Published in final edited form as:

J Neurosci Methods. 2008 January 30; 167(2): 160–166.

Bidirectional synaptic plasticity in the dentate gyrus of the awake freely behaving mouse

Jessica L. Koranda^{1,2}, Susan A. Masino^{2,3}, and J. Harry Blaise^{1,2}

¹Department of Engineering, Trinity College, Hartford, CT 06106

²Neuroscience Program, Trinity College, Hartford, CT 06106

³Department of Psychology, Trinity College, Hartford, CT 06106

Abstract

There is significant interest in *in vivo* synaptic plasticity in mice due to the many relevant genetic mutants now available. Nevertheless, use of *in vivo* models remains limited. To date long-term potentiation (LTP) has been studied infrequently, and long-term depression (LTD) has not been characterized in the mouse *in vivo*. Herein we describe protocols and improved methodologies we developed to record hippocampal synaptic plasticity reliably from the dentate gyrus of the awake freely behaving mouse. Seven days prior to recording, we implanted microelectrodes encapsulated within a lightweight, low-profile headstage assembly. On the day of recording, we induced either LTP or LTD in the awake freely behaving animal and monitored subsequent changes in population spike amplitude for at least 24 hrs. Using this protocol we attained 80% success in inducing and maintaining either LTP or LTD. Recording from a chronic implant using this improved methodology is best suited to reveal naturally occurring brain activity, and avoids both acute effects of local electrode insertion and drifts in neuronal excitability associated with anesthesia. Ultimately a reliable freely behaving mouse model of bidirectional synaptic plasticity is invaluable for full characterization of genetic models of disease states and manipulations of the mechanisms implicated in learning and memory.

Keywords

Synaptic plasticity; freely behaving mouse; hippocampus; dentate gyrus; perforant pathway; long term potentiation; long term depression; chronic recording

1. Introduction

It is widely accepted that changes in synaptic strength underlie the brain's ability to learn and remember. Synaptic plasticity phenomena such as long-term potentiation (LTP) and long-term depression (LTD) have been used successfully as models to test neurophysiological and neurobiological mechanisms associated with changes in the efficacy of synaptic transmission (Bliss and Lomo, 1973; Mockett et al., 2002; Abraham and Williams, 2003; Pastalkova et al., 2006; Whitlock et al., 2006). LTP is observed as a sustained increase in synaptic efficacy while

Corresponding author: J. Harry Blaise, Trinity College, Neuroscience Program/Engineering Department, 300 Summit Street, Hartford, CT 06106-3100, Tel: 1.860.297.2225, Fax: 1.860.297.3531, Email: harry.blaise@trincoll.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

LTD is a sustained decrease in synaptic efficacy. These synaptic changes provide a high-capacity, bidirectional, and synapse-specific framework for information processing and memory consolidation and storage in the nervous system.

Both hippocampal LTP and LTD can be studied *in vitro* or *in vivo*. An overwhelming majority of studies of LTP/LTD in the hippocampus are conducted in *in vitro* slices due to several favorable factors, including the higher success rate associated with hippocampal slices compared to *in vivo* recordings. Other advantages include the suitability of hippocampal anatomy for yielding multiple slices from a single animal and the excellent electrophysiological and pharmacological control of and electrophysiological access to isolated hippocampal slice tissue.

Despite these advantages, laboratory protocols differ widely in physiological and mechanical treatment of slices. Differences may include varying buffer composition used to store and record in slices of brain tissue, diverse physical layout and construction of recording chambers, and the varying stimulation paradigm and recording criteria employed. Recently, it has been suggested that such differences may generate varying and sometimes conflicting results (Moore et al., 2003; Kukley et al., 2005). Unavoidably, slices *in vitro* exist with an overall loss of connectivity and within a significantly altered milieu compared to *in vivo* preparations, both of which may influence synaptic plasticity and cell excitability (Freudenthal et al., 2004). Disparate findings in *in vivo* vs. *in vitro* preparations may be due to the absence of important afferents (locus coeruleus, dorsal and median raphe, and medial septum) in the hippocampal slice which provide neuromodulatory regulation of dentate granule cell excitability (Hargreaves et al., 1990; Bordi et al., 1997; Blaise and Bronzino, 2003).

Commonly employed *in vivo* methodologies, including acute and chronic preparations, also present some drawbacks. Both anesthetized and freely behaving *in vivo* techniques suffer from surgical trauma and local tissue damage due to electrode insertion; some effects on neuronal function may persist for several hours or days (Gilbert and Mack, 1999). Anesthetized models suffer additionally from alterations in neuronal excitability due to the presence of anesthetics, which often have an overall depressing effect on LTP (Simon et al., 2001; Wei et al., 2002; Izaki et al., 2004; Takamatsu et al., 2005) and upward drift effects on tonic neuronal excitability (Gilbert and Mack, 1999). Chronic *in vivo* recordings in freely behaving animals avoid some of these limitations and complications (summarized in Table 1). Although this technique is inherently more difficult and time-consuming, *in vivo* freely behaving preparations may provide the most accurate and holistic view of what is naturally occurring within the brain as animals explore their environment.

Previously we developed a freely behaving rat model to interpret the influence of factors such as age, stress, and protein malnutrition on LTP/LTD within the dentate gyrus (DG) and basal lateral amygdala (Bronzino et al., 1996; Bronzino et al., 2001; Blaise and Bronzino, 2003). Aside from these environmental factors, both LTP and LTD are also influenced by endogenous factors such as genetics and disease states. More recently the literature concerning the study of the effects of various endogenous factors on LTP/LTD has been dominated by the use of genetically manipulated animal models of physiology including both constitutive and inducible knockin and knockout gene models (Kuenzi et al., 2003; Jeon et al., 2004; Kim et al., 2005; Giménez-Llort et al., 2005; Yun et al., 2005; Fisch 2006; Hu et al., 2006; Spalloni et al., 2006; Tamura et al., 2006a,b). At the present time, transgenic animal models aimed at understanding basic neurophysiology, neurobiology and neuropathology are developed most commonly in mice. Both *in vitro* and *in vivo* studies involving gene knockin/out mice can greatly increase our ability to explore and understand the molecular underpinnings of LTP/LTD. Therefore, it is important to test and confirm important conclusions found in slice preparations in the whole mouse model whenever possible.

Previous studies have reported on LTP in the freely moving mouse (Davis et al., 1997; Jones et al., 2001), while others have reported on spontaneous neuronal firing as measured by the EEG and unit activity recordings (Buhl et al., 2003; Buzsáki et al., 2003; Nolan et al., 2004). However, these studies used either a microdrive (Buhl et al., 2003; Buzsáki et al., 2003; Nolan et al., 2004) or a headstage dual junction field effect transistor (JFET) amplifier to record dentate field evoked potentials (Davis et al., 1997; Jones et al., 2003). Either of these configurations adds weight to the assembly on the head of the mouse. Here we describe an improved, lightweight and simplified methodology for inducing LTP and LTD in freely behaving mice which does not require either a microdrive or a headstage JFET and permits unrestricted movement of mice during testing.

2. Materials and Methods

2.1 Animals and Surgery

Male and Female C57BL/6 adult mice (3–18 months; body weight 30–50 g) were housed in a colony maintained on a 12-hour/12-hour light/dark schedule at 22°C with 55% humidity and allowed access to food and water ad lib. 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.

Electrode implantation and recording procedures used in adult mice were adopted and customized from procedures used to record neuronal signals successfully from awake behaving neonatal rats in our laboratories (Bronzino et al., 1996; Bronzino et al., 2001; Blaise and Bronzino, 2003). Adult mice were anesthetized intraperitoneally (IP) with 65 mg/kg sodium pentobarbital (Abbott Laboratories, Chicago, IL) and placed in a stereotaxic frame outfitted with infant rat ear cuffs (David KOPF Instruments, Tujunga, California) to immobilize the head. Body temperature was continually monitored and maintained with thermostat-controlled heating lamps. An incision was made along the midline of the head revealing the underlying skull and connective tissue. The skin was retracted, the connective tissue scrapped away and the skull was air dried completely. A layer of cyanoacrylate (Krazy Glue, Columbus, OH) was added to completely cover the skull and strengthen it. Bregma was located and used to determine the coordinates of the location of the dentate gyrus (DG) of the hippocampus (AP = −2.0 mm, LAT = +0.5 mm from Bregma and 1.5–2.0 mm DV) while Lambda was used to locate the perforant pathway (PP) of the angular bundle leading from the entorhinal cortex (EC) to the hippocampus (AP = +0.5 mm, LAT = +3.2 mm from Lambda and 1.5–2.0 mm DV). Coordinates were adapted from Namgung et al. (1995). A dental drill (Foredom Electrical Co., Bethel, CT) was then used to drill ipsilateral holes (~0.5 mm diameter) in the skull above both the DG and PP. Two additional bur holes were drilled on the contralateral skull plate for the placement of ground and reference electrodes. Figure 1A–B shows a schematic of the skull and electrode placements and coordinates.

A bipolar stimulating electrode was lowered into the PP. The bipolar electrode was constructed from a 29-gauge stainless steel hypodermic tubing (Small Parts Inc., Miami Lakes, FL) which was completely insulated with Epoxylite (P.D. George Co., Atlanta, GA) except at the flat-point tip. A fully insulated 32-gauge stainless-steel wire (Small Parts Inc., Miami Lakes, FL) was inserted into the hypodermic tubing so that the tip separation equaled approximately 0.5 mm. The only uninsulated portion of the wire insert was the flat-point tip where it was cut to the appropriate length.

A monopolar recording electrode consisting of an Epoxylite-insulated single-strand tungsten wire (0.152-mm diameter; Small Parts Inc., Miami Lakes, FL) was lowered into the granule cell layer of the dentate gyrus hilus in the hippocampus to record extracellular field potentials evoked by stimulation of the PP. Ground and reference electrodes consisting of uninsulated

stainless steel machine screws were positioned contralaterally on the cortical surface of the parietal cortex. The recording and stimulating electrodes were lowered until the maximal evoked response was visually confirmed on a digital oscilloscope (Nicolet Instruments, Madison, WI). Electrodes were further lowered an additional 0.1 mm to ensure they were not pulled out of position when cemented in place. A typical PP-DG signal is pictured in Figure 1C.

Once the appropriate signal was located, a second layer of cyanoacrylate was placed on the skull surrounding the electrodes. Individual electrode wires were then connected to individual male contact pins (Electronic Connector Corp., Chicago, IL). and the assembly was fixed to the skull with fast-drying dental acrylic (Lang Dental Manufacturing, Wheeling, IL). Animals were allowed to recover for approximately 7 days prior to any subsequent electrophysiological testing.

2.2 Electrophysiology

On the day of electrophysiological testing, mice and their cages (42 cm × 22 cm × 21 cm) were placed in a sound-attenuating recording chamber (Faraday cage) in order to minimize electromagnetic noise effects. After one hour of acclimation time the exposed male contact pins were attached to the bottom of a 360° rotating six-channel commutator (Plastics One Inc., Roanoke, VA), permanently attached to the inside wall of the recording chamber, via low noise connector cables (Plastics One, Inc., Roanoke, VA) cut to approximately 40.0 cm in length and outfitted with female contact pins (Electronic Connector Corp., Chicago, IL). The nature of the commutator allows the animals to explore their cages in the recording chamber unimpeded. A second set of connector cables led from the top of the commutator and split so that the three cables corresponding to the recording and ground/reference electrodes led to an amplifier while the two cables corresponding to the bipolar electrode led to a pair of photostimulus isolation units connected to the recording apparatus. The entire apparatus allows for free movement throughout the chamber. Animals also were allowed food and water *ad libitum*. Once connected, animals were allowed an additional 1 hour to habituate to the recording environment and recordings were acquired only when animals were in a vigilance state of quiet waking to minimize evoked response variability (Bronzino et al., 1997; Blaise and Bronzino, 2000; Abraham et al., 2001; Blaise and Bronzino, 2003).

Electrical stimulation was provided by a Grass S-88 stimulator and consisted of biphasic square-wave pulses (total pulse width = 0.25 ms, 50% duty cycle) passed through a pair of Grass PSIU-6 photostimulus isolation units to provide constant current (Astro-Med, Inc., West Warwick, RI). Evoked responses were amplified (gain = 1000), bandpass filtered from 1 Hz - 3kHz (DAM-50, World Precision Instr., Sarasota, FL), passed to a digital storage oscilloscope for visual inspection and then to a custom-designed LabView (National Instruments, Austin, TX) data acquisition and analysis system (Blaise and Bronzino, 2003). Figure 1C shows a representative evoked response recorded in the dentate gyrus and the methods used to quantify the population spike amplitude (PSA). Figure 2A shows a freely behaving mouse connected to the low noise cabling.

After verifying an appropriate evoked response from the chronic implant, an input/output (I/O) curve was constructed for each animal by averaging 10 population spike amplitude (PSA) responses evoked by single-pulse current stimuli applied to the PP at varying current intensities (200, 400, 600, 800, 1000, 1200 and 1400 μ A) (Figure 3A). A characteristic triphasic dentate response and an appropriate I/O curve were verified and required from each animal prior to any induction of synaptic plasticity. From the constructed I/O curve, the stimulation intensity required to evoke a population response between 25–50% of the maximum evoked response was determined and this current intensity was then used for the remainder of the recordings. To establish a baseline response amplitude, five evoked responses at the 25–50% current

stimulus intensity were recorded and averaged every minute for 15 minutes. This was followed by tetanization consisting of a single train of 900 pulses delivered at a frequency of either 1 or 100 Hz (to induce LTD and LTP, respectively). All animals were monitored closely for signs of seizure using both visual behavioral signs and electrographic criteria (Bronzino et al., 1999; Blaise and Bronzino, 2000; 2003). Population responses were recorded and analyzed for the first 15 minutes as well as 30 min, 60 min, 120 min, 180 min and 24 hrs after tetanization. The percent change in post-tetanization PSA relative to baseline was quantified and subjected to statistical analysis using a Student's t-test. Following experimentation animals were deeply anesthetized and sacrificed and electrode placement was confirmed histologically (Figure 2B).

3. Results

Both LTP and LTD were reliably induced and maintained in the perforant path to dentate gyrus synapse in freely behaving mice for at least 24 hours. Animals receiving 100 Hz tetanization ($n = 6$) exhibited a significant enhancement in the PSA ($+47.3 \pm 5.2\%$; $p < 0.001$; Figure 3B) relative to baseline after the first 180 minutes, indicating LTP had been reliably induced. LTP persisted at least 24 hours ($+38.3 \pm 6.7\%$). Likewise, a significant depression ($-36.2 \pm 8.1\%$; $p < 0.001$; Figure 3C) in synaptic responses was observed in animals receiving 1 Hz tetanization ($n = 5$), indicating LTD had been reliably induced. LTD also persisted at least 24 hours ($-29.61 \pm 2.5\%$). No overt changes in animal behavior were observed either during or after LTP or LTD induction.

Previous work in our laboratories to develop methodologies to record in the awake freely behaving neonatal rat contributed to the success of this adult mouse preparation (Blaise and Bronzino, 2003). Small details such as an initial coating of cyanoacrylate on the skull prior to drilling and again prior to affixing the electrodes in place with dental cement were important to help the chronic implant to adhere more permanently to the skull. In addition, electrodes were allowed to settle for at least 20 minutes prior to affixing the chronic implant permanently to the skull. The chronic implant weighed 0.3 g on average, totaling approximately 0.75% of the animal's body weight. Animals were then allowed to recover for 7 days before any electrophysiological recordings were taken. Recovering animals were monitored daily and no overt changes in animal behavior or health was observed during that period nor were any overt changes in activity observed when the animals were placed in the recording chamber.

In initial experiments, animals undergoing both the LTP and LTD induction protocols were tetanized at the current intensity that elicited 50% of the maximal evoked response. However, the 50% current intensity was found to be too intense for animals receiving train pulses at 100 Hz. Subsequent customization of the protocol used a current intensity level ranging between 25–50% the individual maximal baseline I/O evoked response and we established a final criteria of 25% for those animals receiving tetanization at a frequency of 100 Hz. In general, animals that showed either behavioral or electrographic signs of seizure were discarded from the study. A total of eleven mice are included in the final data set (LTD was successfully induced in 5 animals while LTP was successfully induced in 6 animals). The rate of successful recordings in this freely behaving mouse model was approximately 80%. Animals were then deeply anesthetized and sacrificed immediately following the 24 hr recording mark to confirm electrode placement via histology (Figure 2B). However, a few mice were retained as teaching tools and in these mice both the headstage assembly and the recorded signal remained viable for up to 4 months following initial electrode implantation.

4. Discussion

Here we establish a reliable protocol for chronic *in vivo* mouse recordings of bidirectional synaptic plasticity. Thus far, some electrophysiological investigations in freely behaving mice

have recorded multiunit EEG spike and field activity employing the use of a microdrive in the headstage assembly outfitted with an array of 2 to 8 electrodes to investigate the collective behavior and ongoing frequency patterns of neuronal networks (Buhl et al., 2003; Buzsáki et al., 2003; Nolan et al., 2004). Other investigations of synaptic plasticity in the freely behaving mouse model have measured evoked PSA but only examined LTP (Davis et al., 1997; Jones et al., 2001). Previous EEG studies used a microdrive electrode apparatus, whereas previous LTP studies used a complex headstage assembly. In latter case, electrodes were connected to a dual differential low noise JFET input operational amplifier (op-amp) integrated circuit embedded within the headstage assembly encapsulating the implanted electrode leads and fixed to the mouse's skull. However, the inclusion of the dual JFET op-amps requires a battery power supply as well as two miniature resistors and two miniature bypass capacitors to reduce noise. These components made the headstage assembly relatively heavy (approximately 2–4 % of average body weight) and cumbersome while recording in behaving animals (Davis et al., 1997). In contrast, in the present study we describe a much improved and simplified methodology to affix encapsulated electrode leads directly to the skull using a layer of dental acrylic with connecting wires passing through a commutator, thereby permitting free and undisturbed movement of behaving animals during electrophysiological recordings. Stimulating and recording wires from the commutator are then led directly to stimulating and recording instrumentation. Therefore, our methodology ensures that the headstage assembly remains light (approximately 0.75% of average body weight compared to 2–4% reported in Jones et al. (2001) and Davis et al. (1997), respectively) and low profile, yet strong enough to withstand damage and breakage. These features, along with unrestricted movements, minimize disturbances to the behaving animal during recording sessions.

In the present study, we successfully induced LTP and LTD in a chronic animal preparation using a freely behaving mouse model. Both LTP and LTD of the PSA were induced in the dentate gyrus seven days following electrode implantation and were maintained reliably for at least 24 hours. In general, the magnitude of both LTP- and LTD-induced changes observed in the adult mouse dentate gyrus are consistent with that obtained previously in the freely behaving adult rat dentate gyrus (Blaise and Bronzino, 2003). For instance, previous studies in our laboratories using freely behaving adult male rats undergoing LTD-inducing tetanization protocols of 1 Hz showed a depression in PSA relative to baseline of approximately 30%, similar to the magnitude of LTD observed here (36.2%). In that same study, freely behaving adult rats undergoing either 50 Hz or 200 Hz sustained stimulation showed approximately a 15 to 20% increase in PSA, somewhat less than the 47.3% obtained in this study (Blaise and Bronzino, 2003). Our present results in mice expand our capability beyond previous LTP/LTD studies in freely behaving neonatal rats to now explore models of hippocampal synaptic plasticity in a wide range of genetic knockout models currently available exclusively in the mouse.

It is widely accepted that LTP and LTD work together as co-mechanisms to create a dynamic gain control system. This bidirectional plasticity permits the widest range of alterations in activity-dependent synaptic responses in the repertoire of an organism faced with environmental challenges and/or novel situations (Abraham et al., 2001; Blaise and Bronzino, 2003). To our knowledge this is the first published report of LTD in the awake freely behaving mouse. It is well known that LTD is difficult to obtain in adult rat hippocampal slices (Bliss and Schoepfer, 2004; Liu et al., 2004), and a previous study by Milner and colleagues (2004) failed to demonstrate either the induction or maintenance of LTD in *in vitro* adult mouse hippocampal slices. Therefore, the improved methodology we describe here for chronic recordings in freely behaving mice may present a better protocol to induce LTD in adult mice. Moreover, we feel that the model we have developed may be better suited to and perhaps even more sensitive to detecting alterations in synaptic plasticity as a result of genetic mutation or pharmacological manipulation.

In spite of achieving a reliable and consistent change in synaptic plasticity, it is important to point out that the degree of potentiation observed in the present study does not match previously published data obtained in the mouse hippocampus. Even though the magnitude of LTP is larger than what we have observed previously in the adult rat (Blaise and Bronzino, 2003), our present results show a smaller degree of LTP when compared with previous results obtained by other investigators (Namgung et al., 1995; Davis et al., 1997; Matsuyama et al., 2005; Chen et al., 2006; Nagy et al., 2006; Tamura et al., 2006a,b). For example, both Namgung et al. (1995) and Matsuyama et al. (2005) reported potentiation of PSA amplitude of more than 100% in the dentate gyrus of anesthetized mice, and Davis et al., (1997) showed a similar change in degree of potentiation in awake freely behaving mice. Differing tetanization protocols likely contribute to the differences reported in those studies compared to ours. Namgung et al. (1995) and Matsuyama et al. (2004) used a 5 Hz bursting paradigm (theta burst stimulation-TBS) to induce LTP and Davis et al., (1997) used 0.1 Hz bursting paradigm while ours used sustained stimulation (900 total pulses). Our sustained stimulation protocol seeks to generate a frequency-response profile of bidirectional hippocampal plasticity and thus keeps the number of pulses in the LTP induction protocol the same as in the LTD protocol. In this way, it can be determined that the expression of LTP in the chronic mouse preparation results from high frequency stimulation (HFS) and LTD results from low frequency stimulation (LFS) rather than changes in the stimulation pattern or in the number of pulses. Another factor that may account for the differences seen in previous studies of PSA amplitude may be suppression of various neuronal projections to and from the hippocampus by anesthetics (Gilbert and Mack, 1999).

In summary, we have developed improved methodologies to successfully insert chronic implants to record evoked responses and synaptic plasticity in the dentate gyrus of freely behaving mice. Beyond the improved methods, we have demonstrated successfully the reliable induction and maintenance of strong LTP and LTD lasting at least 24 hours. This freely behaving mouse model of bidirectional hippocampal synaptic plasticity uses a lightweight, simplified headstage assembly and a one week recovery period between electrode implantation and recording. Ultimately this mouse model will be useful in confirming, characterizing and clarifying the effects of pharmacological or genetic manipulation on synaptic transmission and synaptic plasticity within neural circuitry implicated in learning and memory.

Acknowledgements

Support for this research was provided in part by funding from the NSF BES 0451285, NIH NS 29173, NASA CT Space Grant Consortium and a Howard Hughes Medical Institute Grant to Trinity College.

References

- Abraham WC, Mason-Parker SE, Bear MF, Webb S, Tate WP. Heterosynaptic metaplasticity in the hippocampus *in vivo*: a BCM-like modifiable threshold for LTP. *Proc Natl Acad Sci U S A* 2001;98:10924–9. [PubMed: 11517323]
- Abraham WC, Williams. Properties and mechanisms of LTP maintenance. *Neuroscientist* 2003;9:463–74. [PubMed: 14678579]
- Blaise JH, Bronzino JD. Modulation of paired-pulse responses in the dentate gyrus: effects of normal maturation and vigilance state. *Ann Biomed Eng* 2000;28:128–34. [PubMed: 10645796]
- Blaise JH, Bronzino JD. Effects of stimulus frequency and age on bidirectional synaptic plasticity in the dentate gyrus of freely moving rats. *Experimental Neurobiology* 2003;182:497–506.
- Bliss TVP, Lømo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol (Lond)* 1973;232:357–74. [PubMed: 4727085]
- Bliss TV, Schoepfer R. Controlling the ups and downs of synaptic strength. *Science* 2004;304:973–4. [PubMed: 15143268]

- Bordi F, Reggiani A, Conquet F. Regulation of synaptic plasticity by mGluR1 studied *in vivo* in mGluR1 mutant mice. *Brain Res* 1997;761:121–6. [PubMed: 9247074]
- Bronzino JD, Blaise JH, Austin-LaFrance RJ, Morgane PJ. Studies of dentate cell modulation: paired-pulse responses in freely moving rats at three ages. *Dev Brain Res* 1996;96:277–80. [PubMed: 8922689]
- Bronzino JD, Austin-LaFrance RJ, Mokler D, Morgane PJ. Effects of prenatal protein malnutrition on hippocampal long-term potentiation in freely moving rats. *Exp Neurol* 1997;48:317–23. [PubMed: 9398474]
- Bronzino JD, Blaise JH, Mokler DJ, Galler JR, Morgane PJ. Modulation of paired-pulse responses in the dentate gyrus: effects of prenatal protein malnutrition. *Brain Res* 1999;849:45–57. [PubMed: 10592286]
- Bronzino JD, Kehoe P, Mallinson K, Fortin D. Increased extracellular release of hippocampal NE is associated with tetanization of the medial perforant pathway in the freely moving adult male rat. *Hippocampus* 2001;11:423–9. [PubMed: 11530847]
- Buhl DL, Harris KD, Hormuzdi SG, Moyner H, Buzsáki G. Selective impairment of hippocampal gamma oscillations in connexin-36 knockout mouse *in vivo*. *J. Neurosci* 2003;23:1013–8.
- Buzsáki G, Buhl DL, Harris KD, Csicsvari J, Czeh B, Morozov A. Hippocampal network patterns of activity in the mouse. *Neuroscience* 2003;116:201–11. [PubMed: 12535953]
- Chen AP, Ohno M, Giese KP, Kuhn R, Chen RL, Silva AJ. Forebrain-specific knockout of B-raf kinase leads to deficits in hippocampal long-term potentiation, learning, and memory. *J Neurosci Res* 2006;83:28–38. [PubMed: 16342120]
- Davis S, Bliss TV, Dutrieux G, Laroche S, Errington ML. Induction and duration of long-term potentiation in the hippocampus of the freely moving mouse. *J Neurosci Methods* 1997;57:107–18. [PubMed: 7791361]
- Fisch, GS. Transgenic and knockout models of psychiatric disorders: introduction, history and assessment. In: Fisch, GS.; Flint, J., editors. *Transgenic and Knockout Models of Neuropsychiatric Disorders*. Humana Press; Totowa, New Jersey: 2006. p. 3-20.
- Freudenthal R, Romano A, Routtenberg A. Transcription factor NF-kappaB activation after *in vivo* perforant path LTP in mouse hippocampus. *Hippocampus* 2004;14:677–83. [PubMed: 15318326]
- Gilbert ME, Mack CM. Field potential recordings in dentate gyrus of anesthetized rats: stability of baseline. *Hippocampus* 1999;9:277–87. [PubMed: 10401642]
- Giménez-Llort L, Masino SA, Diao L, Fernández-Teruel A, Tobeña A, Halldner L, Fredholm BB. Mice lacking the adenosine A1 receptor have normal spatial learning and plasticity in the CA1 region of the hippocampus, but they habituate more slowly. *Synapse* 2005;57:8–16. [PubMed: 15858837]
- Hargreaves EL, Cain DP, Vanderwolf CH. Learning and behavioral-long-term potentiation: Importance of controlling for motor activity. *J Neurosci* 1990;10:1472–8. [PubMed: 2332791]
- Hu D, Serrano F, Oury TD, Klann E. Aging-dependent alterations in synaptic plasticity and memory in mice that overexpress extracellular superoxide dismutase. *J Neurosci* 2006;26:3933–41. [PubMed: 16611809]
- Izaki Y, Takita M, Nomura M, Akema T. Effects of ventral hippocampal long-term potentiation and depression on the gamma-band local field potential in anesthetized rats. *Exp Brain Res* 2004;157:147–51. [PubMed: 15112114]
- Jeon D, Yang YM, Jeong MJ, Philipson KD, Rhim H, Shin HS. Enhanced learning and memory in mice lacking Na⁺/Ca²⁺ exchanger 2. *Neuron* 2004;38:965–76. [PubMed: 12818181]
- Jones MW, Peckham HM, Errington ML, Bliss TVP, Routtenberg A. Synaptic plasticity in the hippocampus of awake C57BL/6 and DBA/2 mice: interstrain differences and parallels with behavior. *Hippocampus* 2001;11:391–6. [PubMed: 11530843]
- Kim CH, Takamiya K, Petralia RS, Sattler R, Yu S, Zhou W, Kalb R, Wenthold R, Huganir R. Persistent hippocampal CA1 LTP in mice lacking the C-terminal PDZ ligand of GluR1. *Nat Neurosci* 2005;8:985–7. [PubMed: 16007085]
- Kuenzi F, Rosahl TW, Morton RA, Fitzjohn SM, Collingridge GL, Seabrook GR. Hippocampal synaptic plasticity in mice carrying the rd mutation in the gene encoding cGMP phosphodiesterase type 6 (PDE6). *Brain Res* 2003;967:144–51. [PubMed: 12650975]

- Kukley M, Schwan M, Fredholm BB, Dietrich D. The role of extracellular adenosine in regulating mossy fiber synaptic plasticity. *J Neurosci* 2005;25:2832–7. [PubMed: 15772343]
- Liu L, Wong TP, Pozza MF, Ligenhoehl K, Wang Y, Sheng M, Auberson YP, Wang YT. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. *Science* 2004;304:1021–4. [PubMed: 15143284]
- Matsuyama S, Aihara K, Nishino N, Takeda S, Tanizawa K, Kuroda S, Horie M. Enhanced long-term potentiation *in vivo* in dentate gyrus of NELL2-deficient mice. *NeuroReport* 2005;15:417–21. [PubMed: 15094495]
- Milner AJ, Cummings DM, Spencer JP, Murphy KP. Bidirectional plasticity and age-dependent long-term depression at mouse CA3-CA1 hippocampal synapses. *Neurosci Lett* 2004;367:1–5. [PubMed: 15308285]
- Mockett B, Coussens C, Abraham WC. NMDA receptor-mediated metaplasticity during the induction of long-term depression by low-frequency stimulation. *Euro J Neurosci* 2002;15:819–26.
- Moore KA, Nicoll RA, Schmitz D. Adenosine gates synaptic plasticity at hippocampal mossy fiber synapses. *Proc Natl Acad Sci USA* 2003;100:14397–402. [PubMed: 14608033]
- Nagy V, Bozdagi O, Matynia A, Balcerzyk M, Okulski P, Dzwonek J, Costa RM, Silva AJ, Kaczmarek L, Huntley GW. Matrix metalloproteinase-9 is required for hippocampal late-phase long-term potentiation and memory. *J Neurosci* 2006;26:1923–34. [PubMed: 16481424]
- Namgung UK, Valcourt E, Routtenberg A. Long-term potentiation *in vivo* in the intact mouse hippocampus. *Brain Res* 1995;689:85–92. [PubMed: 8528709]
- Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsáki G, Siegelbaum SA, Kandel ER, Morozov A. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. *Cell* 2004;119:719–32. [PubMed: 15550252]
- Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton AA, Sacktor TC. Storage of spatial information by the maintenance mechanism of LTP. *Science* 2006;313:141–4.
- Spalloni A, Geracitano R, Berretta N, Sgobio C, Bernardi G, Mercuri NB, Longone P, Ammassari-Teule M. Molecular and synaptic changes in the hippocampus underlying superior spatial abilities in pre-symptomatic G93A/+ mice over expressing the human Cu/Zn superoxide dismutase (Gly93 --> ALA) mutation. *Exp Neurol* 2006;197:505–14. [PubMed: 16309674]
- Simon W, Hapfelmeier G, Kochs E, Zieglansberger W, Rammes G. Isoflurane blocks synaptic plasticity in the mouse hippocampus. *Anesthesiology* 2001;94:1058–65. [PubMed: 11465598]
- Takamatsu I, Sekiguchi M, Wada K, Sato T, Ozaki M. Propofol-mediated impairment of CA1 long-term potentiation in mouse hippocampal slices. *Neurosci Lett* 2005;389:129–32. [PubMed: 16112456]
- Tamura H, Fukada M, Fujikawa A, Noda M. Protein tyrosine phosphatase receptor type Z is involved in hippocampus-dependent memory formation through dephosphorylation at Y1105 on p190 RhoGAP. *Neurosci Lett* 2006;399:33–8. [PubMed: 16513268]
- Tamura H, Ishikawa Y, Hino N, Maeda M, Yoshida S, Kaku S, Shiosaka S. Neuropsin is essential for early processes of memory acquisition and Schaffer collateral long-term potentiation in adult mouse hippocampus *in vivo*. *J Physiol* 2006;570:541–51. [PubMed: 16308352]
- Wei H, Xiong W, Yang S, Zhou Q, Liang C, Zeng BX, Xu L. Propofol facilitates the development of long-term depression (LTD) and impairs the maintenance of long-term potentiation (LTP) in the CA1 region of the hippocampus of anesthetized rats. *Neurosci Lett* 2002;324:181–4. [PubMed: 12009518]
- Whitlock JR, Heynen AJ, Shuler MG, Bear MF. Learning Induces Long-Term potentiation in the hippocampus. *Science* 2006;313:1093–7. [PubMed: 16931756]
- Yun SH, Park KA, Sullivan P, Pasternak JF, Ladu MJ, Trommer BL. Blockade of nicotinic acetylcholine receptors suppresses hippocampal long-term potentiation in wild-type but not ApoE4 targeted replacement mice. *Neurosci Res* 2005;52:771–7.

Figure 1. Electrode placement in the adult mouse hippocampus
A. Schematic of electrode position based on skull landmarks. B. Stereotaxic coordinates locating electrode position (stimulating: relative to Lambda, recording: relative to Bregma). Abbreviations: dentate gyrus (DG), perforant path (PP), anterior-posterior (AP), and lateral (LAT) C. A representative trace of dentate evoked response and the quantification of the population spike amplitude (PSA).

Figure 2. *In vivo* experimental setup of an awake freely behaving mouse and subsequent histology
A. An awake freely behaving mouse showing lightweight headstage assembly and unimpeded movement during recording. B. Histological confirmation of recording electrode location in the dentate gyrus (DG) following experimentation.

Figure 3. *In vivo* electrophysiological recordings from freely behaving mice

A. Representative Input/Output curve from a single animal showing the population spike amplitude (PSA) as a function of stimulus current intensity. The current intensity yielding 25–50% maximal PSA was determined from the I/O of each animal and used in all subsequent recordings. B. Long-term potentiation in the awake freely behaving mouse. Mean percent change in PSA amplitude shows a significant potentiation relative to baseline ($+47.3 \pm 5.2\%$; $p < 0.001$; $n = 6$). 900 train pulses were administered at 100 Hz using the current intensity that elicited a response equal to 25% the maximal evoked response. *Inset*: Sample traces of evoked responses prior to (solid line) and 120 minutes after tetanization (dotted line). C. Long-term depression in the awake freely behaving mouse. Mean percent change in PSA amplitude shows

a significant depression relative to baseline ($-36.2 \pm 8.1\%$; $p < 0.001$; $n = 5$). 900 train pulses were administered at 1 Hz using the current intensity that elicited a response equal to 50% the maximal evoked response. Inset: Sample traces of evoked responses prior to (solid line) and 120 minutes after tetanization (dotted line).

Table 1
Complications associated with various animal models of synaptic plasticity

	in vitro	in vivo anesthetized	in vivo freely behaving
tissue damage	✓	✓	✓
loss of connectivity	✓		
drug interference		✓	
varying buffer composition	✓		
varying recording methods	✓	✓	✓