Video Article

Long-term Potentiation of Perforant Pathway-dentate Gyrus Synapse in Freely Behaving Mice

J. Harry Blaise[1]

[1]Department of Engineering and Neuroscience Program, Trinity College

Correspondence to: J. Harry Blaise at harry.blaise@trincoll.edu

URL: https://www.jove.com/video/50642
DOI: doi:10.3791/50642

Keywords: Behavior, Issue 81, freely behaving animal, mouse, dentate gyrus, hippocampus, long term potentiation, electrophysical research technique

Date Published: 11/29/2013

Citation: Blaise, J.H. Long-term Potentiation of Perforant Pathway-dentate Gyrus Synapse in Freely Behaving Mice. *J. Vis. Exp.* (81), e50642, doi:10.3791/50642 (2013).

Abstract

Studies of long-term potentiation of synaptic efficacy, an activity-dependent synaptic phenomenon having properties that make it attractive as a potential cellular mechanism underlying learning and information storage, have long been used to elucidate the physiology of various neuronal circuits in the hippocampus, amygdala, and other limbic and cortical structures. With this in mind, transgenic mouse models of neurological diseases represent useful platforms to conduct long-term potentiation (LTP) studies to develop a greater understanding of the role of genes in normal and abnormal synaptic communication in neuronal networks involved in learning, emotion and information processing. This article describes methodologies for reliably inducing LTP in the freely behaving mouse. These methodologies can be used in studies of transgenic and knockout freely behaving mouse models of neurodegenerative diseases.

Video Link

The video component of this article can be found at https://www.jove.com/video/50642/

Introduction

The development of technology to manipulate genes has produced transgenic and knockout mouse models of almost every neurodegenerative and neurological diseases. This has necessitated translation of electrophysiological research techniques previously used in larger rodent species to the mouse animal model. One such neurophysiological investigation technique is the use long-term potentiation (LTP) to test the efficacy of synaptic connections within neuronal networks involved in various neuropathological disorders. This protocol describes techniques for reliable electrophysiological investigation of LTP in freely behaving mice. The advantage of this protocol over others is that it is simple and easy to implement; it is also rather less costly as it does not require neither the use of expensive computer-controlled microdrive systems nor field effect transistor headstages; and, to our knowledge, is the first video protocol of chronic electrophysiological recordings to study LTP in freely behaving mice. To this end, we describe in this article simple methodologies for studying long-term potentiation in freely behaving mice. These methodologies can readily be translated to transgenic and knockout mouse models of neuropathological disorders.

Protocol

This protocol is appropriate for mice of 3 and 18 months of age and approximate body weight of 30-50 g). Mice can be obtained from The Jackson Laboratory (Bar Harbor, ME). All surgical and experimental protocols were approved by the Trinity College Animal Care and Use Committee and were in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

1. Animal Preparation and Surgical Procedures

1. Prepare anesthesia cocktail containing ketamine (25 mg/ml), xylazine (2.5 mg/ml) and acepromazine (0.5 mg/ml). Inject 1 ml/kg body weight in the lower right quadrant of the abdomen supplemented by 0.2 ml/kg injections every 45 min thereafter to maintain a stable depth of anesthesia using the pedal withdrawal reflex.
2. Prepare anesthetized mouse for surgery by shaving the fur on its cranium using an electric shaver. The shaved area should include the middle of the eyes to the middle of the ears. Make sure not to shave the whiskers as they are part of the mouse barrel sensory system.
3. Use cotton swabs to apply isopropyl alcohol followed by Betadine to the shaved area on the head. Then, also using cotton swabs, apply a small amount of mineral oil on the eyes to prevent the eyeballs from drying.
4. Use infant rat ear cuffs instead of regular ear bars to mount the animal's head on the stereotaxic frame apparatus. To do this, first mount the left ear on the left ear cuff. Then gently ease the right ear onto the right cuff by gently supporting the animal's head with one hand and advancing the ear cuff with the other hand. Next, place a heating pad under the animal's body and set it to 86 °F to maintain body temperature.

5. Then, check for bilateral rigidity by attempting to move the head side to side. The animal is properly mounted on the stereotaxic frame when the head is rigid and cannot be moved side to side but can easily pivot up and down.
6. Gently rest the mouse's snout on the nose/tooth bar assembly making sure that the incisors are resting gently but securely within the tooth aperture of the nose/tooth bar.
7. Using a sterile scalpel, make a midline incision starting from the middle of the eyes to the middle of the ears. Make sure the scalpel is be held at a 45° angle and apply enough pressure to cut through the underlying fascia but not through the skull as that will cause excessive bleeding.
8. Use cotton swabs to separate the fascia and expose the skull. Skull landmarks bregma and lambda should be clearly visible (**Figure 1A**). Ensure that the animal is in the skull flat position by adjusting the nose/tooth bar such that bregma and lambda landmarks are at same the dorsoventral (DV) position (± 0.1 mm).
9. Apply a small amount of sterile saline to the exposed area and use cotton swabs to gently clean the skull. Then wait 2-3 min for the skull to completely air dry before proceeding.
10. Mount a needle on the left stereotaxic arm to measure the DV position of bregma and lambda. The DV positioning of these two landmarks should be within 0.1 mm of each other. If not, the nose bar of the stereotaxic frame can be adjusted up and down to achieve this.
11. Position the needle on bregma to record its anterior-posterior (AP), lateral (LAT) and dorsoventral (DV) measurements. Be sure the needle just touches the skull and does not penetrate it.
12. Use a mouse brain atlas, such as "The Mouse Brain in Stereotaxic Coordinates"[1], to determine the coordinates for the target structures: medial perforant pathway (mPP) in the angular bundle and dentate gyrus (DG) of the hippocampus, relative to lambda and bregma, respectively (also see **Table 1**). Then, use a fine-point pen or pencil to mark these points on the skull.
13. Also, mark two more points on the contralateral side of the skull. These points which will serve as ground (GND) and reference (REF) should be about 3 mm from the midline and positioned longitudinally relative to each other (**Figure 1A**, also **Table 1**).
14. Remove the needle marker from the left stereotaxic arm and replace it with an electric dental drill equipped with a stereotaxic mount. Position the drill over each marked point and make small burr holes of approximately 0.5 mm in diameter. When drilling, use a repetitive up-and-down motion and check frequently whether the drill has penetrated the skull to expose the brain surface.
15. Gently but firmly drive a screw electrode (#0-80 1/8 in stainless steel machine screws, slotted fillister head) in each of the contralateral holes (GND and REF) such that they just touch the cortical surface without penetrating it (**Figure 1A**). These two screw electrodes serve as ground and reference.
16. Mount stimulating and recording electrodes in electrode holders on the left and right stereotaxic arms, respectively.
17. Connect the stimulating electrode terminals to an electrophysiological stimulator with current isolation and the recording electrode terminal to an electrophysiological differential amplifier (gain = 1,000, bandpass filter = 1 Hz–3 kHz) and then to a digital oscilloscope for visual inspection of evoked responses. Also, connect GND and REF electrode terminals to the differential amplifier.
18. Gently and slowly lower both stimulating and recording electrodes in 0.5 mm increments while visually monitoring the evoked response to a stimulus shock of 600-800 uA. Continue to lower each electrode until they reach their respective target DV position and a stereotypical signal is observed on the oscilloscope (**Figure 1B**).
19. Afterward, use dental acrylic cement to make a cap to hold the electrode terminals in place; any dental cement that touches the skin should be wiped off immediately. Once the dental cement is completely cured transfer the animal from the stereotaxic frame to a clean rodent cage. Use a heating lamp or pad to maintain core temperature.
20. Monitor the animal every hour until it regains consciousness. An injection of flunixin may be administered as an analgesic.

2. LTP Induction

1. Allow the animal 5-7 days to recover from surgery. Place the animal in the recording environment consisting of a faraday cage (122 cm x 43 cm x 43 cm) outfitted with soundproofing material and a 5-channel rotating commutator connecting the electrode leads to the stimulating/ recording setup.
2. Allow the animal to acclimate for 1-2 hr in the recording environment before connecting the electrodes to the stimulating and recording instruments (refer to step 1.17).
3. Set the stimulator controls to output 400 uA and record the amplitude of an average of 10 evoked responses making sure that at least 10 sec elapse between stimuli. Use the method shown in **Figure 1C** to quantify the evoked response. Repeat this procedure for 600, 800, 1,000, 1,200, and 1,400 uA.
4. Construct an input/output curve by plotting the average amplitude of the evoked response versus stimulus intensity. From this plot determine the stimulus intensity which corresponds to 50% of the maximum amplitude measured. Use the 50% intensity for the remainder of the experiment.
5. Then, using the 50% stimulus intensity, obtain a baseline by recording the average amplitude of 5 evoked responses every minute for 15 min. Again make sure that at least 10 sec elapse between stimuli.
6. Subsequently deliver the tetanic stimulation consisting of 10 bursts of 10 pulses delivered at 400 Hz with a burst rate of 5 Hz to the medial perforant pathway. Monitor the animal closely for signs of seizure, including wet dog shakes. If the animal has a seizure the experiment should be terminated and all data from that animal should be excluded from the study results.
7. Then, continue to record the average amplitude of 5 evoked responses every minute for 30 min posttetanization. After that, compare this amplitude to the baseline amplitude obtained in step 2.5 by calculating the percent change from baseline (**Figure 2**).
8. Following the conclusion of experiments, the animal is euthanized by means of inhalation of isoflurane—a method which is consistent with the Guidelines on Euthanasia of the American Veterinary Medical Association.

Representative Results

Table 1 shows the coordinates for DG and mPP as used in this protocol. **Figure 1A** shows the markings for the target structures on the skull; also shown are the location of the ground and reference electrodes. **Figure 1B** illustrates representative evoked response traces both pre- and posttetanization in the same animal. Note that the posttetanization evoked response is larger than the pretetanization response which is indicative of LTP induction[2]. **Figure 1C** illustrates the method used to quantify the response amplitude. Indeed, **Figure 2** shows percent change in response amplitude over a time course spanning both pre- and posttetanization time periods. It is to be noted that peak LTP values exceeded

100%. These results of enhanced response amplitude following tetanization indicate that this protocol is successful and therefore reliable for studying LTP in the freely behaving mouse model.

Structure	AP (mm)	LAT (mm)	DV (mm)
DG	-2.0	+1.5	-2.0
mPP	+0.5	+3.2	-2.0
REF	-1.5	-3.0	0.0
GND	+1.5	-3.0	0.0

Table 1. Coordinates for target structures. Anterior-posterior (AP) coordinates for dentate gyrus (DG) and REF are given relative to bregma while those for medial perforant path (mPP) and GND are relative to Lambda. All DV coordinates are relative to the cortical surface below dura.

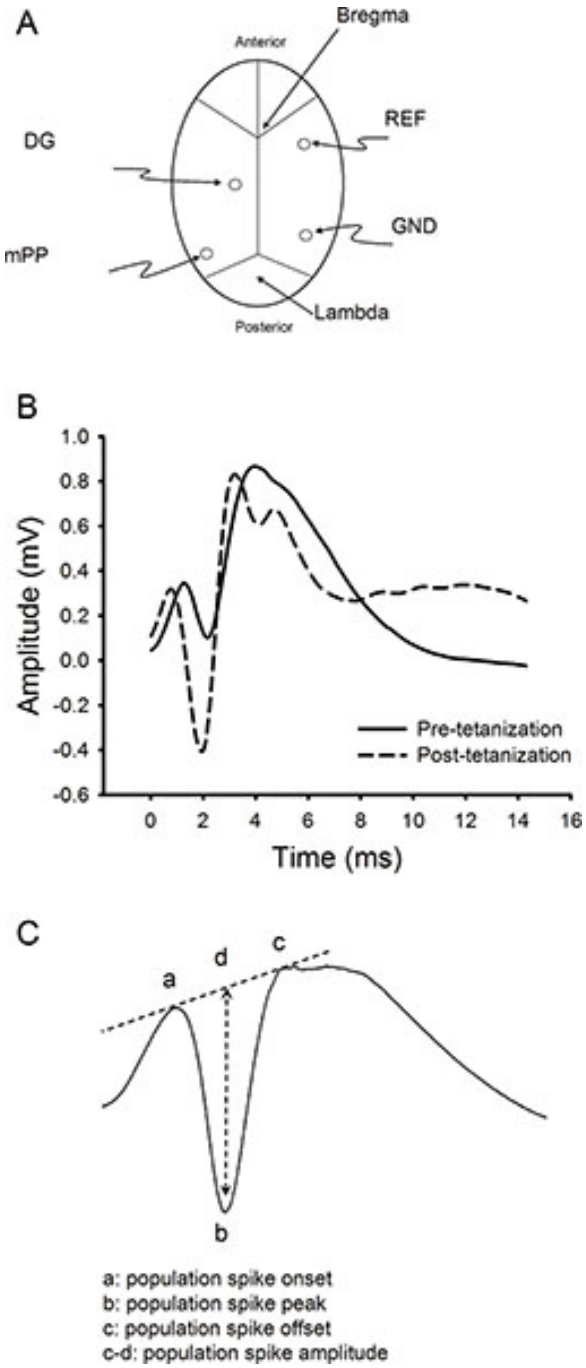

Figure 1. Electrode location and representative traces of the evoked response. A) Diagrammatic illustration of the relative location of electrodes on the mouse skull. **B**) Typical traces of the evoked response both pre- and posttetanization. **C**) Algorithm used to quantify the amplitude of the evoked response.

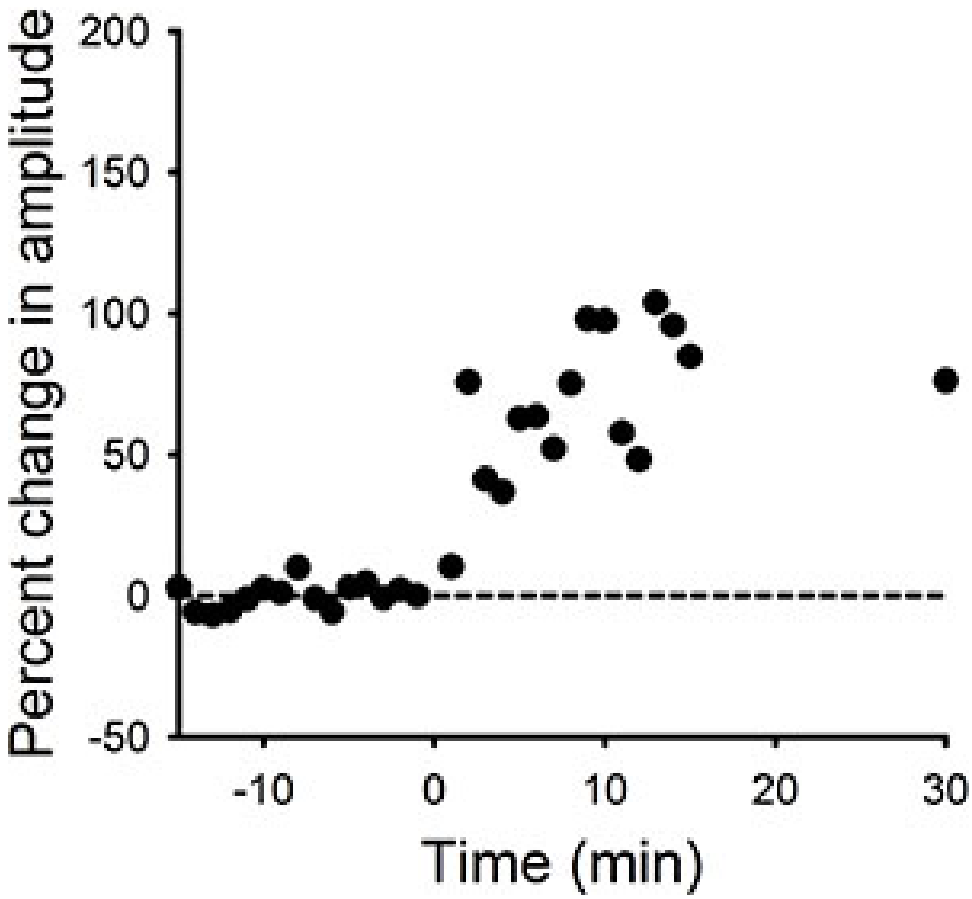

Figure 2. LTP in the medial perforant path-dentate gyrus synapse. Representative result of LTP induction in mPP-DG synapse of a freely behaving mouse. Note posttetanic stimulation enhancement of the evoked response amplitude indicative of LTP induction.

Discussion

In this protocol, we have demonstrated a reliable and simple method for studying LTP in DG in freely behaving mice. While many studies of LTP in awake rats have been performed[3,4], very few have been conducted in awake mice primarily due to the technical complexity posed by the limited cranial real estate in mice and the weight of electrode headstages relative to the average weight of mice[5]. The few studies that have demonstrated LTP in DG in freely behaving mice utilized either microdrive electrode systems or junction field effect transistor (jFET) preamplifiers integrated in the headstage which necessarily adds to the electrode payload burden to the animal[6-10]. The significance of the present protocol is that it represents an improvement over existing methods for inducing LTP in the DG in freely behaving mice as it avoids the use of microdrive electrode systems or a headstage JFET's.

It is important to highlight the critical steps of this protocol. These include: 1) mounting of the mouse's head in the stereotaxic frame such that it is bilaterally rigid (this step may be the most difficult as it requires practice and familiarity with the technique); 2) optimal positioning of electrodes to maximize response magnitude can be enhanced by insuring that bregma and lambda are in the same plane which can usually be achieved by adjusting the stereotaxic frame tooth bar such that the difference in dorsoventral positioning of bregma and lambda is no greater than 0.1 mm; 3) during the recording phase of the experiment, it is recommended that animals be allowed time to habituate to the recording environment because the novelty of the recording environment may insinuate fluctuations in the evoked response data collected; 4) suitable selection of electrodes will improve signal quality and fidelity: bipolar electrodes (stainless steel hypodermic tubing with 0.2 mm stainless steel wire insert with a tip separation of 0.5 mm) are preferred for stimulating while monopolar electrodes (epoxylite-insulated single strand tungsten wire) are used for recording in nervous tissue; and 5) mice can be anesthetized using an intraperitoneal injection of a mixture of ketamine (25 mg/ml), xylazine (2.5 mg/ml) and acepromazine (0.5 mg/ml). This anesthetic mixture should then be administered at a dosage of 1ml/kg which is usually effective in about 20 min supplemented by 0.2 ml/kg every 45 min to maintain a stable depth of anesthesia.

There are a few limitations of this protocol which bear mentioning. This protocol does not give any insight on ion channel mechanisms or receptor protein synthesis that may subserve LTP. Therefore scant information is available concerning the actual numbers of neurons in the population being recorded. Another limitation is that since evoked responses are being collected in awake animals it is difficult to ascertain and dissect out the effect of factors such as stress, handling, or contamination of the recorded signal by spurious sensorimotor activity. These limitations can be overcome by ensuring that evoked responses are recorded only when the animal is the inactive but alert state, otherwise known as the quiet waking vigilance state.

Nonetheless, the techniques described in this protocol provide the most physiologically relevant platform for investigating brain electrical activity underlying behavior. Following the steps outlined in this protocol, any brain structure can be targeted by using the proper coordinates as given

by an atlas of the mouse brain[1]. It is important to note that the adult rat stereotaxic frame can be used in mice provided that suitable infant rat ear cuffs are used instead of the regular ear bars. The ear cuffs will immobilize the head without damaging the mouse's ears. Finally, the methodologies presented here can be readily translated to electrophysiological investigations in transgenic and knockout mouse models of a host of neurological disorders.

Disclosures

Authors have nothing to disclose.

Acknowledgements

The authors wish to acknowledge the following: Dr. Joseph Bronzino, Dr. Khamis Abu-Hassaballah, Mr. R.J. Austin-LaFrance, and Ms. Jessica Koranda.

References

1. Paxinos, G. & Franklin, K. B. J. *The mouse brain in stereotaxic coordinates*. Compact 2nd edn. Elsevier Academic Press (2004).
2. Bliss, T. V. P. & Lomo, T. Long-lasting potentiation of synaptic transmission in the dentate area of the anesthetized rabbit following stimulation of the perforant path. *J. Physiol.* **232** 331-356 (1973).
3. Blaise, J. H. & Bronzino, J. D. Effects of stimulus frequency and age on bidirectional synaptic plasticity in the dentate gyrus of freely moving rats. *Exp. Neurol.* **182**, 497-506, doi:S0014488603001365 [pii] (2003).
4. Blaise, J. H., Koranda, J. L., Chow, U., Haines, K. E. & Dorward, E. C. Neonatal isolation stress alters bidirectional long-term synaptic plasticity in amygdalo-hippocampal synapses in freely behaving adult rats. *Brain Res.* **1193**, 25-33, doi:S0006-8993(07)02852-1 [pii] 10.1016/j.brainres.2007.11.049 (2008).
5. Koranda, J. L., Masino, S. A. & Blaise, J. H. Bidirectional synaptic plasticity in the dentate gyrus of the awake freely behaving mouse. *J. Neurosci. Methods.* **167**, 160-166, doi:S0165-0270(07)00404-9 [pii] 10.1016/j.jneumeth.2007.08.001 (2008).
6. Cooke, S. F. *et al.* Autophosphorylation of alphaCaMKII is not a general requirement for NMDA receptor-dependent LTP in the adult mouse. *J. Physiol.* **574**, 805-818, doi:jphysiol.2006.111559 [pii] 10.1113/jphysiol.2006.111559 (2006).
7. Davis, S., Bliss, T. V., Dutrieux, G., Laroche, S. & Errington, M. L. Induction and duration of long-term potentiation in the hippocampus of the freely moving mouse. *J. Neurosci. Methods.* **75**, 75-80, doi:S0165-0270(97)00053-8 [pii] (1997).
8. Jones, M. W., Peckham, H. M., Errington, M. L., Bliss, T. V. & Routtenberg, A. Synaptic plasticity in the hippocampus of awake C57BL/6 and DBA/2 mice: interstrain differences and parallels with behavior. *Hippocampus.* **11**, 391-396, doi:10.1002/hipo.1053 (2001).
9. Zhang, T. A., Tang, J., Pidoplichko, V. I. & Dani, J. A. Addictive nicotine alters local circuit inhibition during the induction of *in vivo* hippocampal synaptic potentiation. *J. Neurosci.* **30**, 6443-6453, doi:10.1523/JNEUROSCI.0458-10.2010 30/18/6443 [pii] (2010).
10. Tang, J. & Dani, J. A. Dopamine enables *in vivo* synaptic plasticity associated with the addictive drug nicotine. *Neuron.* **63**, 673-682, doi:10.1016/j.neuron.2009.07.025 S0896-6273(09)00580-7 [pii] (2009).

