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Applying a Unified Model of Fiber Dissection, Tractography, Microscopic Anatomy and Plastination Techniques for Basic Neuroanatomy Education: Hacettepe University Experience

Aplicación de un Modelo Unificado de Disección de Fibras, Tractografía, Anatomía Microscópica y Técnicas de Plastinación para la Educación Básica en Neuroanatomía: Experiencia de la Universidad Hacettepe

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SUMMARY: Anatomy education has gathered together a great many of many new modalities and was modified from classical lecture-based and laboratory practice system to the blended modules. In the scope of the present study, we develop a new, practical, cost-effective and efficient three dimensional (3D) educational model, which aimed to be helpful for the detection and better understanding of basic neuroanatomy education. Tractographic imaging, fiber dissection, microscopic anatomy and plastination techniques were applied to the white matter regions of the two brains. After the photographs that were taken were converted to 3D images, the specimens were plastinated. By way of establishing an educational model as a whole, we applied it to 202 second-year medical students. The students were separated into two groups when they attended to the theoretical lecture. Group 1 took the classical laboratory education; on the other hand, Group 2 received the newly designed educational model. Pre and post-tests were introduced to each group before and after laboratory sessions, respectively. The success scores were put to comparison. The average achievement scores of each group showed increase significantly ($p<0.05$) after the laboratory sessions, besides the increase in the post-test results of Group 2 was more statistically significant ($p<0.05$). Consequently, this new educational model enriched by newly designed unified methods could be regarded as useful for grasping and improving the basic neuroanatomy knowledge.

KEY WORDS: Fiber dissection; Medical education; Neuroanatomy; Plastination; Tractography.

INTRODUCTION

Anatomy education has been one of the essential components of medical education starting from the beginning. When the willingness to dominate the human body that has been continuing throughout all human history is combined with the aesthetic value of the body and the desire to recognize it, the basic motivation of anatomy education emerged (Ersoy, 1998). However, this basic motivation does not make the anatomy course easy for medical students. Anatomy is considered as one of the most challenging courses for medical students (Bandyopadhyay & Biswas, 2017). Some students and young physicians are even afraid of neuroanatomy and some researchers call this state as "neurophobia" (Zinchuk *et al*., 2010).

The 3D layout of central nervous system elements, their relative positions, their functions, and the related diseases have been considerably difficult subjects for students to grasp and keep in mind. Besides, it is difficult to demonstrate the anatomical structures in the nervous system by way of separating them through dissection in anatomy laboratories (Familiari *et al*., 2013). While some researchers propose computer-based education models instead of traditional methods, some have stated that essential cadaver training should be upgraded (Familiari *et al*., 2013). Increasing numbers of students, difficulties in procuring cadaver, together with the developing technology have brought in many new assessments regarding anatomy education. The use the educational modality is generally offered for use in which the traditional cadaver training method is enlarged by use of visual materials as much as possible, particularly with the use of computer technology (Sugand *et al*., 2010; Turkish Society of Anatomy and

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Clinical Anatomy, 2013; Hackett & Proctor, 2016; Schleisman *et al.*, 2018).

Understanding of the white matter anatomy of the brain is important not only for neuroscientists but also for the medical students (Familiari *et al*., 2013). White matter anatomy has been moved away from its inaquaintence in the past through progress in computer-based imaging techniques and has gained an increasing interest. By detecting that the connections in the white matter are responsible for some diseases and the spread patterns of the white matter fibers of some tumour formations are related to one another, all brain researchers, particularly neurosurgeons and neurologists have a great desire for comprehending the anatomy of white matter.

Also during the Covid-19 pandemic period, we became aware of the fact that distance learning applications became necessary as face-to-face teaching was hindered. Nowadays, the development of computer-based systems and their placement in educational programs have become highly important (Iwanaga *et al*., 2021).

This study aims to combine some educational techniques from the scratch by taking advantage of the applications currently in use, to obtain a compact anatomy knowledge out of them and to measure the effectiveness of a new model. For this purpose; the imaging modalities, that are more suitable for white matter structures, including tractographic imaging, fiber dissection, microscopic anatomy and plastination were made use of in the scope of the study. The said modalities have already been in use in anatomy education (Arnts *et al*., 2014). In the present study, some educational components such as 3D images, microscopic anatomy sessions were added into them and all the given modalities were applied to the student sample that was selected.

MATERIAL AND METHOD

Student Sampling. The study was approved by the Non-interventional Clinical Research Ethics Board of Hacettepe University (Decision number: GO 17/582-22). Participation in the study was voluntary. A total of 202 students, 104 of whom were studying in 2017 (33 % of total students in class 2017) and 98 of those, who were studying in 2018 (31 % of total students in class 2018), participated in the study at Hacettepe University Faculty of Medicine's (HUFM) Turkish Medicine Department. 108 students were female (53.4 %) and 94 were male (46.6 %). Participants were divided into two groups on a random basis.

Study Design. During the study, the students participated in the course titles of “Brain Hemispheres: White Matter", which was a 50-minute long (1 lesson hour) class. Then, the students were taken into HUFM Macroscopic Anatomy Laboratory to carry on with the practical application of the theoretical course that they participated in (The schematic design of the study is shown in Figure 1). To the first group of students, 50-minute laboratory practice was applied through the models that are currently used in laboratory education and are demonstrated every year. While the first 20 minutes of the session were devoted to the examination of the model and the next 20 minutes to the examination of the cadaver, the last 10 minutes to the discussion. To the second group, the training model, which was put forward in this study, was applied in 50 minutes. The content of this application was as follows: an examination of images obtained by fiber dissection method for 10 minutes, an examination of tractography images for 10 minutes, an examination of microscopic anatomy images for 10 minutes, an examination of plastinated samples for 10 minutes, and lastly, discussion for 10 minutes. A preliminary test was applied to the students before the practical application got started, and their initial knowledge was gathered up. The same test was repeated after the application was completed. The tests were made up of 10 multiple choice questions in line with the educational objectives.

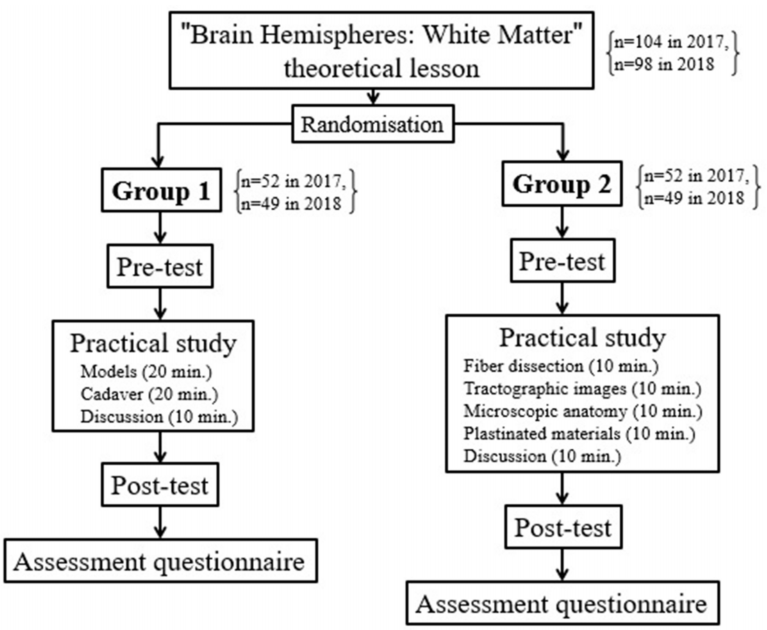

Fig. 1. The schematic design of the study.

Then, with a 12-question questionnaire, the students' opinion about the practices were obtained (after demonstrating the new model to Group 1 additionally). The questionnaire was prepared as a Likert Scale questionnaire in which the answers were "Strongly disagree, Disagree, Neither agree nor disagree, Agree and Strongly agree". Thus, the effects of two different education models on students' achievement were compared, and their ideas about different education models as well as the said models' effects on learning motivations were made evaluation.

Material Preparation. Two human and ten bovine brains were put to use as study materials. All the brains were fixed with 10 % formaldehyde solution.

Tractography. Imaging was performed using a 3T (Philips Healthcare, Ingenia, Netherlands) MRI device using a 4-way head-coil. MR tract of white matter pathways was performed in a PC-centered workstation using FACT (fiber separation with continuous tracking) algorithm. No suitable images of the brains were obtained in tractography, and the signal reduction was observed in T2-weighted images due to the effect of formaldehyde. Since no appropriate tractography images could be obtained as a result of the procedure, the images of pathways compatible with the dissection were selected from the images of the patients, who had been selectively tractographed in the HUFM Picture Archiving and Communications System (PACS) (Fig. 2). Although the postmortem interval of the brains used in our study was as short as had been recommended (<24 hours), the long scan and processing times suggested that prevention of this negativity could not be obtained on MRI devices at the time of routine clinical use.

Fiber dissection. Preparations were made by Klingler method for the brain dissection (Agrawal *et al*., 2011). In compliance with the Klingler method, brains were fixed in 10 % formalin solution for three months. After meningeal membranes and vascular structures around them were eliminated, the brains were washed under tap water and frozen at -17ºC for 14 days. Then, using the dissection set and wooden spatulas and under a surgical microscope (Carl Zeiss, Opmi Pico) and magnifier (Atersan 8069 LED), the fiber dissection was applied to cortical regions (Fig. 2a).

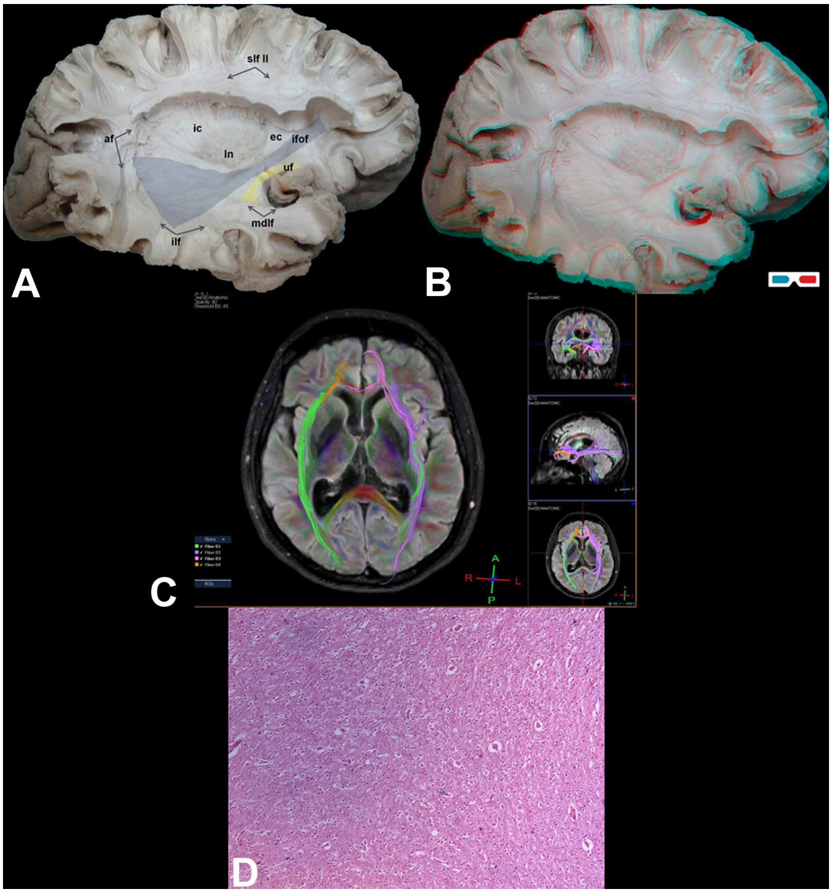

Fig. 2. Samples of the association fibers. A. Dissected brains show the association fibers. B. 3D photo of the samples. C. Tractographic image of the association fibers. D. microscopic anatomy of the corona radiata.

The dissections made were photographed in 3D. The Sony Cyber-shot DSC-W290 camera was used for photography. 3D photography of the graded dissection was applied according to the method of obtaining anaglyphic picture (Shimizu *et al*., 2006). The resulting binary images were rendered red-blue anaglyph using Adobe Photoshop CS6 (Adobe Systems Incorporated, San Jose, CA, USA) (Fig. 2b). While the lens was shifted, the camera was mounted on a wheeled and angular stabilizer.

Microscopy. Samples taken from the dissections were embedded in paraffin blocks, and microscopic anatomies of the sections were examined using a light microscope (Nikon, Optiphot). Samples were taken from the frontal lobe to show the 6-layered cortical structure of the brains, from the cingulum as it was easier to separate it in isolation, and from the corona radiata to observe the white matter as a whole (Fig. 2d). Nerve fibers and their relations with surrounding tissues were evaluated. While applying this method, brain sections were stained with Luxol Fast Blue and Hematoxylin-Eosin as staining methods.

Plastination. Cold silicone plastination technique was applied for plastination (DeJong & Henry, 2007). After the dissection of the brain tissues was completed, samples were plastined by applying dehydration, defatting, forced impregnation and gas curing methods, respectively. The plastination process was completed after 41 days by following up the given procedures.

Data Analysis. SPSS 22.0 package was used for data analysis. Firstly, descriptive statistical methods were applied to analyze data. "Shapiro-Wilk normality tests" were applied to evaluate whether the distribution of test results was normal or not. For abnormally distributed data, Wilcoxon Signed-Rank Test and the Mann-Whitney U Test were made use of to carry out comparisons between variables. The significance level was set at $p < 0.05$.

RESULTS

According to the results, the average achievement score of the participants in Group 1 was 3.87 ± 1.75 in 2017 and 4.59 ± 2.31 in 2018. After the standard laboratory practice training was provided for the same group. the average achievement score increased to 5.38 ± 1.74 in 2017 and 5.94 ± 1.97 in 2018. The average scores of the participants in Group 2 were 3.69 ± 1.91 in 2017 and 4.71 ± 1.89 in 2018. After the practical training was provided for the same group using the new laboratory model. the average achievement score showed increase up to 6.23 ± 1.75 in 2017 and 6.76 ± 1.92 in 2018 (Fig. 3).

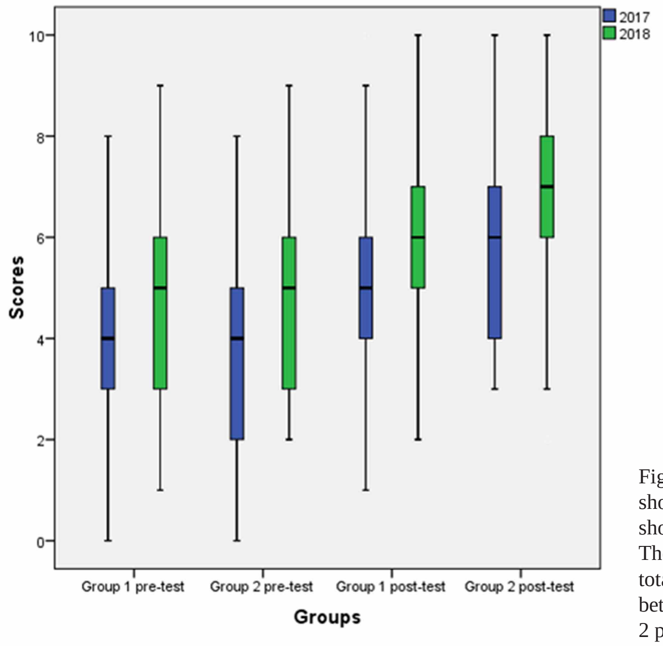

Fig. 3. A boxplot of students' scores in the exams. Segment inside the boxplot shows the median. Blue boxplots show the scores in 2017 and green boxplots show the scores in 2018. Mean values of the boxplots shown on the figure. There was no difference between the Group 1 pre-test and Group 2 pre-test in total ($p > 0.05$); both in 2017 and 2018. There was a significant difference between Group 1 pre-test and Group 1 post-test ($p<0.001$); also between Group 2 pre-test and Group 2 post-test ($p<0.001$); both in 2017 and 2018.

When the statistical comparison of the groups was made after these results, the achievements of Group 1 and Group 2 in the pre-test were measured to be very close to each other both in 2017 and 2018 ($p > 0.05$).

The achievement of the students in Group 1 showed increase significantly after standard laboratory practice training that was applied both in 2017 and 2018 ($p < 0.001$).

After the practical training as based on the new laboratory model, the achievement of the students in Group 2 increased significantly both in 2017 and 2018 ($p < 0.001$).

The achievement of the students in Group 2 after the practical training as based on the new laboratory model increased significantly compared to the ones in the Group 1 after standard laboratory practical training that was applied both in 2017 and 2018 ($p < 0.05$).

In addition, when the achievement scores of the participants in the pre-test and post-tests were compared separately for each participant, it was found out that in 2017, the achievement scores increased when it comes to 45 (86.53 %) of the participants in Group 1, did not change for 7 (13.47 %) of them and none of the participants had any decreased scores. In 2018 the achievement scores increased for 45 (91.83 %) of the participants in Group 1, did not change for 4 (8.17 %) of them and none of the participants had decreased scores (Table I). In Group 2. in 2017 it was found out that the success scores of 49 (94.24 %) students showed increase, the success scores of 2 (3.84 %) did not show a change, and decreased for one (1.92 %) of them; in 2018 it was found out that the success scores of 42 (85.71 %) students increased. the success scores of 7 (14.29 %) did not show a change and none of the participants decreased their scores (Table I).

According to the questionnaire through which we aimed to evaluate the students' opinion about the method that we developed in our study, the majority of students are not willing to get taught only on a theoretical basis (83 %). Almost all (98 %) considered that different methods should be applied in the courses, 96 % considered that the training model applied in the study increases their motivation, 93 % considered that their success would increase depending on the use of the said training model. Additionally, 98 % stated that they benefited from the training. On the other hand, it was found out that the education model that students utilized most was 3D pictures with 65 %, followed by plastination models with 20 % and tractographic images with 9 % (Table II). Cronbach's alpha coefficient value amounted to 0.81 for the questionnaire answers panel.

Table I. Evaluation of the pre-test and post-test success of the students in Group 1 and 2.

		2017		
		N	Percentage	N
Grup1post-test – Grup1pre-test	Negative rows	0[a]	0,00	0[a]
	Positive rows	45[b]	86,53	45[b]
	Equal	7[c]	13,47	4[c]
	Total	52	100	49
Grup2post-test – Grup2pre-test	Negative rows	1[a]	1,92	0[a]
	Positive rows	49[b]	94,24	42[b]
	Equal	2[c]	3,84	7[c]
	Total	52	100	49

a. Grup post-test < Grup pre-test. b. Grup post-test > Grup pre-test. c. Grup post-test = Grup pre-test. N: Number of the cases.

Table II. Students' view of the educational model.

Question 1: Lectures are sufficient to explain theoretically.	1.	Strongly disagree	114 (% 56,4)
	2.	Disagree	60 (% 29,6)
	3.	Neither agree or disagree	14 (% 7)
	4.	Agree	7 (% 3,5)
	5.	Strongly agree	7 (% 3,5)
Question 2: It is better both practical and theoretical education combined.	1.	Strongly disagree	4 (% 2,0)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	3 (% 1,5)
	4.	Agree	59 (% 29,2)
	5.	Strongly agree	136 (% 67,3)
Question 3: Different methods should be applied in practicing the courses.	1.	Strongly disagree	0 (% 0)
	2.	Disagree	4 (% 2,0)
	3.	Neither agree or disagree	0 (% 0)
	4.	Agree	55 (% 27,2)
	5.	Strongly agree	143 (% 70,8)
Question 4: I took advantage of 3-D glasses.	1.	Strongly disagree	0 (% 0)
	2.	Disagree	7 (% 3,5)
	3.	Neither agree or disagree	25 (% 12,4)
	4.	Agree	72 (% 35,6)
	5.	Strongly agree	98 (% 48,5)
Question 5: I took advantage of computer usage.	1.	Strongly disagree	7 (% 3,5)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	20 (% 9,9)
	4.	Agree	87 (% 43,1)
	5.	Strongly agree	88 (% 43,5)
Question 6: I took advantage of plastinated materials.	1.	Strongly disagree	7 (% 3,5)
	2.	Disagree	7 (% 3,5)
	3.	Neither agree or disagree	35 (% 17,3)
	4.	Agree	64 (% 31,7)
	5.	Strongly agree	89 (% 44,0)
Question 7: I took advantage of microscopic models.	1.	Strongly disagree	0 (% 0)
	2.	Disagree	2 (% 0,9)
	3.	Neither agree or disagree	15 (% 7,4)
	4.	Agree	110 (% 54,6)
	5.	Strongly agree	75 (% 37,1)
Question 9: I took advantage of tractographic images.	1.	Strongly disagree	3 (% 1,5)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	14 (% 6,9)
	4.	Agree	82 (% 40,6)
	5.	Strongly agree	103 (% 51)
Question 8: This training increased my motivation for learning.	1.	Strongly disagree	4 (% 2,0)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	4 (% 2,0)
	4.	Agree	81 (% 40,1)
	5.	Strongly agree	113 (% 55,9)
Question 10: If this training model is implemented, my performance increases.	1.	Strongly disagree	4 (% 2,0)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	29 (% 14,4)
	4.	Agree	71 (% 35,1)
	5.	Strongly agree	98 (% 48,5)
Question 11: I took advantage of this educational model.	1.	Strongly disagree	4 (% 2,0)
	2.	Disagree	0 (% 0)
	3.	Neither agree or disagree	4 (% 2,0)
	4.	Agree	61 (% 30,2)
	5.	Strongly agree	133 (% 65,8)
Question 12: The most useful model is…	1.	3-D images	132 (% 65,3)
	2.	Plastinated materials	42 (% 20,8)
	3.	Microscopic images	3 (% 1,5)
	4.	Tractography images	20 (% 9,9)
	5.	Fixed cadaver	5 (% 2,5)

DISCUSSION

Educational Modalities. With the recent technological advances, the use of a kind of computer software has led to the introduction of neuroanatomy education, which is challenging for students, with computer-based and especially 3D education models. The said models, which are also useful in providing solutions for cadaver supply problems, do not diminish the importance of dissection, which is one of the traditional anatomy education methods and which still constitutes to be the basis for this education. Their use as part of the education further contributes for the student's achievement over the procedures allowing 3D modelling.

The techniques that were applied in the present study were used before in Arnst et. al's combined model, except for the microscopic anatomy. They suggested that the said modalities could be made use of as an educational tool for improving the learning skills (Arnts *et al*., 2014).

In some studies where new 3D education models were compared with traditional methods, like Familiari *et al*. (2013) (260 participants), Macchi *et al*. (2007) (40 students monitored each year for 5 years), Peterson & Mlynarczyk (2016) (56 students), Elizondo-Omaña *et al*. (2004) and Ocak & Topal (2015), it was observed that students' 3D anatomy perceptions, course achievements, retention skills and learning motivations showed increase.

According to the results obtained by applying the method that we developed in our study, the achievement scores of the students showed an increase significantly both with our traditional education model and the new education model that we applied based on the tests after the theoretical course. Besides, a significant increase in student achievement was observed in the scope of the new model. The given results, in line with some studies indicated that the new model as based on a higher amount of visual materials increases the success when compared to the traditional model (Elizondo-Omaña *et al*., 2004; Macchi *et al*., 2007; Familiari *et al*., 2013; Ocak & Topal, 2015; Allen *et al*., 2016; Peterson & Mlynarczyk, 2016).

In our study, the achievement scores of the participants in the pre-test and post-tests were compared separately for each participant (Tables II and III). No such data was found in the studies that have been reviewed.

Spatial (3D) perception of the Neuroanatomical Structures. Described by the fact that there are two different visual points due to the distance between the two eyes, stereoscopic images make it easier to understand 3D perception and spatial relationship between objects. Since the standard cameras have a working principle with a single lens, the images are therefore 2D. It is possible to obtain a 3D photograph of the object by taking another photograph as close as a second eye distance in the horizontal image of the same image and processing these photographs both in colour and image. Studies on the use of these 3D images are available as part of medical education.

According to the results obtained by way of applying the method that was developed in our study for students; the achievement scores of the students showed increase significantly both in our traditional education model and in the new education model when compared with the theoretically explained test after the course. Also, as a result of the new education model that we applied, a significant increase was observed in student achievement when compared to the traditional model. The given results indicate that 3D visualization increases anatomy success in accordance with some studies (Luursema *et al*., 2008; Hilbelink, 2009; Estevez *et al*., 2010).

As a result of our study, just like Goodarzi *et al*. (2017), Faria *et al*. and Kockro *et al*. (2015), that compared the effects of two different visual models on anatomical knowledge, it became evident that both visual models increase the success of both models (de Faria *et al*., 2016). However, in the said studies, there was no statistical difference between the 3D model and the old model, moreover in our study, it was found out that the new model showed increase in the success when compared to the previous one.

Student's Opinions related to Educational Models. In their study, Lujan & DiCarlo (2006) made an evaluation of the opinions of 166 first-year medical students about education models and found out that the majority of the students (64 %) made a preference for multiple education models that included visual, auditory and tactile elements.

According to Estevez *et al*. (2010) study that included 101 students, it was reported that the education with 3D colour-coded model increased the students' achievement more than the 2D education and that the students were willing to get trained through similar models in the future following this training.

The results of the present study indicate that students are satisfied with the implementation of the new educational model in line with the work of Lujan & DiCarlo (2006) and Estevez *et al*. (2010).

Limitations of the study. The present study includes some limitations. The model is not applied to all second-year medical students. Further validation of the framework in larger study groups is needed. If a higher number of cases had been included, safer conclusions could have been obtained.

CONCLUSION

In our study, a combined model of fiber dissection, tractography, microscopic anatomy, and plastination methods was established in basic neuroanatomy education, and this education model was compared to the current education model, on 202 students. Our results can be summarized as follows: after the education that was applied, avarage achievement scores of students have increased significantly when compared to just the theoretical course. After the new education model, the success of the students has increased significantly when compared to the current education model (theoretical and practical education). Divergent techniques can be made use of together in basic neuroanatomy education, and this increases students' success and motivation for learning. The findings that were observed during fiber dissection of the brains are consistent with the studies in the existing literature. Fiber dissection method is an important educational tool in the comprehension of 3D brain anatomy. It is not possible to obtain postmortem tractography images by making use of imaging devices as part of routine clinical procedure. Fixation method, the time between death and imaging, temperature and scanning time are decisive in this regard. Plastination method has been successfully applied to the brain. This method can be used effectively both to reduce the harmful effects of formaldehyde on health and to provide long-term storage of samples. In the scope of the education models, inexpensive but effective methods can be used to obtain increase of the visuality, such as taking 3D photographs. 3D photography is the method with which students are satisfied mostly. After our study, this combined model started to get used in the medical class practical anatomy education in the second year in Hacettepe University, Faculty of Medicine in 2019. In the duration of the Covid-19 pandemic, the given methods are integrated into the distance learning system as a module. There is a need for obtainment of new goals in the future.

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RESUMEN: La educación en anatomía ha reunido una gran cantidad de nuevas modalidades, modificándose el sistema clásico de la práctica del laboratorio y de las clases basadas en conferencias, hacia los módulos combinados. En el ámbito del presente estudio, desarrollamos un modelo educativo tridimensional (3D) nuevo, práctico, rentable y eficiente, que pretendía ser útil para la detección y una mejor comprensión de la educación básica en neuroanatomía. Se tomaron imágenes tractográficas, disección de fibras, anatomía microscópica y técnicas de plastinación en los cerebros. Después de convertir las fotografías que se tomaron en imágenes 3D, se plastinaron los especímenes. A modo de establecer un modelo educativo en su conjunto, lo aplicamos a 202 estudiantes de segundo año de medicina. Los estudiantes fueron separados en dos grupos cuando asistieron a la clase teórica. El Grupo 1 tomó la educación clásica de laboratorio; por su parte, el Grupo 2 recibió el nuevo modelo educativo diseñado para el estudio. Se introdujeron pruebas previas y posteriores a cada grupo, antes y después de las sesiones de laboratorio. Se compararon las puntuaciones. Los puntajes promedio de rendimiento de cada grupo mostraron un aumento significativo ($p<0,05$) después de las sesiones de laboratorio. Además, se obtuvo un aumento en los resultados positivos, posteriores a la prueba del Grupo 2, siendo estadísticamente significativo ($p<0,05$). En consecuencia, este modelo educativo, enriquecido por métodos unificados de nuevo diseño, podría considerarse útil para captar y mejorar los conocimientos básicos de neuroanatomía.

PALABRAS CLAVE: Disección de fibras; Educación médica; Neuroanatomía; Plastinación; Tractografía.

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