PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci1625.com. Email: managingeditorpjms1625@gmail.com.

A LOW-COST, CUSTOM-BUILT FEAR CONDITIONING SYSTEM FOR RODENTS: DESIGN, CONSTRUCTION AND VALIDATION

***^Abdulmajeed Wahab Imam, **Abdulrasheed Kamil Muhammad, *Adedeji Tayyib Adekunle, *Osho Oluwabukola Daniel, *Adesanya Fuhad Babajide, *Yusuf Sekinat Funmilayo, *Akinlabi Islamiyah Adenike, *Nureni Mardiyat Arinola, *Oseni Nuriyat Oyindamola, *Amin Abdulbasit, *Ayinla Maryam Tayo**

*Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.
**Department of Computer Engineering, Faculty of Engineering, University of Ilorin, Ilorin, Nigeria

^Corresponding author: abdulmajeed.w@unilorin.edu.ng

Submitted: *January 2026. Accepted: March 2026*

A LOW-COST, CUSTOM-BUILT FEAR CONDITIONING SYSTEM FOR RODENTS: DESIGN, CONSTRUCTION AND VALIDATION

***^Abdulmajeed Wahab Imam, **Abdulrasheed Kamil Muhammad, *Adedeji Tayyib Adekunle, *Osho Oluwabukola Daniel, *Adesanya Fuhad Babajide, *Yusuf Sekinat Funmilayo, *Akinlabi Islamiyah Adenike, *Nureni Mardiyat Arinola, *Oseni Nuriyat Oyindamola, *Amin Abdulbasit, *Ayinla Maryam Tayo**

*Department of Physiology, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Nigeria.
**Department of Computer Engineering, Faculty of Engineering, University of Ilorin, Ilorin, Nigeria

^Corresponding author: abdulmajeed.w@unilorin.edu.ng

Submitted: *January 2026. Accepted: March 2026*

ABSTRACT

A major goal in behavioral neuroscience is to identify the neural mechanisms underlying learning and memory. Fear conditioning models, including contextual and cued conditioning, are widely used to assess associative learning in rodents; however, commercially available systems for these experiments are often expensive (> $4000), limiting accessibility and reproducibility across laboratories. Here, we present a low-cost (about $100), modular fear conditioning platform for rodents that enables precise control of experimental parameters while maintaining compatibility with standard behavioral protocols.

The fear conditioning system consists of conditioning chambers equipped with stainless-steel grid floors, a custom-built constant-current foot-shock generator**,** and an auditory cue delivery section, all synchronized with video-based behavioral recording. Shock intensity, duration, and timing are fully programmable, and the platform supports both contextual and cued fear conditioning paradigms. To validate the setup, we designed three well-established experiments and demonstrated that this low-cost fear conditioning system produces reliable and reproducible fear conditioning behavior comparable to established commercial systems while substantially reducing system cost. Its open and flexible design provides an accessible alternative for laboratories seeking standardized fear conditioning experiments, promoting broader adoption of behavioral assays in resource-limited settings.

Keywords: Conditioning, Fear, Foot-shock, Memory, Learning, Low-cost

INTRODUCTION

Fear conditioning is a well-established behavioral model for studying associative learning and memory in rodents and has been extensively used to investigate the neural mechanisms underlying emotional learning and psychiatric disorders such as post-traumatic stress disorder and schizophrenia [1 - 3]. Standard implementations of contextual and cued fear conditioning rely on specialized hardware, including conditioning chambers with electrified grid floors, auditory stimulus

generators, and programmable shock delivery units, all of which must operate with high temporal precision and reliability.

Commercially available fear conditioning systems provide robust and standardized solutions; however, they are often cost-prohibitive, limiting accessibility for laboratories with constrained resources and reducing experimental scalability. Furthermore, proprietary hardware and software designs may restrict flexibility in protocol customization, integration with external devices, or adaptation to novel experimental needs. These limitations have motivated the development of custom-built and open hardware solutions for behavioral neuroscience, aimed at improving accessibility while maintaining experimental rigor [4,5].

A critical component of fear conditioning hardware is the foot-shock generator, which must deliver stable, reproducible electrical stimulation at low current levels through stainless-steel grid floors while ensuring animal safety and experimental consistency. Commercial shock generators, such as the SGS-003DX Shock Generator Scrambler, are commonly used as reference standards in laboratories and provide programmable constant-current output and grid scrambling to distribute current across electrodes. While effective, such systems substantially increase the overall cost of a fear conditioning setup.

In this work, we introduce a low-cost, modular fear conditioning system designed to replicate the core functional requirements of established systems while reducing cost and complexity. The platform integrates conditioning chambers with stainless-steel grid floors, a custom-designed constant-current shock generator, and an auditory cue delivery system under unified software control. We experimentally validated the system and demonstrated that the hardware successfully supports standard contextual and cued fear conditioning protocols, while providing precise control over shock intensity, duration, timing, and synchronization with behavioral recordings.

By situating our design within the context of widely used fear conditioning hardware, this system aims to facilitate standardized, reproducible behavioral experiments while lowering barriers to adoption. The system complements existing behavioral neuroscience methodologies and provides an accessible alternative for laboratories seeking flexible, open, and cost-effective experimental tools, especially in developing countries.

MATERIALS AND METHODS:

Hardware Description:

The Controller:

The controller unit was implemented as an auxiliary control circuit based on an ATmega328P microcontroller mounted on a custom printed circuit board (PCB). The system is powered by a 12 V DC external power adapter, with on-board voltage regulation provided by a linear 7805 regulator to supply a

stable 5 V rail for the microcontroller and peripheral components (Figure 1A1 – A3).

The microcontroller governs the timing and sequencing of foot-shock delivery according to user-defined experimental parameters. Specifically, the controller manages the onset and offset of shock stimuli, allowing precise control over shock duration and inter-stimulus timing. Shock parameters are selected by the user before the experiment and executed autonomously by the controller during the conditioning session.

An integrated liquid crystal display (LCD) provides real-time feedback during experiments, displaying key parameters such as cycle count, selected shock intensity, and system status while a test is ongoing. This enables experimenters to monitor protocol execution without interrupting the conditioning procedure. The controller also interfaces with an auditory cue module, triggering a buzzer used for cued fear conditioning paradigms.

Auditory stimulus timing is synchronized with shock delivery under microcontroller control to ensure consistent temporal relationships between conditioned and unconditioned stimuli.

The system supports programmable shock current amplitudes in the range of 0.1 mA to 4 mA, covering the range commonly used in rodent fear conditioning experiments.

The Shocker Rig:

The shock delivery unit, or Shocker Rig, is responsible for generating the actual foot shocks, complementing the controller, which manages input and timing. The system begins with a step-down transformer that reduces the AC mains voltage from 200 – 250 V to 110 V AC. The output is then rectified using a KBP206G bridge rectifier and filtered with a 400 V, 47 µF capacitor to provide a stable DC supply. Given the low-current requirements of the system, the voltage is further reduced to a safe output range of 40 –75 V, suitable for delivering reproducible foot shocks to rodents.

This configuration ensures that the shocks are precisely controlled, stable, and safe, while the low-current design minimizes risk to the animals and allows the unit to operate reliably over repeated cycles. By separating the control logic (managed by the ATmega328P controller) from the high-voltage output stage, the design maintains both safety and modularity.

The Conditioning Chamber:

The fear conditioning chamber was custom designed to provide a durable and transparent environment for rodents while allowing reliable shock delivery. The base consisted of a wooden platform fitted with 5 mm stainless steel rods positioned at 12 mm center-to-center intervals to create the electrifiable grid floor. The walls were made from clear acrylic, allowing unobstructed observation and video recording of behavioral responses. The overall dimensions of the

chamber are 300 mm × 320 mm × 300 mm, and the structure weighs approximately 2.5 kg, making it both lightweight and stable during experiments (Figure 1B). This design ensures consistent foot-shock delivery to support both contextual and cued fear conditioning paradigms and provides visibility for automated or manual behavior scoring, while maintaining a low-cost and easily reproducible construction.

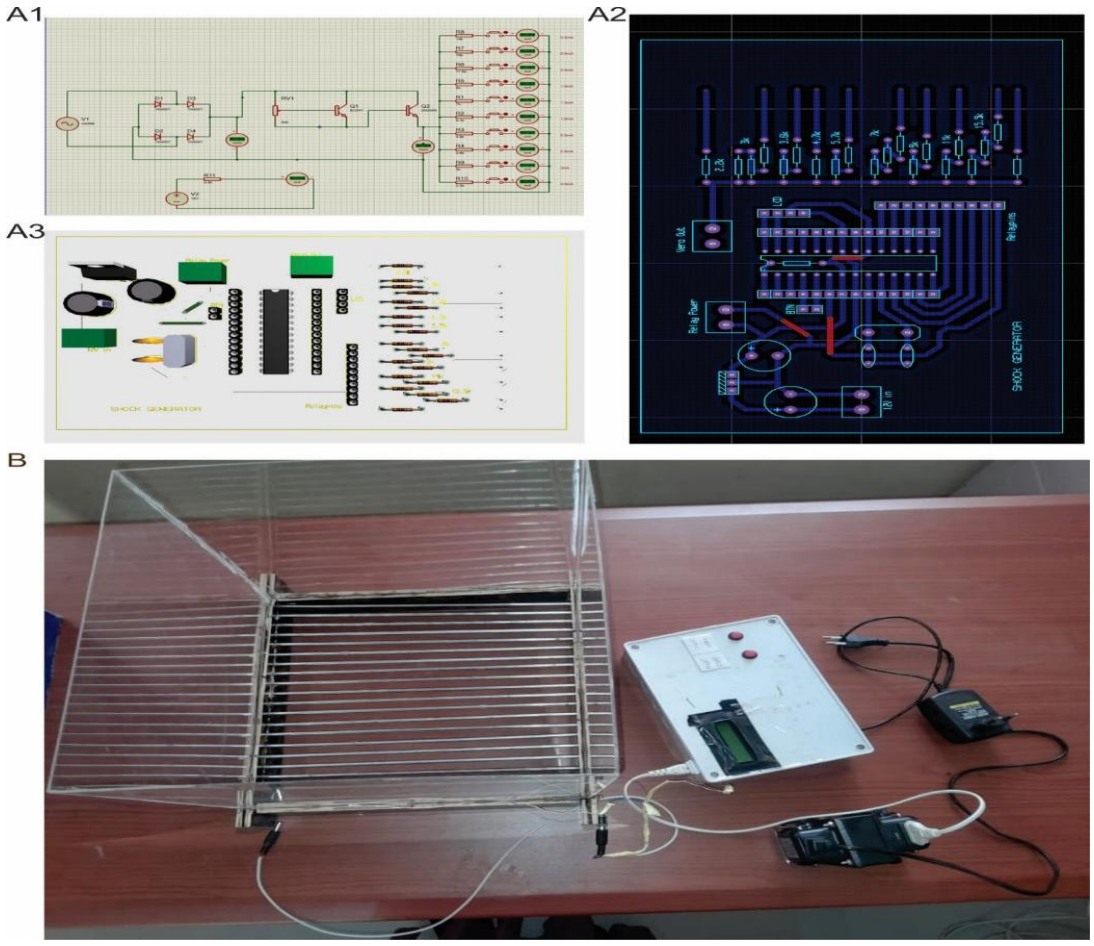

Figure 1: Hardware construction (A1) Schematic diagram of current control circuit (A2) Printed circuit board (PCB) layout showing the microcontrollers (ATMEGA328P) and their connections (A3) A 3D view of the printed circuit board (PCB) design. (B) Fear conditioning chamber

Animals:

Forty-two (42) healthy adult rats weighing 150 – 230 g were used for validation experiments. Animals were housed under standard laboratory conditions, with controlled temperature and a 12-hour light/dark cycle and were provided with food and water *ad libitum*. All procedures were conducted in accordance with the guidelines for the care and use of animals as approved by the University of Ilorin's ethical committee, Ilorin, Nigeria.

Fear conditioning protocol:

The protocol was as previously described [6] with slight modifications. We employed a 2-day conditioning protocol. On the conditioning/training\ day (DAY 1), each rat was placed in Context A and allowed to explore for 180 seconds before stimulus presentation (baseline, BL). Each rat received repeated pairings of an auditory tone (conditioned stimulus, CS) with a foot shock (unconditioned stimulus, US). Each tone presentation was co-terminated with a foot shock. Each rat was exposed to five conditioning trials (T1 – T5) with a 20-second tone and a 2-second foot-shock (55 dB; 1.5 mA) delivered at 1-minute intervals. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

To assess contextual fear memory, animals were re-exposed to the conditioning chamber 24 hours after the acquisition without tone and shock presentation. Freezing behavior was recorded continuously during the 8-minute session. Two hours after the context test, the cue test was conducted. Rats were placed in a neutral context (Context B). This box is similar in dimensions to those of the shock chamber (context A, Figure 1 B). This new chamber, however, was made highly different from the shock chamber. Two of its walls were transparent and had no metal grid. Each rat was allowed to explore the new context for 180 seconds before the tone presentation (baseline, BL). This phase consists of five trials (T1 – T5), with 1-minute intervals. Each rat received an auditory cue for 20 seconds every minute without a foot shock. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

The experimenter recorded the behaviors of the rats as freezing or not. Freezing behavior was defined as the inhibition, absence, or suppression of movement, beyond that required for autonomic nervous system functioning [7]. Head scanning and sleeping were not included as freezing. Freezing behavior was scored in the conditioned and unconditioned groups during the final 20 s of the first minute (baseline, BL), the 20 s before each foot-shock (T1–T5) and the final 20 s of the last minute (TL; [8]).

Sleep deprivation:

Sleep deprivation was induced using a modified multiple-platform technique [9,10]. The rats were placed in a plastic cage (23 × 23 cm) containing several small platforms (5 cm diameter) positioned 7 cm apart.

Food and water were provided *ad libitum* via an attached acrylic compartment and drinking bottles. When the animals entered the rapid eye movement (REM) sleep, muscle atonia caused them to lose balance and contact the water beneath the platforms, forcing them to awaken. This method selectively disrupted REM sleep while allowing non-REM sleep.

The rats underwent 18 hours of sleep deprivation daily (from 16:00 to 10:00 the next day) for seven consecutive days before behavioral testing.

After each deprivation period, the animals were gently dried with towels and returned to their home cages.

Scopolamine treatment:

To test the effect of post-training scopolamine injection on fear Conditioning, on the conditioning/training day (DAY 1), each rat was placed in Context A and allowed to explore for 180 seconds before the presentation of tone (baseline, BL). Each rat was exposed to five conditioning trials (T1 – T5) with a 20-second tone and a 2-second foot-shock (55 dB; 1.5 mA) delivered at 1-minute intervals. After the final trial, rats remained in the chamber for 90 seconds. Immediately, test rats were injected with scopolamine intraperitoneally (10 mg/kg, i.p) and returned to their home cages. The control animals received an intraperitoneal injection of normal saline. The contextual and cued tests were carried out on day 2 as described above.

On the second day, we assessed contextual fear memory by re-exposing the rats to the conditioning chamber without tone or shock presentation. Freezing behavior was recorded continuously during the 8-minute session. Two hours after the context test, the cue test was conducted. Rats were placed in a neutral context (Context B) where each rat was allowed to explore the new context 180 seconds before the tone presentation (baseline, BL). This phase consists of five trials (T1 – T5), with 1-minute intervals. Each rat received an auditory cue for 20 seconds every minute without a foot shock. After the final trial, rats remained in the chamber for 90 seconds before being returned to their cages.

Data Analysis:

These data were analyzed by a two-way ANOVA and subsequently, Bonferroni's multiple comparison tests were applied when appropriate. Data were represented as mean ± standard error of mean and the level of significance was set at $p < 0.05$.

RESULTS

Experiment 1: Low-Cost Rodent Fear Conditioning Setup: Acquisition and Expression of Tone-Shock, Contextual, and Cued Fear Memories:

The first experiment tested whether our low-cost fear conditioning apparatus could reliably demonstrate associative learning in rodents through the pairing of a tone (conditioned stimulus) with foot shock (unconditioned stimulus). Baseline freezing levels were comparable between the tone-only (control) group and the tone + shock (paired) group (n = 5/group), remaining low and consistent with typical pre-conditioning values in standard auditory fear conditioning paradigms. Following the pairing phase, however, the tone + shock group displayed significantly elevated freezing during tone presentation compared to the tone-only group (2-Way ANOVA, $F_{1,8} = 10.08$, $p = 0.0131$). This marked increase in the percentage

of freezing time spent indicates a robust conditioned fear response specific to the tone-shock association, as the tone-only group showed no such elevation. These results confirm that the rats in the paired condition successfully acquired and expressed intact Pavlovian associative learning, validating the effectiveness of the low-cost setup for detecting fear conditioning in rodents.

The context test, conducted 24 hours after the training session, involved re-exposing the rats to the original conditioning chamber for an 8-minute period without any tone or foot shock presentation. Consistent with established findings in the literature on contextual fear conditioning, the tone + shock (paired) group exhibited significantly higher levels of freezing compared to the tone-only (control) group (2-Way ANOVA, $F_{1,8} = 20.54$, $p = 0.0019$) during this exposure [11,12]. This elevated freezing response indicates the successful formation of a robust contextual fear memory, which is widely recognized as primarily dependent on the hippocampus [13,14]. These results provide further evidence that the low-cost setup reliably elicits and detects hippocampus-dependent associative learning in rodents, complementing the tone-specific (cued) fear conditioning observed in the earlier test and supporting its utility for studying fear memory mechanisms.

The cued test was performed 2 hours after the context test. Rats were placed in a novel, neutral chamber distinct from the original conditioning box (See methods). After a 180-second baseline period, the tone was presented for 20 seconds every minute, repeated five times. Results revealed significantly higher percentage freezing during tone presentations in the tone + shock (paired) group compared to the tone-only (control) group (2-Way ANOVA, $F_{1,8} = 11.07$, $p = 0.0104$). This selective increase in freezing to the tone demonstrates successful formation of a cued (auditory) associative fear memory, which is primarily dependent on the amygdala [1,2,13]. Collectively, these findings confirm that our low-cost fear conditioning setup effectively captures and quantifies key forms of associative learning in rats, offering a reliable, accessible tool for studying fear memory mechanisms.

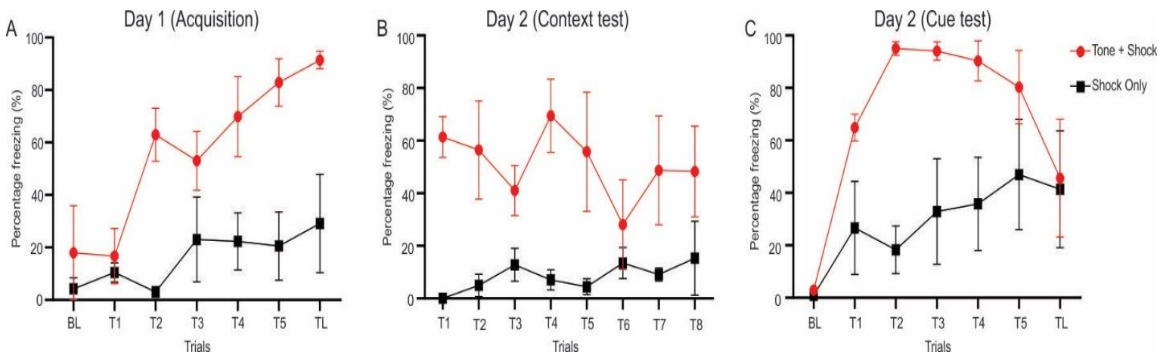

Fig. 2. Rats learned associations between the conditioned stimulus (tone) and the aversive unconditioned stimulus (foot shock). Averaged percentage of freezing displayed by rats exposed to shock and tone or tone only in the fear-conditioning paradigm (A) Training (B) context test (C) cue test. Error bars represent standard error of mean. *n* = 5; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

Experiment 2: Selective Impairment of Hippocampus-Dependent Contextual Fear Memory Following Sleep Deprivation in Rats:

To further validate the utility of our low-cost setup for fear conditioning studies involving sleep manipulation, twenty-two rats were randomly assigned to two groups (n = 11/group): a control group and a sleep-deprived (SD) group. Testing began 2 hours after the completion of the sleep deprivation protocol (or at the equivalent time point for controls). During acquisition on day 1, both the control and SD groups exhibited progressive increases in freezing across trials, indicating gradual acquisition of the tone-shock association. Although the SD group displayed modestly lower freezing levels overall, this difference did not reach statistical significance (2-Way ANOVA, $F_{1,20}$ = 1.94, p = 0.1804). These observations suggest that pre-training sleep deprivation did not substantially impair the initial acquisition of fear learning.

On day 2, in the context test, rats were re-exposed to the original conditioning chamber for 8 minutes without tone or shock presentation. Freezing percentages were significantly higher in the control group compared to the SD group (2-Way ANOVA, $F_{1,20}$ = 21.66, p = 0.0002). This finding aligns with extensive evidence that sleep deprivation selectively impairs the consolidation and/or expression of contextual fear memory, a process heavily reliant on hippocampal function [15,16]. In contrast, during the subsequent cued test (conducted in a novel neutral chamber), both groups displayed robust and comparable freezing responses to tone presentations, with no significant differences between control and SD animals (2-Way ANOVA, $F_{1,20}$ = 0.0043, p = 0.9476). This pattern indicates that sleep deprivation did not affect the formation or expression of cued (auditory) fear memory, which is primarily amygdala-dependent and hippocampus-independent [15,16]. Taken together, these results demonstrate a selective impairment of contextual fear conditioning following sleep deprivation, while sparing cued conditioning.

This is consistent with the differential neural substrates involved (hippocampus for context vs. amygdala for cue). This further confirms the sensitivity and reliability of our low-cost fear conditioning setup for detecting hippocampus-dependent memory deficits in rodent models.

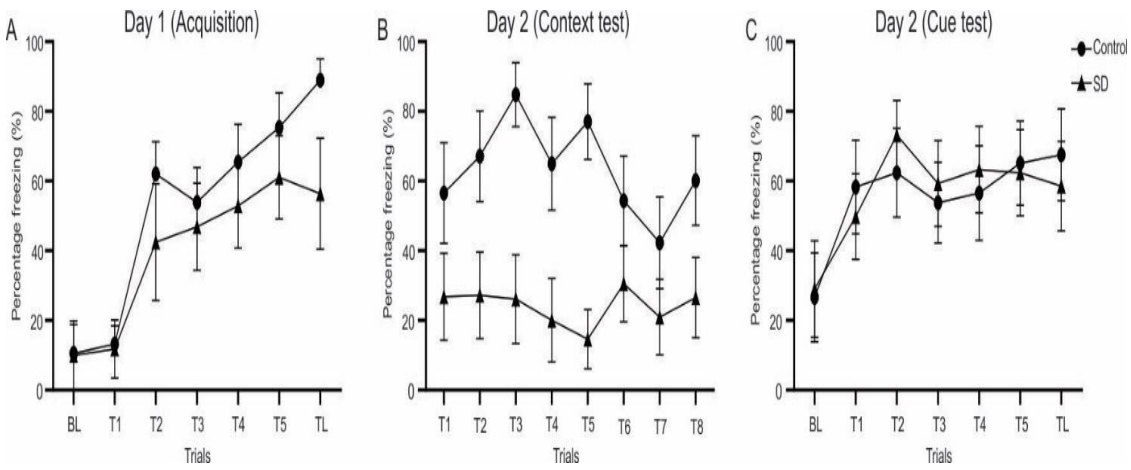

Fig. 3: Sleep deprivation selectively impairs contextual fear memory retrieval. Average percentage freezing displayed by the control or sleep-deprived rats in the fear-conditioning paradigm (A) Training (B) context test (C) cue test. Error bars represent standard error of mean. *n* = 11/group; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

Experiment 3: Effects of Post-Training Scopolamine on Hippocampal-Dependent Contextual and Amygdala-Dependent Cued Fear Conditioning in Rats:

To further validate the pharmacological sensitivity of our low-cost fear conditioning setup, we examined the effects of post-training scopolamine administration, a muscarinic cholinergic antagonist known to disrupt memory consolidation in fear paradigms. Twelve (12) rats underwent fear conditioning via tone-shock pairings and were then randomly assigned to either a control group or a scopolamine group (n = 6/group). Immediately following training, the scopolamine group received an intraperitoneal injection of scopolamine (10 mg/kg), while the control group received an equivalent volume of normal saline intraperitoneally. On day 2, during the context test, freezing levels were significantly higher in the control group compared to the scopolamine-treated group (2-Way ANOVA, $F_{1,10} = 5.04$, $p = 0.0485$). In contrast, no significant differences in freezing were observed between the two groups during the cued test (2-Way ANOVA, $F_{1,10} = 0.22$, $p = 0.6482$). These results indicate that post-training scopolamine selectively impaired the consolidation of hippocampus-dependent contextual fear memory, while sparing amygdala-dependent cued (auditory) fear conditioning, consistent with selective hippocampal disruption in contextual but not discrete-cue fear following cholinergic blockade [6,17]. Collectively, these pharmacological

findings, alongside the prior behavioral and sleep-deprivation validation experiments, demonstrate that our low-cost apparatus reliably replicates established effects on fear memory formation and consolidation, making it a robust, accessible tool for studying cholinergic modulation of hippocampal- versus amygdala-dependent associative learning in rodents.

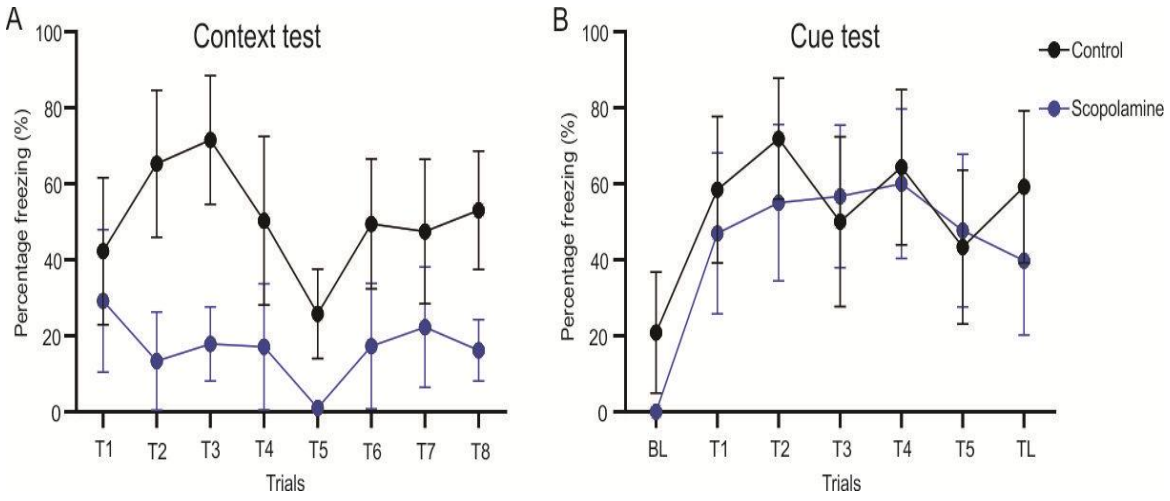

Fig. 4: Post-training injection of scopolamine affected contextual fear memory recall. Averaged percentage freezing displayed by control or scopolamine-treated rats in the fear-conditioning paradigm (A) context test (B) cue test. Error bars represent the standard error of mean. *n* = 6/group; data are represented as Mean ± standard Error of mean. BL, baseline; TL, freezing level during the last trial.

DISCUSSION:

In the present study, we successfully replicated core phenomena from classical fear conditioning paradigms, including robust tone-shock associative learning, contextual fear expression, and pharmacologically induced memory impairment by employing a novel, low-cost rodent fear conditioning system assembled from affordable, readily available components. These outcomes not only confirm the reliability and sensitivity of our economical apparatus but also highlight its viability as an accessible substitute for expensive commercial systems, thereby expanding opportunities for fear-related behavioral neuroscience research in resource-constrained environments.

In Experiment 1, our low-cost setup demonstrated effective associative fear learning in rats via a tone-shock pairing paradigm. Animals in the paired (tone + shock) group exhibited significantly elevated freezing during both acquisition and retrieval phases compared to the tone-only control group. This behavioral dissociation clearly indicates that freezing reflects a learned association between the conditioned stimulus (tone) and the unconditioned stimulus (foot shock), rather than nonspecific reactions to the auditory cue or

general arousal. Acquisition trials revealed progressive increases in freezing in the paired group, mirroring the classic pattern of Pavlovian fear conditioning where repeated CS-US pairings yield robust conditioned responses [2,7]. The tone-only group, by contrast, displayed persistently low freezing levels, consistent with prior work showing that an unpaired neutral cue fails to elicit fear without reinforcement [18,19]. Retrieval tests further substantiated memory formation: paired animals showed sustained freezing to the tone in a neutral context, while controls did not, aligning with evidence that cued fear memories are amygdala-dependent and retrievable independently of the original context [3,13]. Minimal freezing in the tone-only group across all phases ruled out baseline anxiety, habituation effects, or nonspecific stress as confounders, reinforcing freezing as a valid, specific index of associative fear [7]. Overall, these results clearly replicate canonical tone-shock conditioning outcomes, providing essential validation of the paradigm's reliability prior to more advanced manipulations.

In Experiment 2, pre-training sleep deprivation selectively impaired contextual fear memory retrieval while leaving cued fear memory intact. Both control and sleep-deprived (SD) groups acquired the tone-shock association comparably during training, with only modest, non-significant reductions in freezing among SD animals. However, during day-2 contextual testing, SD rats exhibited significantly lower freezing than controls, suggesting a targeted disruption of hippocampus-dependent processes rather than broad deficits in fear learning. This selective impairment accords with reports linking sleep deprivation to altered Hypothalamic-Pituitary-Adrenal axis activity, reduced corticosterone, and diminished phosphorylated cyclic AMP response element-binding protein (pCREB) in hippocampal CA1, which compromise synaptic plasticity and contextual memory [15,16]. In contrast, both groups displayed comparable, robust freezing to the tone in the cued test, indicating preservation of amygdala-dependent cued conditioning. This dissociation highlights the differential vulnerability of hippocampal versus amygdala circuits to sleep loss [15,16]. Additional factors, such as circadian disruptions from altered training/testing timing or varying SD durations, may modulate these effects, with longer deprivation periods more likely to induce pronounced hippocampal dysfunction [15]. Finally, in Experiment 3, post-training scopolamine (10 mg/kg, i.p.) selectively impaired contextual fear memory consolidation while sparing cued conditioning. Both control (saline) and scopolamine-treated groups tolerated training without overt motor or behavioral abnormalities, confirming the setup's tolerability. On day 2, scopolamine-treated rats showed significantly reduced freezing in the context test compared to controls, but equivalent freezing during cued tone presentations in a neutral chamber. This pattern demonstrates

cholinergic involvement in post-training memory processing, particularly for hippocampus-dependent contextual fear, consistent with evidence that systemic muscarinic blockade shortly after training disrupts later contextual memory [6,20]. The robust freezing in controls for both context and tone underscore associative learning, while the scopolamine-induced deficit validates the setup's sensitivity to established amnestic interventions. Collectively, the three experiments closely mirror findings in established literature. These congruences affirm the low-cost fear conditioning apparatus as a dependable, cost-effective platform for investigating associative fear mechanisms, neural substrates, and modulatory influences in rodents.

Acknowledgement:

The authors acknowledge Ms. Dada Mary for her valuable comments on the earlier version of the manuscript.

Authors Contribution:

Abdulmajeed Wahab Imam conceptualized the idea, designed the experiments, supervised the experiments and prepared the manuscript; Abdulrasheed Kamil Muhammad designed and built the hardware, wrote the codes and drafted the manuscript; Adedeji Tayyib Adekunle, Osho Oluwabukola Daniel, Adesanya Fuhad Babajide, Yusuf Sekinat Funmilayo, Akinlabi Islamiyah Adenike, Nureni Mardiyat Arinola and Oseni Nuriyat Oyindamola carried out the experiments and analyzed data; Amin Abdulbasit and Ayinla Maryam Tayo designed the experiments and wrote the manuscript.

REFERENCES

1. LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, 23, 155–184.
2. Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Annual Review of Neuroscience*, 24, 897–931.
3. Tovote, P., Fadok, J. P., & Lüthi, A. (2015). Neuronal circuits for fear and anxiety. *Nature Reviews Neurosci*, 16, 317–331.
4. Akam, T., Lustig, A., Rowland, J.M., Kapanaiah, S.K.T., Esteve-Agraz, J., Panniello, M., Márquez, C., Kohl, M.M., Kätzel, D., Costa, R.M., Walton, M.E. (2022). Open-source, Python-based, hardware and software for controlling behavioural neuroscience experiments. *eLife* 11: e67846.
5. Voigts, J., Siegle, J. H., Pritchett, D. L., & Moore, C. I. (2013). The flexDrive: An ultra-light implant for optical control and highly parallel chronic recording of neuronal ensembles. *Frontiers in Systems Neuroscience*, 7, 8.
6. Rudy, J.W. (1996). Scopolamine administered before and after training impairs both contextual and auditory-cue fear conditioning. *Neurobiology of Learning and Memory,* 65(1): 73 - 81.
7. Fanselow, M. S. (1980). Conditioned and unconditioned components of post-shock freezing. *The Pavlovian Journal of Biological Science*, 15, 177–182.
8. Chaaya, N., Jacques, A., Belmer, A., Beecher, K., Ali, S.A., Chehrehasa, F., Battle, A.R., Johnson, L.R., Bartlett, S.E. (2019). Contextual Fear Conditioning Alter Microglia Number and Morphology in the Rat Dorsal Hippocampus. *Front Cell Neurosci.*, 14;13:214.
9. Morden B, Mitchell G, Dement W. (1967). Selective REM sleep deprivation and compensation phenomena in the rat. *Brain Res.*, 5(3):339-49.
10. Suchecki D, Tufik S. (2000). Social stability attenuates the stress in the modified multiple platform method for paradoxical

sleep deprivation in the rat. *Physiol Behav.,* 68(3):309-316.
11. Kim, J.J., Fanselow, M.S. (1992). Modality-specific retrograde amnesia of fear. *Science,* 1; 256(5057): 675-677.
12. Curzon, P., Rustay, N.R., Browman, K.E. (2009). Cued and Contextual Fear Conditioning for Rodents. In: Buccafusco, J.J., editor. Methods of Behavior Analysis in Neuroscience. 2nd ed. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 2.
13. Phillips, R.G., LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neuroscience*, 106(2): 274-85.
14. Anagnostaras, S.G., Gale, G.D., Fanselow, M.S. (2001). Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus*, 1;11(1):8-17.
15. Graves, L. A., Heller, E. A., Pack, A. I., & Abel, T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learning & Memory,* 10(3), 168–176.
16. Ruksin, V., Seeman, P., & Ruskin, D. N. (2004). Sleep deprivation affects hippocampus-mediated contextual learning but not amygdala-mediated cued learning. *European Journal of Neuroscience, 19*(11), 3215–3221.
17. Wallenstein, G.V., Vago, D.R. (2001). Intrahippocampal scopolamine impairs both acquisition and consolidation of contextual fear conditioning. *Neurobiol Learn Mem*., 75(3):245-252.
18. Maren, S., Fanselow, M.S. (1996). The amygdala and fear conditioning: has the nut been cracked? *Neuron*; 16(2): 237-40.
19. LeDoux, J.E. (2014) Coming to terms with fear. *Proc Natl Acad Sci U S A*.; 111(8):2871-2878.
20. Anagnostaras S.G., Maren S., & Fanselow M.S. (1995). Scopolamine selectively disrupts the acquisition of contextual fear conditioning in rats. *Neurobiology of Learning and Memory*, 64, 191-4.