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ARTICLE

Low-Cost Classroom and Laboratory Exercises for Investigating Both Wave and Event-Related Electroencephalogram Potentials

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Electroencephalography (EEG) has given rise to a myriad of new discoveries over the last 90 years. EEG is a non-invasive technique that has revealed insights into the spatial and temporal processing of brain activity over many neuroscience disciplines, including sensory, motor, sleep, and memory formation. Most undergraduate students, however, lack laboratory access to EEG recording equipment or the skills to perform an experiment independently. Here, we provide easy-to-follow instructions to measure both wave and event-related EEG potentials using a portable, low-cost amplifier (Backyard Brains, Ann Arbor, MI) that connects to smartphones and PCs, independent of their operating system. Using open-source software (SpikeRecorder) and analysis tools (Python, Google Colaboratory), we demonstrate tractable and robust laboratory exercises for students to gain insights into the scientific method and discover multidisciplinary neuroscience research. We developed 2 laboratory exercises and ran them on participants within our research lab (N = 17, development group). In our first protocol, we analyzed power differences in the alpha band (8-13 Hz) when participants alternated between eyes open and eyes closed states (n = 137 transitions). We could robustly see an increase of over 50% in 59 (43%) of our sessions, suggesting this would make a reliable introductory experiment. Next, we describe an exercise that uses a SpikerBox to evoke an event-related potential (ERP) during an auditory oddball task. This experiment measures the average EEG potential elicited during an auditory presentation of either a highly predictable ("standard") or low-probability ("oddball") tone. Across all sessions in the development group (n=81), we found that 64% (n=52) showed a significant peak in the standard response window for P300 with an average peak latency of 442ms. Finally, we tested the auditory oddball task in a university classroom setting. In 66% of the sessions (n=30), a clear P300 was shown, and these signals were significantly above chance when compared to a Monte Carlo simulation. These laboratory exercises cover the two methods of analysis (frequency power and ERP), which are routinely used in neurology diagnostics, brain-machine interfaces, and neurofeedback therapy. Arming students with these methods and analysis techniques will enable them to investigate this laboratory exercise's variants or test their own hypotheses.

Keywords: Electroencephalography (EEG); cognitive neuroscience; neural oscillations; event-related potentials (ERPs); low-cost; open-source; P300; education: alpha waves.

The German psychiatrist Hans Berger (1873-1941) was determined to discover how he was able to use "spontaneous telepathy" to convey information about his frightful military horse-riding incident to his sister, prompting her to telegram him from kilometers away (Stone and Hughes, 2013). While he failed to determine a telepathic medium, he made a historical breakthrough by non-invasively discovering voltages arising from the brain in the form of an electroencephalogram (EEG). Ever since Berger's first recordings in 1929, the electroencephalogram has continued to be a rich source of information for investigations into the brain, including signals that predict the onset of atypical states. Electroencephalograms are indispensable in the clinical setting to diagnose epilepsy (Rajendra *et al.*, 2015), sleep disorders (Tan *et al.*, 2012), depth of anesthesia (Marchant *et al.*, 2014), and brain death (Cavinato *et al.*, 2009); in a neural engineering setting for brain-machine interfaces such as assistance spellers (Guy *et al.*, 2018) or cursor control (Wolpaw and McFarland, 1994); and in neuroscience research for motor planning (Libet *et al.*, 1983), visual decoding (Bötzel and Grüsser, 1989), and attentional processing (Herrman and Knight, 2001; Klimesch, 2012; Picton, 1992; Polich and Herbst, 2000). EEGs have been used to detect concealed information with surprising accuracy (Rosenfeld, 2019). The US legal system has shown interest in how this technology, specifically qualitative EEGs, can influence legal proceedings (Jones et al., 2013). The clinical and research importance of the EEG makes it imperative that this technology be made available to undergraduates in a neuroscience laboratory. This report describes instructional laboratory exercises using open-source, low-cost EEG hardware as a teaching tool for introducing basic neuroscience principles in the classroom. For a review of EEG options in the classroom, see Hatton *et al.* (2023).

The EEG signal relays information about underlying neural processes in low-voltage oscillations or rhythms recorded electrically from the scalp. This oscillatory activity summates activity across large populations of neurons and provides an excellent lab exercise to introduce students to EEG neurophysiology. The alpha wave (as first described by Hans Berger) is the dominant oscillation in the brain, occurring during a resting state at a frequency of 8-13 Hz (Arnal and Giraud, 2012; Herrmann and Knight, 2001). Alpha waves can be induced during tasks that cause a state of "cortical idling," in which the visual cortex receives little or no input (Basar *et al.*, 1997). One popular method for evoking alpha waves is to alternate between eyes-open and eyes-closed states (Woodman, 2010), which is not a location (although strongest above visual cortex) or material-dependent activity. Using this task as an initial exercise teaches students the "how" of conducting a scientific experiment, collecting data, and interpreting results while removing the need for supplementary materials.

While the spatial resolution is relatively poor, temporal information can be extracted from the EEG by averaging over repeated time-locked events, identified as Event Related Potentials (ERPs). ERPs provide an opportunity to measure responses from neural populations. ERPs can describe more complex stimulus responses, as they are elicited from neuronal populations in response to sensory, cognitive, or motor events (Luck, 2005). One well documented ERP is the P300, a positive potential evoked from the parietal lobe roughly 300 milliseconds following the onset of a novel stimulus. The P300 is commonly evoked in an attentional activity called the Oddball Task, in which a participant is exposed to a series of repeating stimuli with a deviant novel stimulus, or "oddball stimulus," interjected at a low probability (Picton 1992; Polich and Herbst, 2000; Li *et al.*, 2019). The Oddball Task can take many forms, including auditory or visual discrimination tasks or relevant stimulus recognition in different sensory modalities (Bennington and Polich, 1999; Fischer et al., 2008; Krigolson et al., 2017; Herrmann and Knight, 2001). The most prominent is the auditory oddball task, which has been found to reliably predict recovery of consciousness in vegetative or minimally conscious patients (Cavinato *et al.*, 2009; Fischer *et al.*, 2008). Due to its ease in task construction and real-world application, the Oddball Task is a useful classroom exercise for introducing students to the concept of ERPs.

Success in teaching complex topics like neurophysiology can be improved by providing students with hands-on learning experiences (Freeman *et al.*, 2014; Gage, 2019; Oliver-Hoyo et al., 2004; Segawa, 2019). Evidence has demonstrated the utility of hands-on learning in improving students' procedural understanding and enabling improved knowledge transfer between disciplines (Michael, 2006). Additionally, experiments that tackle questions of consciousness and translate to real-world circumstances engage to students. Demonstrating neuroscientific investigational techniques in the classroom, however, is challenged by the high cost and limited access to necessary equipment; low-cost EEGs cost at least $400 USD and have limited flexibility and interpretability (Ledwidge *et al.*, 2018; Shields *et al.*, 2016). Historically, the equipment used to study neurophysiology has been limited to professional researchers for these reasons. Therefore, there is a need for accessible technology for teaching neuroscience in the classroom.

Here, we report on a low-cost, simple-to-use EEG for teaching about brain waves and ERPs in a neuroscience laboratory and classroom setting. Nine of 14 laboratory participants showed alpha waves, and 13 of 17 showed statistically significant P300s using the Heart and Brain SpikerBox. In the classroom, 20 out of 30 participants showed a clear P300 signal. By participating in these experiments, students understood how a scientific study was conducted, including variability between participants and trials, and how brain signals were analyzed statistically. Numerous different classroom experiments could be designed to induce alpha waves, ERPs (such as P300), test variables that influence the parameters of the signal, and provide hands-on experience in the design, data collection, and analysis of neurophysiological recordings. Once students gain experience conducting an experiment and collecting and interpreting data, they have the tools to ask and answer questions independently.

MATERIALS AND METHODS

Laboratory Alpha Wave and P300 Data Collection

Experiments were conducted using components of the Backyard Brains Heart and Brain SpikerBox ($190 USD). SpikerBoxes with filter settings of 1-129 or 0.6-105 Hz were used. For each experiment, the SpikerBox was connected to the computer via a USB cable (See Figure 1A for schematic diagram), and the electrodes in the headband were connected to recording hardware via an electrode cable. For the P300 experiment, we developed custom code for the Arduino on the Heart and Brain SpikerBox. This code produced a train of short (300ms) tones separated by 2 seconds. Two tones were delivered, a low-pitch "Standard" tone (300Hz) at 90% probability, and a high pitched "Oddball" tone (500Hz) at 10% probability. Each tone was associated with a unique time-stamped event marker that allowed the EEG signal to be synchronized to the tone onset. The open-source software package SpikeRecorder (Backyard Brains, Ann Arbor, MI) was used to visualize and analyze the signal in real-time to identify any noise or signal quality issues.

Classroom P300 Data Collection

The Human SpikerBox ($300, Backyard Brains, Ann Arbor, MI) was used to collect data at Minnesota State University, Mankato (Mankato, MN) as it can run the P300 protocol without modifications (Figure 1B). The filter settings for EEG were set to 0-50Hz in SpikeRecorder to reduce high-frequency noise in an active classroom setting. Upon the initiation of the experiment in the SpikeRecorder software, the train of tones began with synchronized event markers.

Laboratory Alpha Wave Acquisition

Participants placed two stainless steel electrodes embedded in a custom EEG headband at locations O1 and O2 (Figure 2b) according to the International 10–20 electrode system (Klem et al., 1999). The participant's hair

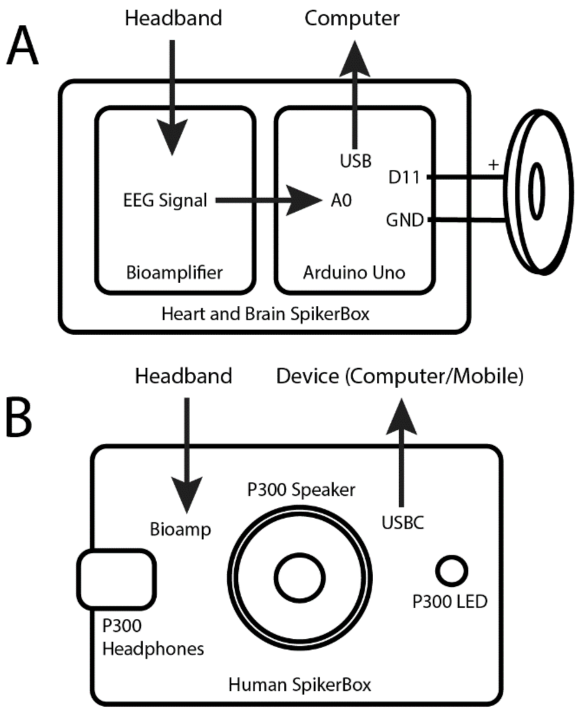

Figure 1. Schematics of the SpikerBoxes used in the laboratory and classroom experiments. *A.* Schematic of the Heart and Brain SpikerBox including the addition of external speaker (102-1554-ND from DigiKey, Thief River Falls, MN) connected via header connector piece (SAM1092-01-ND from DigiKey, Thief River Falls, MN) at D11 and Gnd pins. B. Schematic of the Human SpikerBox, which includes a built-in speaker and headphone jack for delivering P300 audio stimuli (High vs. Low tones), and a P300 LED for delivering visual stimuli (Green vs. Red).

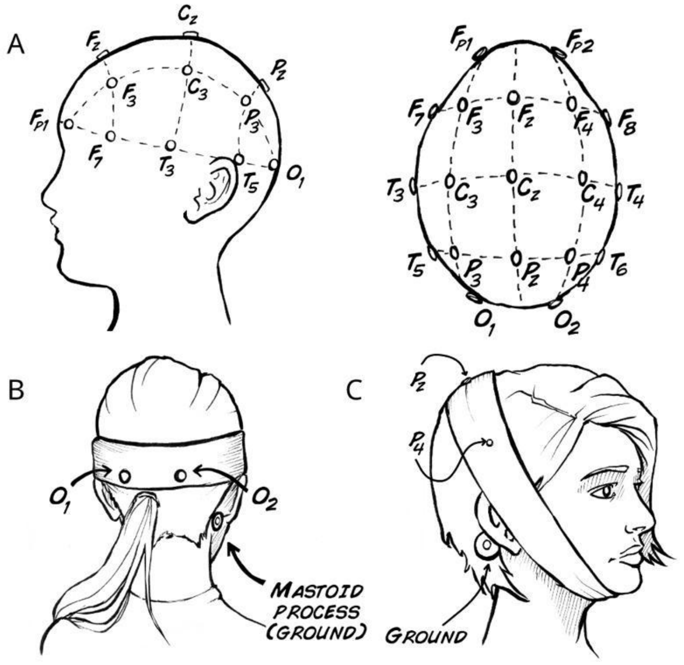

Figure 2. Electrode configuration for classroom and laboratory exercises. *A.* Standard electrode placement locations according to the 10-20 electrode system. *B.* For the alpha wave experiments, electrodes were placed at locations O1 and O2, using an adhesive electrode at the mastoid process as ground. *C.* Electrodes placed at locations Pz and P4 for the Laboratory Oddball Task, with a ground electrode at the mastoid process. For Classroom exercises, one recording electrode was sacrificed, and instead, an additional mastoid ground was used. We recorded from Pz comparing to the two references, on left and right mastoid processes.

was parted beneath the stainless-steel electrodes, and conductive gel was placed between the electrodes and the scalp to improve signal detection. The red alligator clips of the orange electrode cable were clipped to the stainless-steel electrodes and plugged into the recording hardware. An adhesive electrode serving as a ground was placed on the mastoid process connected to the black alligator clip.

Signal quality was examined and remedied if noisy by parting the hair at the electrode-scalp interface or adding more conductive gel. Once a quality signal was obtained, participants were instructed to alternate between eyes open, and eyes closed states every five seconds while recording in the SpikeRecorder application. Event markers were inserted into the recording to signify each transition by pressing the number keys (1-close, 2-open) on the recording laptop. Participants were requested to minimize movement, especially in the jaw.

Laboratory P300 Acquisition

Participants placed the electrodes attached to the electrode-fitted headband at locations Pz and P4 by donning the headband like a chinstrap (Figure 2c, 3). As done in the alpha wave experiment, the hair was parted beneath the electrodes where conductive gel was placed. For participants with long hair, the hair was parted at the line between electrode locations and tied up. The red alligator clips were connected to each stainless-steel electrode, and the black ground clip was connected to the adhesive electrode at the mastoid process. This electrode cable was connected to the SpikerBox, and the signal was visualized in the SpikeRecorder software. Troubleshooting the signal was done as previously described.

Before starting the oddball task, instructors can incorporate a lesson on verification techniques into the exercise. For students who demonstrate a visible alpha wave during the eyes open/closed task, this can serve as a way to confirm that the equipment is functioning properly and the signal is legitimate. It is important to note, however, that not all individuals will exhibit a strong alpha wave, and its presence is not necessary for obtaining a visible P300 signal. Nonetheless, having students attempt to validate the equipment and expected signal prior to the main experiment provides a valuable teaching opportunity. It demonstrates good laboratory practices and highlights the importance of ensuring the reliability of experimental setup before proceeding with data collection. While this verification step may not be feasible for all students, it offers an additional learning experience when circumstances allow.

Participants were instructed to listen for the oddball tone and keep a tally until 50 were reached, as the P300 signal is expected to reliably appear following 30-40 presented stimuli (Kotchoubey *et al.*, 2005). The recording was

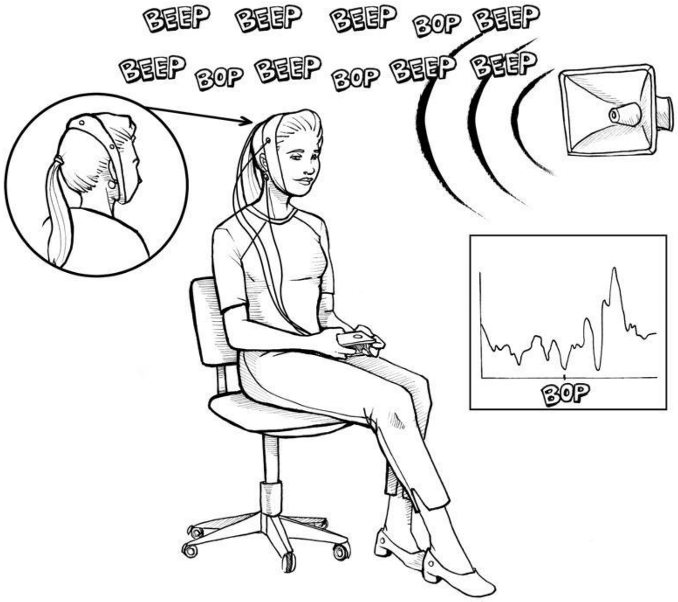

Figure 3. Experimental setup. Depiction of participant holding SpikerBox while listening to the auditory oddball task.

stopped after 50 oddball stimuli were presented. As before, participants were requested to minimize movement during the recording.

Classroom P300 Acquisition

Data was collected from four separate psychology classes at Minnesota State University, Mankato. Participants (n = 30) were shown a short presentation on the basics of the EEG and the P300 signal. All participants wore headphones during the experiment to ensure that tones did not interfere with other participants.

To limit additional signal noise present in the classroom, one electrode lead was switched from P4 and instead connected to a ground electrode pad on the mastoid process. Ground electrode gel pads were placed bilaterally on the mastoid processes behind the ears. The headband was fitted under the participant's chin (Figure 2c). Hair was parted at the location of the electrode, and the conductive gel was placed between the electrode and the scalp. We placed electrodes at approximately Pz according to the 10–20 system (Klem *et al*., 1999). The Human SpikerBox was connected to a laptop via a USB-C cord. One alligator clip was attached to the stainless-steel electrode located at Pz. The black alligator clip, common ground, was attached to the adhesive gel pad behind the right ear, and one red alligator clip was connected to the left mastoid adhesive gel pad. Once alligator clips were connected to their corresponding electrodes, the cable was connected to the SpikerBox.

In SpikeRecorder, the frequency filter was set from 0Hz to 50Hz while the notch filter was set at 60Hz. We tested oculomotor responses to test the strength of the signal. If excessive noise was present, the measures mentioned previously were used. The upper frequency was reduced to limit excessive classroom noise; any movement near the device increased high-frequency oscillations.

The built-in P300 protocol was started by pressing the "Start" button. A second button on the device was pressed once to switch between light (2 colors of an LED) and sound (2 tones) stimuli. Auditory tones began to play and occurred every 2 seconds until the "Stop" button was pressed a subsequent time.

Due to limited class time, participants were asked to tally 25 presentations of the Oddball tone. This also reduced the chances of noise, distraction, and participant fatigue. The total experiment time per participant averaged 10 minutes.

Laboratory Alpha Wave Analysis

The presence of an alpha wave was confirmed using the SpikeRecorder spectrogram (Figure 4a). A band of activity between 8-13 Hz was expected to appear after the eyes closed and disappear after the eyes were opened. This transition from eyes open to eyes closed served as the event (n=137) on which our analyses were conducted. Recordings were analyzed post-hoc using Python in the Google Colaboratory platform. The signal was high-pass filtered at 3 Hz using a 2nd order Butterworth filter. Three seconds on either side of the eyes-closed event (n=137) were analyzed, where one second on either side of the event was omitted to eliminate inter-participant variability in response time. A fast-Fourier transformation was taken for the signals representing each condition, and the resulting FFT was smoothed over seven points using a Savitzky-Golay filter. Obvious artifacts were removed by hand (n=11).

Laboratory P300 Analysis

The P300 is a small ERP, and the signal should be averaged over multiple events to be accurately visualized. Averaging across event responses reduces spontaneous "noise" in the recording. In the Spike Recorder software, when set to thresholding mode, Spike Recorder can use the event marker for the oddball tone (event 2) and average the signal in real time when the event is triggered. This was done separately for the standard and oddball tones, which resulted in a visible P300. To boost excitement for students (n=11), this analysis can be performed during the experiment so that students can visualize a neural response by the time the experiment has finished (Figure 4a).

The data files exported to the hard drive by SpikeRecorder software were analyzed post-hoc using Python in the Google Colaboratory platform. EEG signals in a window of 950 ms surrounding tone onset were analyzed. The grand average of the probe tones was compared to the grand average of the standard tones. Each average was compared to a 95% confidence interval, which considered the noise of the overall EEG recording. If the P300 exceeded the 95% confidence interval and average of the standard tones, then it was deemed a significant response (Figure 4b, bottom). P300 peak responses were calculated during a window of -250ms to 700ms centered on stimulus presentation and by smoothing mean responses with a boxcar filter (length 10 samples). Then, we identified the maximum peak after the stimulus presentation. We only selected peaks for evaluation that occurred after tone presentation and were significantly different from the standard tone and when the P300 peak fell outside the 95% confidence interval for the standard tones.

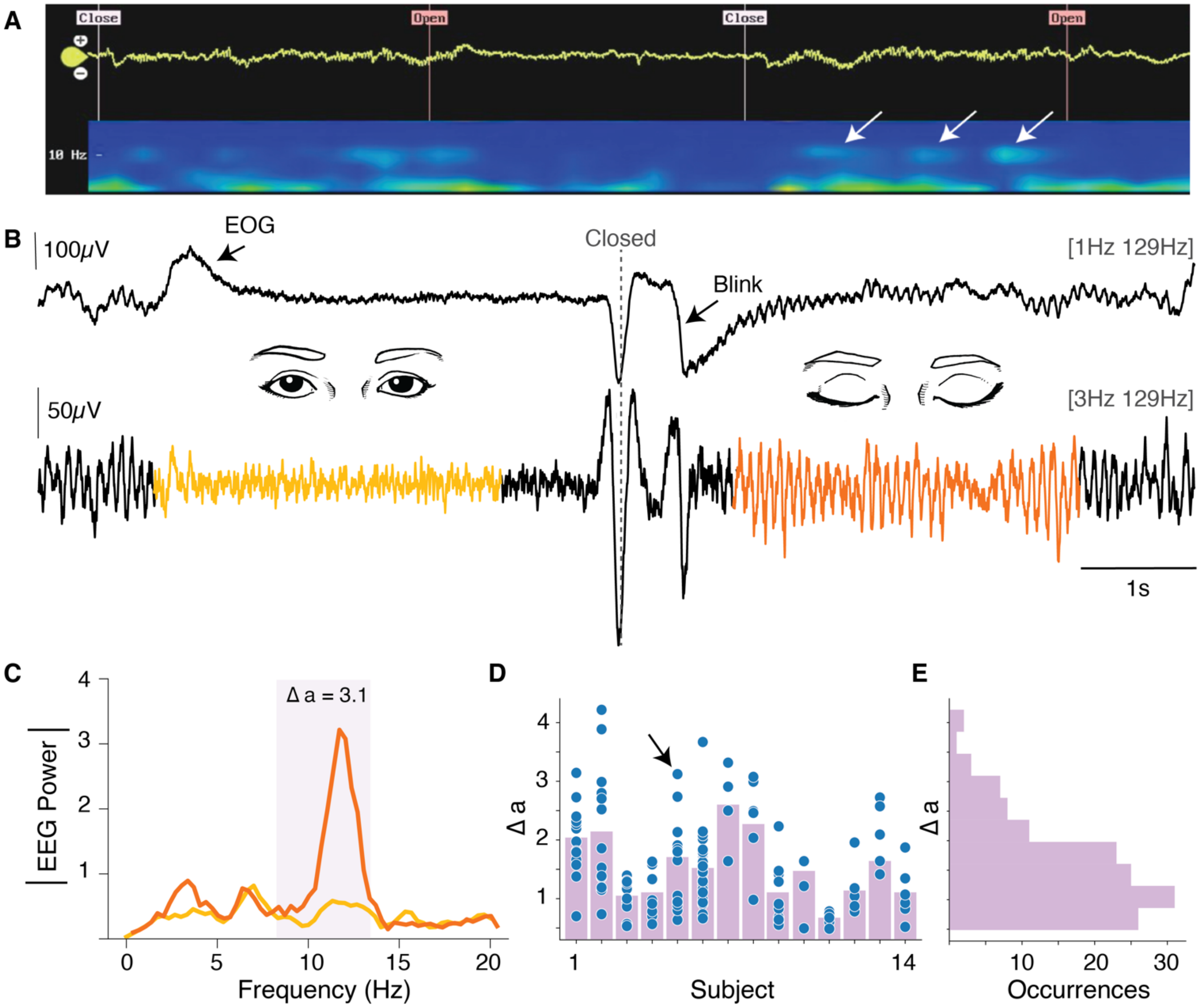

Figure 4. Results of Alpha Wave Experiment. *A*. Real-time analysis in Backyard Brains SpikeRecorder software showing alpha waves (10 Hz, arrow) occurring during the eyes closed condition. *B*. Representative trace of one event, marked by the “closed” event marker (top). The representative trace was filtered and colored to highlight analyzed regions (below). *C*. Differences in frequency power for eyes open (yellow) and eyes closed (orange) conditions (left). Plot showing the ratio of alpha power in eyes closed/eyes open conditions for all events, shown as dots (middle). Arrow identifies the event shown at left. Average change in alpha power per participant shown as bar. Histogram showing the change in alpha power for all recorded events (right).

Classroom P300 Analysis

To analyze the P300 data in a classroom setting, we modified our procedure to account for a nosier environment. Event markers were placed each time a tone occurred. When an oddball tone was present, an event marker labeled “2” would occur in the SpikeRecorder software. A 1Hz software filter was used to compare peak amplitude measures within a larger window of -400ms to 1000ms. We identified positive or negative peaks after tone presentation for the standard and oddball tones. We compared the peak differences of the oddball to the standard tone ERPs. The resulting difference reliably differentiated between oddball tones and standard tones.

We used a Monte Carlo simulation to randomly sample based on the variables provided in the experiment. By generating many random samples, we can determine if oddball and standard tones are significantly different from the innate variability in the EEG ERPs. Both oddball and standard tones were compared to a Monte Carlo shuffle to determine if they were differentiable from noise. If the signal were greater than the 95% confidence interval, it would be considered a real signal different from noise < 5% ($p < 0.05$). If either the oddball or the standard tone was not significantly different from the Monte Carlo simulation and did not fall outside the 95% confidence interval, then neither was considered a significant ERP.

Classroom Survey Data

Following the classroom experiment, participants were asked to complete a short survey consisting of 11 questions related to their general thoughts about EEG and neuroscience. 10 questions were rated on a 5-point scale, 1 meaning “Strongly agree” and 5 meaning “Strongly disagree.” The last question asked participants to rank the survey portion on a scale of 1-10, 1 being “Terrible” and 10 being “Excellent.” Some questions were, “This section increased my interest in studying neuroscience,” and “This section encouraged me to generate and test my own hypothesis.”

RESULTS

We tested the two laboratory protocols described here on 17 total participants across 81 recording sessions spanning 11 days. While a majority of the participants (n=11) were recorded in consecutive sessions on a single day, we tested some participants (n=6) across multiple days (Mean sessions per participant = 3.3, Mean days of recording per participant = 2.6). Classroom participants were recorded over the span of three days for a total of 30 sessions, 1 session per participant.

Laboratory Alpha Wave Experiment

The first stage of alpha wave analysis was performed in real-time within the Backyard Brains SpikeRecorder software. Alpha signal (8-13 Hz) was visible as a light-colored band using the spectrogram function, absent from eyes-open conditions but present when the eyes were closed (Figure 4a). Five seconds on either side of the event are shown in a representative trace in Figure 4b, where electrooculogram (EOG) artifacts are visible in the raw signal (top). EEG signals were filtered by a 2nd order high-pass Butterworth filter at 3 Hz to remove EOG artifacts (Figure 4b, bottom). The left panel of Figure 4c shows the difference in EEG power between eyes closed and eyes opened conditions for the representative event shown in Figure 4b, with said event highlighted by an arrow in the middle panel. Across all events, an average of 53% increase in alpha power for eyes closed conditions was observed. Of the 14 participants used in alpha experiments, nine participants (64%) doubled their alpha power in one or more recording sessions. Six participants (42%) saw an average increase in alpha power during the eyes closed condition of 50% or more across all events (Figure 4c, middle). Of the 137 individual events analyzed, 59 demonstrated a 50% increase in alpha power for the eyes closed condition, and 32 showed alpha power doubling (Figure 4c, right).

Laboratory P300 Experiment

As with the alpha task, the initial analysis was conducted using the Backyard Brains SpikeRecorder software. By performing real-time averaging around tone onset events, the P300 response became visible as a positive potential occurring roughly 300 ms after tone onset by the end of the recording (Figure 5a). Oddball tone responses were extracted, overlayed, and averaged to produce the P300 signal (Figure 5b, top). Aligning the average standard and oddball tone responses shows where the responses are statistically different from one another, as shown in Figure

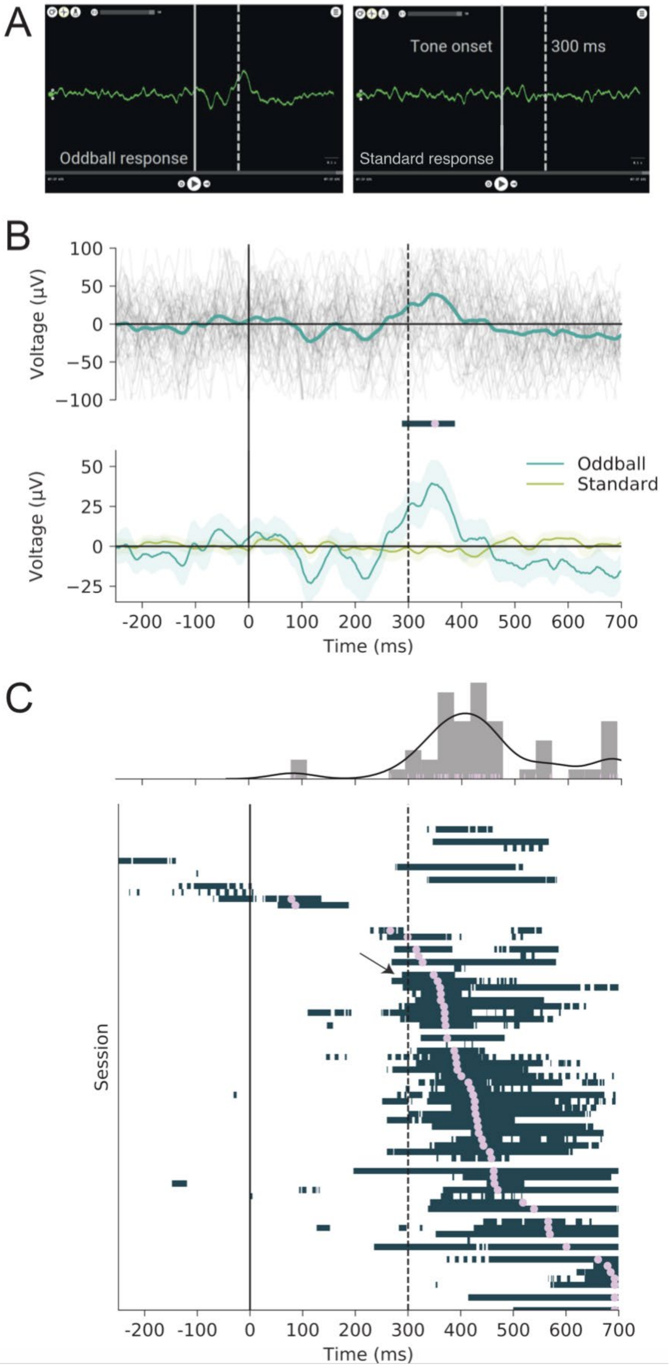

Figure 5. Laboratory P300 Results. *A*. Real-time analysis of the P300 in Backyard Brains SpikeRecorder software, showing averaged responses to oddball (left) and standard (right) tones. *B*. All oddball responses were overlaid (black) and averaged (teal) for a single representative session (top). Average responses for standard and oddball tone are plotted together (bottom), where a navy bar indicates regions where the average oddball response is statistically different from the standard tone response. Peak latencies that fall within the significance window are shown with a purple dot. *C*. Regions of significance and peaks that fall within said region are shown for all 81 recording sessions.

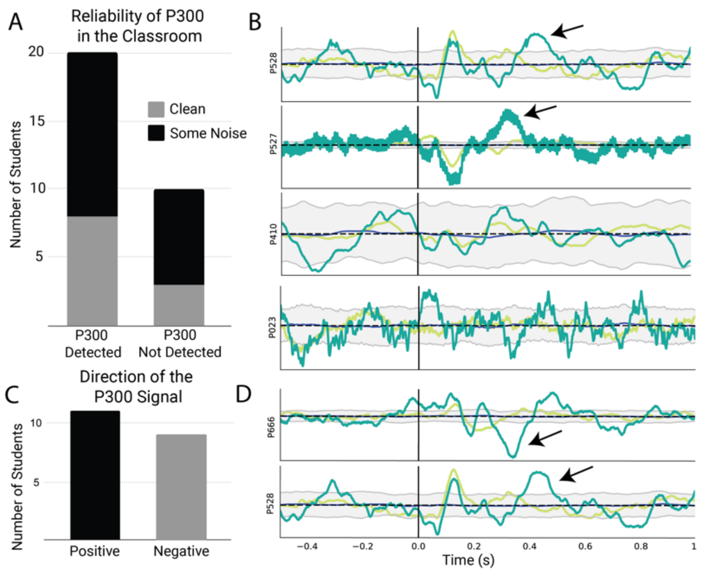

Figure 6. Results of Testing the P300 protocol in 4 undergraduate classes at Minnesota State University. Summary (A) and examples (B) of the reliability of the P300 signal. Two-thirds of the students (66%) could detect a reliable P300, even though 8 of those sessions contained recording noise in the classroom. Roughly half (45%) of the 20 sessions (C) that detected a significant P300 signal were actually in the negative direction (D), indicating that the polarity of the electrodes matters and should be marked. Arrows indicate clear P300 response.

5b (bottom). The maximum amplitude of the P300 detected during the analysis window was denoted by a navy bar with a purple dot to represent the peak latency of the response only if the peak fell within the window of significance. Windows of significance for all recording sessions (n=81) are shown in Figure 5c (bottom). Of the 81 recording sessions, 52 (64%) show significant P300 peaks that fall 250-700ms following tone onset, with an average peak latency of 442ms (Figure 5c, top). With a difference in average peak latency across participants of only 33 ms, our findings are consistent with the literature (Bledowski *et al.*, 2004; Cavinato *et al.*, 2009; Picton 1992).

Classroom P300 Experiment

As mentioned above, the SpikeRecorder software collected the P300 classroom data in real-time. In order to view the P300 wave, averages of the standard tone data were compared to the averages of the oddball tone data. To determine if the P300 signal was significant, we analyzed EEG response to tones during a recording window of 300-650ms. These averaged signals were aligned and compared against a Monte Carlo simulation. If the tone data exceeded the 95% confidence interval calculated from when comparing averages across the tone types (oddball, control, and no tone), the tone data was considered significant. A clear P300 signal was present in two-thirds (n = 20) of the classroom recording sessions (n = 30) at approximately 300 ms. Of those 20 sessions, only four sessions had a P300 that could not be differentiated from noise (Figure 6a, b). EEG traces from 9 of the 20 sessions had inverted signals. However, this did not affect the calculation of the P300 signal or calculation of the 95% confidence intervals from bootstrapped samples (Figure 6c,d).

P300 Classroom Survey

Data from the P300 survey was compiled into a graph scaled from 0-100%; 0% rating reflected no learning/strongly disagreed with the measured outcomes while a 100% rating indicated students strongly agreed with the learning outcomes (Figure 7). Results showed that not only did multiple participants report an increased understanding in neuroscience, but also reported an increased interest in the field. Participants also reported feeling confident in their ability to design their own experiments after using the EEG device. Participants agreed that the EEG device was user-friendly and many participants felt they could configure the headband electrodes and SpikeRecorder settings independently.

DISCUSSION

Our results demonstrate the ability of the Backyard Brains Heart and Brain SpikerBox and Human SpikerBox to record brain waves and event-related potentials (ERPs) in both laboratory and classroom settings. Each experiment yielded significant results, showcasing the effectiveness of these devices for educational purposes with low cost and time investment. Despite some limitations, such as the SpikeRecorder software's ability to average only up to fifty event responses simultaneously, these can be managed through simple workarounds.

Results of the alpha wave experiment show a visually detectable doubling of alpha power during the eyes closed phase in 8 out of 14 participants (57%). Students may have to test several subjects to see a noticeable increase in alpha. Students could also test if machine learning could distinguish the eyes open vs. closed state by training on labelled data.

Regarding the laboratory and classroom P300 data, a compelling line of inquiry is the late latency P300 responses (Figure 5c). While most responses showed a significant peak latency around 400 ms, some occurred much later, suggesting reduced attentional processing. These data were acquired in a workspace with potential distractions, and we suspect that significant P300 responses with later latencies were collected on days with more distractions. Future experiments could investigate conditions like quiet versus distracting environments to address this question.

Interestingly, some participants anticipated the oddball tone before its presentation in about 5 trials (Figure 5C), likely due to the predictable 2-second intervals of the tones. Introducing a random delay (e.g., 2 seconds plus a random 200 ms delay) could mitigate this anticipatory effect.

The inversion of P300 signals due to incorrect electrode placement can be resolved by marking the leads and maintaining consistent electrode positioning. This troubleshooting example offers practical insights into experimental design and constraints.

Our classroom P300 data indicated that clear P300 signals were detected in the majority of recording sessions, even in noisy conditions. Static electricity, attention span, scalp connectivity, and movement artifacts tended to interfere with the P300 signal, but it was still detected in most noisy data (Figure 6a, b). This underscores the robustness of the equipment and its suitability for educational purposes. Students can expand on the introductory exercises for alpha oscillations and P300 by exploring various parameters affecting these signals, as described in the literature (Klimesch, 1999; Haider and Fezel-Rezai, 2017; Polich, 1987; Picton, 1992; Fischer et al., 2008; Bledowski et al., 2004; Herrman and Knight, 2001). They can modify the oddball task or recording technique to observe changes in latency or amplitude. Students may also identify common problems affecting the P300 signal, such as the amount of conductive gel or hair placement (Shields et al., 2016). Designing new tasks, like visual (Bledowski et al., 2004; Polich and Herbst, 2000) or somatosensory (Herrman and Knight, 2001) tasks, can help compare different P300 responses. Investigating electrode locations can provide insights into amplitude and morphological changes in recorded signals (Polich, 1989; Picton, 1992; Polich and Herbst, 2000; Li et al., 2019).

Beyond the P300, students can investigate other ERPs and neurophysiological phenomena, utilizing the flexibility of this technology (Sur and Sinha, 2009; Haider and Fezel-Rezai, 2017; Woodman, 2010; Libet et al., 1983). Early proof-of-concept experiments with the Human SpikerBox can record visually evoked potentials, showing the technology's capability to record low-frequency brain signals. Students can explore components of ERPs like the C1, P1, and N1 in tasks involving perceptual or cognitive analysis (Haider and Fezel-Rezai, 2017; Woodman, 2010). By conducting introductory exercises and designing their experiments, students become empowered to ask and answer questions about brain function, enhancing their understanding through hands-on learning (Segawa, 2019).

Students can move beyond the P300 to investigate other ERPs as well. Early proof-of-concept experiments investigated the ability of the Heart and Brain SpikerShield to record the reflexive elicited flash visually evoked potential (data not shown). This was done by recording a participant's response to a flashing light in a dark room with electrodes placed over the occipital lobe. Not only would this be simple to recreate in a classroom, it shows that this technology is flexible in its ability to record low-frequency brain signals. See Sur and Sinha (2009) for a list of other ERPs to explore. Further, components of an ERP (i.e., the C1, P1, N1, etc.) can be investigated in the oddball task or other tasks involving perceptual or cognitive analysis (Haider and Fezel-Rezai, 2017; Woodman, 2010). Students can use this recording approach to study additional neurophysiological phenomena such as habituation, response versus recognition time, and the response-associated readiness potential as done in the famous Libet experiment (Libet et al., 1983). After conducting introductory classroom

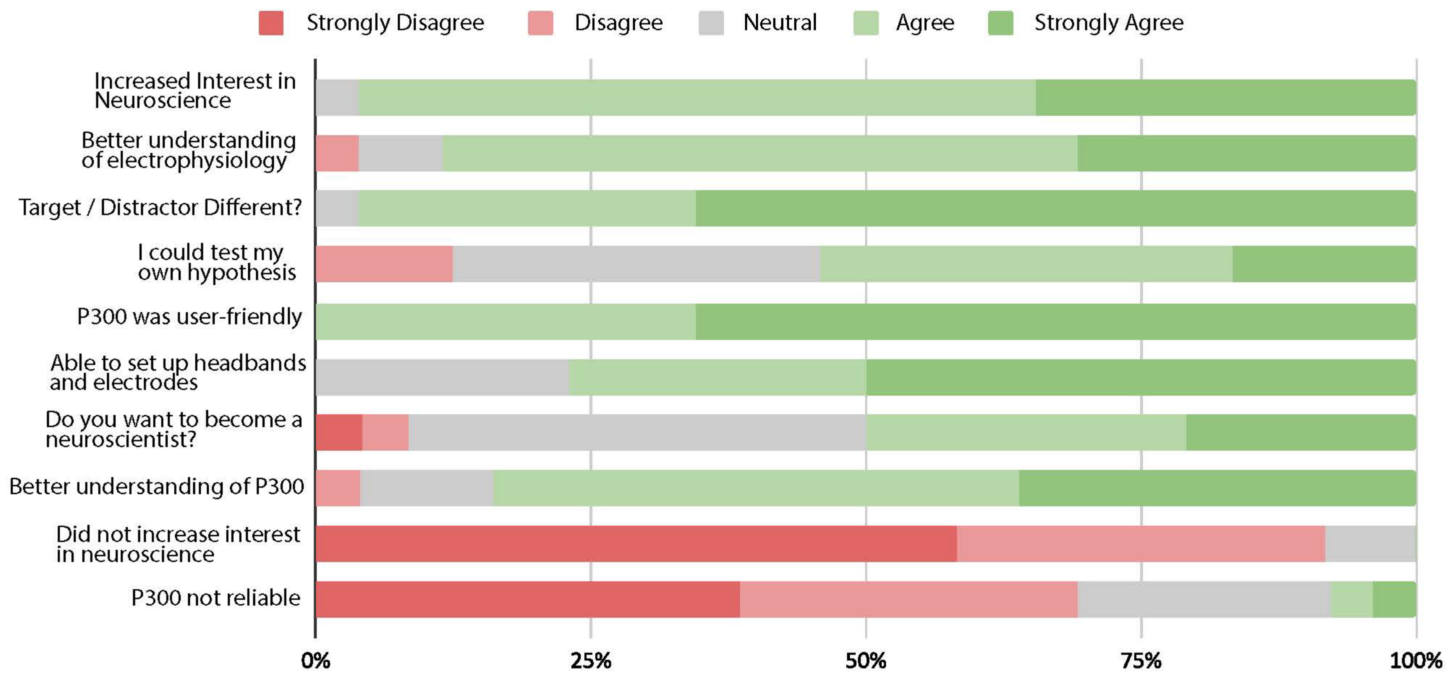

Figure 7. Classroom P300 Survey - Survey results following the P300 classroom experiment. There was a total of 31 survey responses. Overall, participants agreed that the experiment was user-friendly and increased an understanding and interest in neuroscience.

exercises, students could also be encouraged to design, execute, and analyze their own experiments as done by Segawa (2019). By providing students with instruction in the scientific method and a means to put this learning to use, they become empowered to ask and answer their own questions about how the brain works.

CONCLUSION

With the high cost of neuroscience teaching tools and technologies and the significant time required to learn to use such tools, hands-on neuroscience education in the classroom has traditionally been lacking.

Here, we have described two laboratory experiments and have replicated the P300 experiment within a classroom setting to introduce neuroscience students to the basics of brain signaling. The low cost and ease of use of both the Heart and Brain SpikerBox and the Human SpikerBox reduces barriers to access and understanding for novice scientists. Further, providing students with a laboratory exercise allows them to gain familiarity with and practice the scientific method, enabling them to ask and answer their own questions about the brain. By using these devices in the classroom setting, students should be able to identify common problems such as unexpected noise, varying signal strength, connectivity issues, etc., and make the necessary adjustments in order to collect strong data. Hands-on teaching exercises can be especially useful in neuroscience, where concepts about brain function may be challenging for students to grasp. Elucidating the techniques of neuroscience technology and enabling students to see themselves as critical thinkers may embolden more students to choose a STEM field for a career.

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Online Repository: All the data and analysis tools discussed in this paper are available on an online GitHub repository: https://github.com/BackyardBrains/SpikerTools. Instructions are available to generate your own database from SpikerBox recordings, as well scripts to generate the figures used in this paper. Scripts can be modified for your own needs. The Google Collab Notebook used for analysis is found here: https://colab.research.google.com/drive/1wGB_BzXhJRQ7sd8kyAjuyT7PXId7K5WT?usp=sharing

Schematics for the Heart and Brain SpikerBox and Human SpikerBox can be accessed via the BYB website: https://backyardbrains.com. The SpikeRecorder application GitHub repository is at: https://github.com/BackyardBrains/. The modified P300 code for the Heart and Brain can be found at: https://github.com/BackyardBrains/Heart-and-Brain-SpikerBox-Pro.

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Disclosure of Interest: Authors GJG and TCM are co-owners of Backyard Brains, a company that develops and distributes neuroscience education tools, some of which were used in this study. Authors MLMA, SM, and ESK are currently employed by Backyard Brains. While the study used Backyard Brains products, the primary focus of this work is on the educational and scientific value of the experiment rather than the promotion of specific products. The authors declare no other conflicts of interest.

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