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HOW WE TEACH | *Generalizable Education Research*

The effectiveness of TBL with real patients in neurology education in terms of knowledge retention, in-class engagement, and learner reactions

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Alimoglu MK, Yardım S, Uysal H. The effectiveness of TBL with real patients in neurology education in terms of knowledge retention, in-class engagement, and learner reactions. *Adv Physiol Educ* 41: 38–43, 2017; doi:10.1152/advan.00130.2016.—In our medical school, we changed from a lecture-based method to a team-based learning (TBL) method to teach "polyneuropathies" in the neurology clerkship starting from the 2014 to 2015 academic year. Real patients were used instead of written scenarios in TBL sessions. This study aimed to compare former lecture-based and the current TBL methods in terms of knowledge retention, in-class learner engagement, and learner reactions. First, we determined in-class engagement and satisfaction of the students for the lectures given in the 2013–2014 academic year. The following year, besides the same criteria, we also determined individual (IRAT) and group readiness test (GRAT) scores in the TBL group. End-of-clerkship exam scores for both groups were recorded. Additionally, opinions of patients about their experiences throughout the TBL process were determined. One year later (2015 for lecture and 2016 for TBL), both groups sat for an MCQ test to determine their knowledge retention levels. We found no difference between groups regarding end-of-clerkship exam scores. The mean knowledge retention test score of the TBL group was significantly higher than that of the lecture group (5.85 ± 1.74 vs. 3.28 ± 1.70). The differences between IRAT, GRAT, and retention test scores in the TBL group were significant. The mean student satisfaction score on a five-point scale was 3.01 ± 0.9 (median = 3) in the lecture group and 4.11 ± 1.1 (median = 4) in the TBL group. Our results seem encouraging for use of TBL performed with real patients in neurology education to achieve better long-term knowledge retention and higher in-class engagement and student satisfaction.

team-based learning

WITH INCREASING ENROLMENT in health professions programs, there is a growing interest in active learning strategies due to the belief that active learning results in enhanced knowledge retention and skills application (7).

Team-based learning (TBL) attempts to balance the issues of active learning and faculty teaching time. By breaking up a large lecture hall of 100 or more students into small groups, TBL gets students actively learning while requiring only one faculty to facilitate (14, 26).

TBL was developed by Dr. Larry Michaelsen in a business curriculum in the 1970s. The first reported implementation of TBL in health professions education was at the Baylor College of Medicine in 2002 (8). Within 1 yr, 10 medical institutions in the US piloted TBL (26). Currently, TBL is being used at schools of medicine, nursing, dentistry, pharmacy, residency programs, and continuing medical education (9).

Dr. Michaelsen refined the process of TBL over the years to characterize it with three main phases: *1*) advanced preparation by the students, *2*) individual and group readiness assurance, and *3*) application, including team assignments, discussion, and feedback (9). A meaningful peer evaluation process is an additional cornerstone of this learner-centered educational strategy (11). Some other modified models using only a part of these phases also exist (10, 29).

For the preparation phase, some learning resources such as written texts and electronic or web-based material are delivered or suggested to students. A sufficient time period is left for students' self- study. When the large group comes together in the classroom, the class starts with an individual readiness assurance test (IRAT). In this test, the students answer the questions individually. Then, the large group is divided into teams, including five to seven students on each team to perform group readiness assurance tests (GRAT). This time, each team answers the same questions used in IRAT by discussing and sharing opinions. The next process is appeals from the teams and explanations by the instructor about the test content. In the application phase, application exercises that build on the readiness materials are used to encourage students to engage the content at a deeper, more meaningful level. These exercises help students achieve the learning objectives through the careful evaluation of problems or cases that require critical thinking and investigation to solve. Effective application exercises for team-based learning generally follow the "4S" rules. First, application exercises should be designed around problems that are "significant" to the students. When students are able to attach relevance and value to a problem, it becomes significant and meaningful to them and leads to deeper learning. Second, teams should be working on the "same" problem. This allows for discussion among teams following the completion of the exercise. Third, teams should be required to make and defend a "specific" choice. This action helps teams develop consensus-building and critical thinking skills. Finally, teams should "simultaneously" report their choices to the class. This action promotes team accountability and motivates teams to defend their answers. This also eliminates the phenomenon with sequential teams answering where the first team's answer has a potent effect on subsequent answers. Team presentations, discussion by the large group, and feedback from the instructor

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are beneficial to learners for deeper learning. The application phase may be repeated with different assignments for the teams using different problems to achieve learning objectives. At the end of the session(s), each team member evaluates the other students in the team focusing on group dynamics such as contribution of others to team performance, communication and collaboration skills, etc. (9, 17, 19).

In the literature, effectiveness of TBL has generally been evaluated regarding learning outcomes such as improvement in knowledge levels in terms of test scores, knowledge retention, and learner reactions. The results of contribution of TBL to learning outcomes are mixed (4, 5).

Neurology is rated as one of the most difficult clinical fields not only by medical students, but also by physicians specialized in other areas, which leads to a high degree of subjective uncertainty in terms of neurological issues (6, 24, 25, 30, 31). Jozefowicz (12) used the term "neurophobia" to define the fear of neurology among medical students. The most common reasons behind this phenomenon were students' inability to apply their knowledge of basic sciences to clinical situations and poor quality of teaching neurological content (25, 30). Employing active learning methods has been recommended to cope with "neurophobia" among learners (15, 16). A limited number of reports have been published in literature about use of TBL as an active learning method for teaching neurology in undergraduate medical education (2, 3, 27). There is also one publication on TBL use among rehabilitation students to teach neurology (11). These studies compared the TBL approach with passive learning and measured effectiveness of TBL regarding student achievement scores in exams and student satisfaction levels. One study additionally evaluated self-reported student engagement (27). We have not found any article about effectiveness of neurology education using TBL on long-term knowledge retention.

Integration of theoretical knowledge with clinical practice on real patients enhances the skills such as history taking, physical examination, communication, clinical decision making, differential diagnosis, or patient management (20–23). We hypothesized that such an integration with a student-centered learning approach may also enhance long-term knowledge retention. To test this hypothesis, we changed a 4-h lecture-based neurology class to TBL (as a student-centered method), using real patients instead of written scenarios for team assignments (to build better integration of theory and practice), and designed a prospective study. The aim of this study was to compare the former lecture-based class and the current TBL method in terms of knowledge retention, in-class learner engagement, and learner reactions.

METHODS

Setting

The curriculum in the first 3 yr of Akdeniz University Faculty of Medicine is composed of thematic blocks structured on the basis of organ systems. Clinical clerkships are taken up in the 4th and 5th years. The 6th year is an internship period. The neurology clerkship is included in the 5th-year curriculum and repeated six times a year with different student groups, each of which lasts 3 wk. Theoretical knowledge and practical skills of the students are assessed at the end of the clerkship period. A half-day is allocated for the topic of "Polyneuropathies". Lecture had been used as the teaching method in the past. We changed this method to team-based learning in the 2014–2015 academic year. We applied a modified TBL design using a similar but different problem for each team and omitting the peer evaluation phase. The TBL process was explained, and a relevant text was delivered to each student at the beginning of the clerkship (~10 days before "polyneuropathies" class) for individual preparation. In the IRAT and GRAT, we used 10 multiple-choice questions (MCQs) which were extremely challenging to start discussion. One MCQ intentionally included two correct answers to encourage appeals from the teams. At the suggestion of Student Assessment Committee, IRAT/GRAT results were not taken into account while calculating students' final achievement scores for this clerkship. This information was declared to the students at the beginning of the clerkship while the TBL process was introduced. Teams were created by the instructor subsequent to the IRAT according to the seating arrangement in the classroom formed by the students randomly. Generally, three to four students from the front rows and three to four students from the back rows were selected to build a team. Depending on number of available patients and students, we created five to six teams, including five to seven students in each session. We discussed each MCQ with the groups and explained the correct answers with the reasons behind. Then each team was assigned to a volunteer patient with polyneuropathy that had already been invited to the learning environment by the instructor. Among 236 registered patients with a definite diagnosis of polyneuropathy periodically followed up at neuromuscular diseases polyclinics, those considered as suitable to tell their medical history were offered to participate in the study. The ones with the most frequently seen type of polyneuropathy (diabetic polyneuropathy) or those with rarely seen diseases such as hereditary polyneuropathy, cured acute inflammatory polyneuropathy, and chronic inflammatory polyneuropathy but showing characteristic medical history and neurological examination findings were especially preferred. A total of 26 patients had agreed to participate in the study, and five to six of them were invited in each instruction cycle. Assignments of all teams on different patients were the same and included history taking and determining the data relevant to polyneuropathy in the patient's history, performing a complete physical/neurological examination to determine polyneuropathy-related findings, and finally, creating an initial diagnosis list with explanations. Each team prepared a written report, including their findings and explanations, and delivered it to the instructor simultaneously. The last step was the team presentations. Each team presented the data of their patients and explained possible diagnoses to the whole class. The class asked questions and discussed the points they agreed or disagreed with. At the end of each team presentation, the instructor briefly clarified the case and provided some theoretical information if needed. The peer evaluation phase was omitted, because a half-day module was too short for students to observe group dynamics. Feedback from the students was obtained at the end of the session using a written form. The remaining theoretical classes in the neurology clerkship were given using lecture as the teaching method.

Study Design

This was a prospective controlled followup study. First, we determined in-class engagement and satisfaction scores of the students participating in the "polyneuropathies" lectures given in the 2013–2014 academic year. In the following academic year, besides the same criteria, we also determined IRAT and GRAT scores in the TBL (intervention) group. Grade point averages of the lecture and TBL groups for the past 4 yr of medical education and scores attained by the students at the end-of-clerkship theoretical exam, which was made 4 days later than the class in both groups, were recorded as well. Additionally, we asked for opinions of patients about their experiences with the students throughout the TBL process. During the 1st wk of April in following year (2015 for lecture group and 2016 for TBL group), both lecture and TBL groups sat for a test (followup exam) to determine their knowledge retention levels. The students

were not previously informed about the retention exam to avoid any preparation for the test. The test included 10 MCQs about the polyneuropathies that were not identical to those used in the IRAT/GRAT.

Participants and Ethical Issues

One-hundred seventy-nine students participated in the lectures during six cycles in the 2013–2014 academic year. In the 2014–2015 academic year, a total of 194 students participated in the TBL sessions. The number of the students completing feedback forms to express their satisfaction with the classes was 161 in the lecture group and 179 in the TBL group. Feedback forms were anonymous to prevent any possible negative effect on the teacher-student relationship, since one author was also the teacher and assessor of the participants.

IRAT, GRAT, and end-of-clerkship theoretical exam scores of all participants were available. Seventy-eight percent of the control group ($n = 141$) and 81% of the TBL group ($n = 158$) were accessed for followup exams performed in the next academic year. Mean duration between lecture/TBL session and retention test was 12.2 (10–18) mo.

All of the students participating in the classes in both groups were randomly observed by independent observer pairs using an observation tool to determine their engagement levels with the classes.

Approval for the study was granted from Akdeniz University Board of Ethics on Noninvasive Clinical Human Studies.

Instruments

In-class engagement measure. This is a written form for observing and recording behaviors of the instructor and four randomly selected students as snapshots for 5-min cycles in classes. The in-class engagement measure (IEM) was created on the basis of a previously developed observation tool called STROBE (18) and validated in a study conducted among Turkish medical students (1). Instructor and student behaviors were scored 1 to 5 on this tool. The IEM scores were parallel to the degree of behavior's contribution to active student engagement, so higher scores for student and instructor behaviors were associated with more in-class learner engagement. Additionally, the number of questions asked by the instructor and students was recorded. A sample of the IEM is provided in online supplementary materials (1).

Observation process. The observers were trained about observation procedure, description of observable behaviors, how to take a position in the learning environment with different groups, and how to select individuals to observe. A total of five observer pairs observed and recorded the instructor and student behaviors in different classes. The observation unit was a 5-min cycle. The cycle proceeds as follows: First, the observer writes the starting time of the cycle and information about the class (title, instructor's name, and number of students). Next, the observer selects a student from the class and observes the selected learner for 20 s, marking the type of engagement the learner exhibits. This is performed four times with different students in succession. The observer also observes the instructor and marks the instructor's behavior. Then, for the remainder of the STROBE cycle, the observer tallies the number of questions asked by all students, not only the observed ones, and the instructor to have an idea about learner-to-learner and learner-to-teacher interaction level that can be an indicator to show the degree of in-class learner engagement.

Observers independently selected the students and observed and marked their behaviors separately. Generally, the classroom was divided into two, and observers selected the students from their own section. They were asked not to observe the same student repeatedly if possible.

Numbers of the observations performed in the lecture and TBL groups were 234 and 242, respectively.

Feedback Forms

Lecture feedback form. We employed the standard feedback forms used in all clinical clerkships to determine student satisfaction levels about lectures in terms of lecturer performance. On this form, which was delivered at the end of the clerkship, the students are supposed to read statements and give a score for each on a five-item Likert-type scale between 1 (absolutely do not agree) and 5 (absolutely agree). An open-ended part for comments exists at the bottom of the form (Table 1).

TBL feedback form. Since TBL is a new methodology for the students, we used an extensive feedback form created by the authors to have more detailed feedback from students. The form is composed of five parts: *1*) organization, infrastructure, and resources (3 statements); *2*) preparation and readiness (2 statements); *3*) discussion (3 statements); *4*) teacher (3 statements); and *5*) general (6 statements). The students scored each statement on a five-item Likert-type scale between 1 (absolutely not agree) and 5 (absolutely agree). There is also an open-ended part for comments at the bottom of the form (Table 2).

Patient feedback form. We also asked three open-ended and one close-ended written question to the patients to determine their satisfaction with the experience they lived. The open-ended questions were as follows:

1. What do you think about behaviors of the students against you? Do you have any recommendation for them to improve their patient-doctor relation and communication skills?
2. Would you like to live a similar experience again, and why?
3. Did you experience any difficulties about this experience? What would you recommend to overcome such difficulties?

The close-ended item was about overall satisfaction of the patients with this experience, and they gave a score for the statement on a five-item Likert-type scale between 1 (absolutely not satisfied) and 5 (absolutely satisfied).

Data Analyses

We used descriptive statistics to determine mean and median values. The difference between scores of the end-of-clerkship test, retention test, and engagement in two groups was investigated by Student's *t*-test. Repeated-measures ANOVA test was used to explore differences between IRAT, GRAT, and knowledge retention test scores.

P values of <0.05 were set for statistical significance.

RESULTS

Mean age was 24.0 ± 2.9 yr in the lecture group and 24.3 ± 3.2 yr in the TBL group. Male/female ratio was 51:49

Table 1. *Mean satisfaction scores of the lecture group for the instructor's performance*

	Mean	SD
1. Allowed us to be aware of the aim and objectives of his/her lectures and the lecture content met all aims and objectives	3.0	0.7
2. Gave the lectures in a logical order to help us comprehend better.	2.9	0.9
3. Provided/reminded evidence based scientific basis of the topic to build clinical information on it.	3.0	1.0
4. Kept us focused on the subject during whole lecture.	2.4	0.8
5. Created a positive communication environment for us to ask questions without hesitating.	3.3	0.9
6. Started and stopped the lectures on scheduled time	3.7	0.9
7. Was competent on teaching	2.9	1.0
8. Contributed to my learning sufficiently	2.9	0.7
Total	3.01	0.9

Table 2. *Mean satisfaction scores of the TBL group on feedback form*

	Mean	SD
Organization, infrastructure, and resources		
1. Information given at the start of the clerkship about how TBL process runs was sufficient to understand the procedures well.	4.19	0.95
2. Organization of the TBL session (duration, break time, exams, discussion processs, etc.) was good.	3.96	1.08
3. Physical conditions in the learning environment were suitable.	4.03	1.16
Total	4.06	1.07
Preparation and readiness		
4. Self-study materials provided at the start of the clerkship were comprehensive enough to gain required knowledge.	4.17	0.99
5. Individual/team test content was challenging enough to start discussion.	4.03	1.04
Total	4.10	1.02
Discussion		
6. Team assignments (patients) facilitated learning positively.	4.23	0.95
7. Discussing all possible solutions facilitated the learning.	4.11	0.97
8. This method helped us to show a more systematic and logical approach to the patient.	4.25	0.88
Total	4.20	0.94
Teacher		
9. The teacher helped us to better comprehend the subject by providing feedback, discussion, and explanations.	4.25	0.92
10. The teacher supported our learning as much as was done in lectures.	4.26	0.94
11. The teacher managed whole TBL process successfully.	4.47	0.84
Total	4.33	0.91
General		
12. TBL increased my interest in neurology.	3.67	1.18
13. I understood TBL classes better than other neurology lectures.	3.76	1.14
14. I focused on TBL sessions longer than other neurology classes.	3.87	1.11
15. I participated more actively in the TBL classes than other neurology lectures.	4.15	1.00
16. I think the knowledge I gained in this TBL session will be more permanent than what I gained in lectures.	4.20	0.93
17. Overall, I am satisfied with this TBL session	4.08	0.98
Total	3.94	1.08

in lecture group and 50:50 in TBL group. Grade point averages for the first 4 yr of medical education in the lecture and TBL groups were 73.2 ± 6.6 and 72.7 ± 6.4, respectively. There was no significant difference between them ($P = 0.546$, *t*-test).

There was no difference between groups regarding end-of-clerkship exam scores (79.6 ± 4.4 in the TBL and 78.9 ± 3.9 in lecture group, Student's *t*-test, $P = 0.966$). The mean knowledge retention test score in the TBL group was significantly higher than that of the lecture group (5.85 ± 1.74 vs. 3.28 ± 1.70, Student's *t*-test, $P < 0.001$; Table 3). Mean IRAT and GRAT scores were 4.31 ± 1.67 and 7.42 ± 1.22, respectively. The differences between IRAT, GRAT, and retention test scores in the TBL group were significant (repeated-measures ANOVA, Bonferroni test, $P < 0.001$).

The mean student satisfaction score was 3.01 ± 0.9 (median = 3) in the lecture group and 4.11 ± 1.1 (median = 4) in the TBL group. Details of the student satisfaction data for the lecture and TBL are presented in Tables 1 and 2, respectively.

A total of 33 students provided written comments on TBL. These comments and frequency of each were as follows:

"It was the best class I have ever participated in so far" ($n = 14$).

"TBL must be applied in as many classes as possible" ($n = 19$).

"TBL completely integrates theoretical knowledge and practice and facilitates our learning" ($n = 18$).

"I have actively participated in and focused on the class" ($n = 24$).

"Dealing with a real patient, taking history, performing physical examinations, thinking about polyneuropathy-related findings and diagnosis, and discussion on different cases were all beneficial to us" ($n = 30$).

"The instructor built a warm communication and learning environment. This should be inspiring for other instructors" ($n = 17$).

"If a checklist was provided for us to follow while taking history and performing physical examinations, we would feel much more comfortable" ($n = 5$).

"The individual test at the beginning was not helpful for me since I had not read the text, which was too complicated for me to understand" ($n = 4$).

"It would be better to prepare a more simple text, including basic elements of this disease group" ($n = 4$).

"I can learn best by listening to the instructor, so TBL and other student-centered approaches are not the right methods for me. However, I am glad to deal with real patients" ($n = 2$).

"The learning environment was not comfortable enough to deal with patients" ($n = 2$).

The patients were highly satisfied with this experience (mean satisfaction score was 5 over 5), and all of them, without exception, expressed that they would participate in following sessions if needed.

Mean in-class learner engagement scores for the instructor and students in the TBL group were significantly higher than those of the lecture group (4.54 ± 0.65 vs. 1.33 ± 0.67 for the instructor and 4.33 ± 0.91 vs. 2.50 ± 0.88 for the students, $P < 0.001$ for all, Student's *t*-test) The number of questions asked by the instructor and students in the TBL class was higher than that asked in the lecture (3.66 ± 1.50 vs. 2.45 ± 1.86 for the instructor and 3.27 ± 0.45 vs.1.33 ± 0.66 for students, Student's *t*-test, $P < 0.001$ for all; Table 3).

DISCUSSION

This study aimed to reveal the effectiveness of a 4-h TBL activity in a neurology clerkship in terms of knowledge retention, in-class learner engagement, and learner reactions. Com-

Table 3. *End-of-clerkship exam and retention test scores and in-class engagement in TBL and lecture groups*

	TBL	Lecture	*P* Value*
End-of-clerkship exam†	79.6 ± 4.4	78.9 ± 3.9	0.966
Retention test‡	5.85 ± 1.74	3.28 ± 1.70	<0.001
In-class engagement			
Observation scores			
Instructor§	4.54 ± 0.65	1.33 ± 0.67	<0.001
Student§	4.33 ± 0.91	2.50 ± 0.88	<0.001
No. of questions			
Instructor	3.66 ± 1.50	2.45 ± 1.86	<0.001
Student	3.27 ± 0.45	1.33 ± 0.66	<0.001

*Student's *t*-test; †scores > 100; ‡scores > 10; §scores > 5.

pared with lectures on the same topic, we found higher knowledge retention and more in-class learner engagement with high satisfaction in the TBL group.

To our knowledge, this is the first study where the application phase of the TBL was performed with real patients instead of written patient scenarios. This has been the most attractive part of the method for our students for 4 h, which were actually allocated for theoretical classes within the curriculum. Even those who expressed opinions against TBL methodology accepted that studying with real patients had been an excellent experience and very helpful for their learning. Although it was hard and labor-intensive organization, our advantage was having a large patient pool and good physician-patient relations. The TBL day was also arranged as a control date for these chronic patients, and they all were seen by the instructor after the session. However, we acknowledge that organizing such a TBL session with real patients may be seen as highly challenging by others and prevent generalizability of this approach. On the other hand, it may be suggested that medical schools create a patient pool with frequently seen chronic disorders for the sake of such educational purposes. High satisfaction of our patients participating in TBL sessions increases our hope with feasibility of such an arrangement.

The effect of TBL on knowledge retention has already been investigated in studies conducted on fields other than neurology. In a study conducted at Washington University, the student cohort taking a preclinical pediatrics course in a TBL-plus-lecture curriculum was compared with those who received the traditional curriculum the year prior. For both groups, knowledge was assessed at four time points spanning two years. They found significant knowledge gains over the short term for the TBL group, but these gains disappeared in the long term (29). Another study compared psychiatry educators taking a 1-day conference with TBL or lectures. Their knowledge was assessed by immediate posttest and 2-mo remote posttest. The TBL group performed better on the immediate posttest, but there were no differences between groups 2 mo later (28). In contrast, we found no difference between groups at the end-of-clerkship exam performed 4 days later (short term), but we found a difference in favor of the TBL group in knowledge of a retention test performed approximately 1 yr later. The reason may be explained by opportunity for students to prepare for the exams. All students regardless of teaching methodology prepare for the end-of-clerkship exam, the date of which was announced previously. So, it is difficult to find any difference since those who took lectures also self-studied as TBL students did. However, if the exam date was not announced previously and the students sat through the test without any preparation, a difference could have appeared between groups. However, we accept the fact that there may be some confounding factors effective on knowledge retention of the students in both groups. The followup exam was performed almost 1 yr later than the end of the clerkship. In this period, some students might have seen more polyneuropathy patients than others, or some may have been more interested in neurology and personally read more. It is also possible for some students to have selected neurology as an elective clerkship in the internship period.

We found only one report in the literature investigating in-class learner engagement in TBL using an observation tool. Kelly et al. (13) compared lecture, problem-based learning and TBL regarding learner engagement and found that TBL was superior to lecture. Their findings were similar to ours. Our students' satisfaction with TBL seems high, although we could not statistically compare the student satisfaction in TBL and lecture groups since different tools were used to collect data. Only a few students were not satisfied with TBL activities in the classroom, which was due possibly to variety in their learning style preferences. In a study implementing TBL in a supplemental neurology program, the majority of TBL participants stated that TBL had further increased their interest in the subject of neurology (3). In our study, we found a medium mean value (3.68 ± 1.18) for this statement. The reason not to obtain a higher value might be the relatively more complex nature of "polyneuropathies" compared with other topics of neurology. TBL with other neurology classes may further increase the interest of students in the field or at least help them to cope with "neurophobia" better.

In conclusion, we found promising results in favor of the TBL approach performed with real patients in terms of long-term knowledge retention, in-class engagement, and learner reactions. Our study was conducted in only one medical school and on just one topic of the neurology clerkship held in a half-day session. This is the main limitation of this study for generalizability of the results. Another limitation is lack of information about students for the period between the end of clerkship and the knowledge retention test day. Larger studies with larger populations in various medical schools with different curricula are needed to have more reliable data and opinion on the general effectiveness of TBL with real patients. We recommend that researchers create other TBL modules for other neurological conditions to see the effect of this approach on knowledge retention or neurophobia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.K.A., S.Y., and H.U. analyzed data; M.K.A. and S.Y. prepared figures; M.K.A. drafted manuscript; M.K.A., S.Y., and H.U. approved final version of manuscript; S.Y. performed experiments; H.U. interpreted results of experiments; H.U. edited and revised manuscript.

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