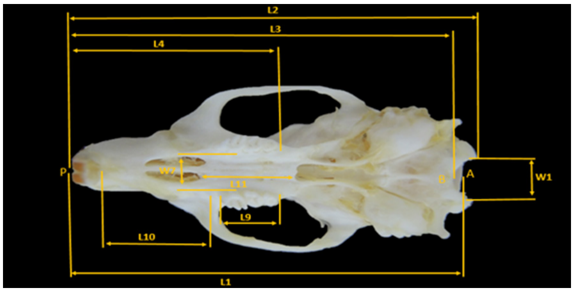

Figure 4. Measurement points on skull (ventral view)

Measurements taken from the rat skull:

L1. Skull length (acrocranium-prosthion), **L2.** Condylobasal length (aboral borders of occipital condyle-prosthion), **L3.** Basal length, **L4.** Dental length (postdentale - prosthion), **L5.** Largest nasal length, **L6.** Parietal length (lambda - bregma), **L7.** Frontal length (bregma - nasion), **L8.** Viscerocranium length (nasion-prosthion), **L9.** Length of the cheektooth row (measured along the alveoli on the buccal side), **L10.** Diestema length, **L11.** Palatal length, **L12.** Widest length between the external acoustic meatus (otion – otion), **L13.** The highest length of mandible (Infradentale-Gonion caudale), **L14.** Length of socket for mandibular inciciva, **L15.** Length of socket for maxillar inciciva, **W1.** Greatest width between the occipital condyles, **W2.** Maximum neurocranium width (euryon – euryon), **W3.** Skull width (distance between the temporal fossae) **W4.** Oral zygomatic width (between the oral parts of zygomatic arch) **W5.** Aboral zygomatic width (between the aboral parts of zygomatic arch), **W6.** Largest nasal width, **W7.** Palatal width, **W8.** Width of sockets for mandibular inciciva, **W9.** The highest width of maxiilar sockets for second molars(Molare-Molare), **W10.** The highest width of mandibular sockets for second molars(Molare-Molare, **H1.** Height of the mandible between M1 and M2, **H2.** Height of mandibular ramus (Gonion ventrale-Coronion) (Figure 1-2).

Craniofacial index values calculated on the skull:

Skull index : Maximum zygomatic width x 100 / skull length

Nasal index : Greatest breadth across the nasals x 100 / greatest length of the nasals

Facial index : Maximum zygomatic width x 100 / viscerocranial length

Neurocranium index : Maximum neurocranium width x 100 / neurocranium length

Basal index : Maximum neurocranium length x 100 / basal length

Data analysis: The suitability of animal lineage–based skull measurements were analyzed with the Shapiro-Wilk test on the normal distribution of each group, and the intra-group distributions of all measurements of each group were found to be normal ($p > 0.05$, all measurements in all groups). As the data showed a normal distribution, the skull measurements of each group were shown as mean ± Sd. One-way ANOVA F test, whether or every each one of this skull measurements was different between these four groups, was analyzed. When the F test was meaningful, the Bonferroni test was performed to identify from which dual group comparison the difference has emanated. Bonferroni test was implemented as because the variances of all the comparison groups were equal. Though the possibility of a high margin of error caused by ANOVA examining variables one by one, discriminant analysis was applied concurrently to minimize the margin of the error. In all analytical comparisons, $p<0.05$ statistical significance was taken as the limit value. However, SPSS 23.0 package program was used for data analysis.

RESULTS

Averages and standard deviations of data obtained from four different zones – skull measurements of the rat lineages are given in Table 1. The mean and standard deviations of the craniofacial indices and the lowest and highest values of the measurements are given in Table 2. The statistical data of the craniofacial indices (nasal index, facial index, skull index, neurocranial index, and basal index) among the groups are shown in Table 3.

Table 1. The averages and standard deviations of rat skull zones measured.

Measurements taken from the skull	Mean±SD	Measurements taken from the skull	Mean±SD
L1	4,81 ± 0,13	**W1**	0,96 ± 0,05
L2	4,84 ± 0,13	**W2**	1,54 ± 0,05
L3	4,59 ± 0,16	**W3**	1,74 ± 0,10
L4	3,45 ± 0,12	**W4**	1,60 ± 0,12
L5	2,24 ± 0,12	**W5**	2,31 ± 0,06
L6	0,72 ± 0,06	**W6**	0,74 ± 0,07
L7	1,41 ± 0,13	**W7**	0,53 ± 0,04
L8	1,91± 0,13	**W8**	0,30 ± 0,01
L9	0,69 ± 0,03	**W9**	0,90 ± 0,00
L10	1,30 ± 0,07	**W10**	0,90 ± 0,00
L11	1,04 ± 0,05	**H1**	0,86 ± 0,05
L12	1,82 ± 0,12	**H2**	1,56 ± 0,09
L13	3,35 ± 0,08		
L14	1,16 ± 0,05		
L15	0,74 ± 0,06		

Table 2. Average and standard deviations of craniofacial indices of all animals and lowest and highest values.

Craniofacial indexes	Mean±SD	Lowest values	Highest values
Nasal index	33,18 ± 2,85	27,27	40,91
Facial index	121,68 ± 9,80	106,98	150,00
Skull index	47,99 ± 2,13	44,90	52,17
Neurocranial index	61,39 ± 14,60	48,39	94,12
Basal index	57,21 ± 10,77	35,42	68,89

Table 3. Comparision of the means of nasal index, facial index, skull index and basal index in animal strains

	Group	Mean±SD	P*	Pairways Comparision Group	P**
Nasal Index	Wistar Brown Lewis Sprague Dawley	32.8±2.3 31.0±2.3 31.2±1.3 36.5±2.4	<0.002	W-B W-SD B-SD L-SD All other pairways	0.979 0.039 0.001 0.023 >0.005
Facial Index	Wistar Brown Lewis Sprague Dawley	113.2±6.4 133.3±8.4 121.0±6.9 119.3±4.9	<0.001	W-L W-SD B-L B-SD L-SD W-B	0.373 0.791 0.029 0.011 0.022 <0.001
Skull Index	Wistar Brown Lewis Sprague Dawley	45.8 ± 0.9 50.5 ± 1.0 48.5 ± 2.1 47.2 ± 0.9	<0.001	W-L W-SD B-L B-SD L-SD W-B	0.013 0.445 0.106 0.003 0.727 <0.001
Basal Index	Wistar Brown Lewis Sprague Dawley	64.0 ± 3.5 62.6 ± 1.1 62.9±2.2 39.4±2.4	<0.001	W-SD B-SD L-SD All other pairways	<0.001 <0.001 <0.001 >0.005

*ANOVA F test: Comparison of the mean of 3 or more groups in the parametric condition
**Bonferoni: Post Hoc multiple comparison test

According to One-way ANOVA F test:

A difference between nasal index averages has been found among the animal strains. It has been determined that this difference has emanated from the average of Sprague Dawley measurement, and it was significantly higher than the averages of Wistar, Brown Norway, and Lewis groups.

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A difference between nasal index averages has been found among the animal strains. It has been determined that this difference has emanated from the average of Brown Norway's measurement, and it was significantly higher than the averages of Wistar, Sprague and Lewis, and Lewis group facial indices were higher than the Sprague group.

It has been determined that the mean skull indexes belonging to four lineages were different. The average of Brown Norway was significantly higher than, respectively, Sprague Dawley and Wistar groups. Again, when the skull index of Lewis lineage was compared to Wistar, the difference between them was found to be significantly higher. No significant difference was found between Brown Norway and Lewis.

It has been determined that the averages of neurocranial indexes belonging to four lineages were different. This difference was found to be emanating from a significantly high Sprague Dawley index average and significantly higher than the respective averages of Brown Norway, Lewis, and Wistar groups. No significant difference has been found between Brown Norway, Lewis, and Wistar groups.

It has been determined that basal index averages belonging to four lineages were different. This difference was found to be emanating from the measurement average of Sprague Dawley and significantly lower than the averages of Brown Norway, Lewis, and Wistar groups. No significant difference has been found between Brown Norway, Lewis, and Wistar groups.

Given the discriminant analysis, nasal index, facial index, neurocranial index, skull index, and basal index parameters were used for discriminant analysis among Wistar, Brown, Spraque Dawley, and Lewis lineages. Five indexes were included in the discriminant analysis for the distinctiveness of four lineages. Due to the high correlation between the basal index and neurocrainial index, the neurocrainial index was excluded from the analysis, and discriminant analysis was performed again among the other four indexes. In this model obtained, the total variance with two functions was found to be 99.5% discriminatory, and the discrimination of the four strains included in the study was determined by four indices.

DISCUSSION AND CONCLUSIONS

In a study conducted on black-hooded rats[5], they found that skull measurements on male rats were significantly greater than on females, but no difference was found between sexes in cranial and facial indices. In this study, significant differences were found between the mean of the measured craniofacial indices on the skulls of male rats belonging to four different lineages but no difference was found between the sexes in cranial and facial indices. In this study, significant differences were determined between the averages of craniofacial indices measured on the skulls of male rats belonging to four different lineages. In the study carried out to reveal possible differences between the cranial structures of Tuj and Purple Karaman sheep, the measurements were taken from 38 different places on the skull, and no significant difference was found between the two species in the measurements other than the smallest width (entorbitale - entorbitale) between the orbitas (16). In another study, it was mentioned that in the head bones of sheep, there are similarities as well as macro-anatomical differences.[20] The skulls of the Polish Heath sheep were found to be shorter and wider than the skulls of the Spanish Xisqueta sheep.[2] As to this study, in the measurements made on the skulls belonging to four distinct lineages, differences at significant levels have been determined among all lineages of the craniofacial index. In the study carried out on the dogs, the skull, cranial, and nasal lengths and the cranial width showed similarities with the adult Kangal dog but exhibited relatively from that of pups of German shepherd (Alsatian) dog.[12] In another morphometric study, a strong positive correlation has been discovered between skull index and facial index in the skull of German shepherd (Alsatian) dogs.[11] In a study accomplished with 5 different rat lineages at similar ages, it has been discovered that the long bones of Wistar and Sprague Dawley rats were greater than the other lineages.[22] Also, in this study, it has been determined that the average facial index belonging to the Brown-Norway lineage was significantly higher than the averages belonging to the other three lineage groups. Again, it has been determined that Brown-Norway skull index averages were significantly higher than Sprague-Dawley and Wistar averages, but it has been determined that there was no statistically significant difference with Lewis lineage. In conclusion, 27 measurements have been actualized in this study carried out on the four different rat skulls, and the craniofacial indices have been computed, and high-level significant differences have been determined in terms of craniofacial indices among the lineages.

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Ethical Statement: This study was approved by the Animal Experiments Local Ethics Committee of Dokuz Eylül University. (Ethics committee approval date: 23.08.2016 Decision number: 43/2016).

Conflict of Interest: The authors declared that there is no conflict of interest.

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