Electrophysiological Techniques for Studying Synaptic Activity In Vivo

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ABSTRACT

Understanding the physiology, pharmacology, and plasticity associated with synaptic function is a key goal of neuroscience research and is fundamental to identifying the processes involved in the development and manifestation of neurological disease. A diverse range of electrophysiological methodologies are used to study synaptic function. Described in this unit is a technique for recording electrical activity from a single component of the central nervous system that is used to investigate pre- and post-synaptic elements of synaptic function. A strength of this technique is that it can be used on live animals, although the effect of anesthesia must be taken into consideration when interpreting the results. This methodology can be employed not only in naïve animals for studying normal physiological synaptic function, but also in a variety of disease models, including transgenic animals, to examine dysfunctional synaptic plasticity associated with neurological pathologies. *Curr. Protoc. Pharmacol.* 64:11.11.1-11.11.17. © 2014 by John Wiley & Sons, Inc.

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INTRODUCTION

The importance of bioelectrical activity in nervous system function was recognized over two centuries ago by Galvani in 1791 (Piccolino, 1998; *UNIT 11.1*). Subsequently, research focused on the mechanisms responsible for the generation of electrical activity and the manner in which it is propagated, communicated, and coordinated at the single-cell level through function-specific neural circuits, and how, ultimately, it relates to animal behavior. Electrophysiological techniques are crucial for monitoring bioelectrical activity in real time. Furthermore, these methods have contributed significantly to our knowledge of electrical activity pathologies, e.g., epileptic phenomena, and to our understanding of neuronal plasticity, a phenomenon critical for learning and memory, but which may be dysfunctional in neurological disorders, e.g., Alzheimer's disease. The hippocampus is a brain region that is critical for memory and learning. A component of the limbic system, the hippocampus plays a key role in spatial navigation and in consolidating the formation of short- and long-term memory. The hippocampus is one of the first brain regions to degenerate in Alzheimer's disease, with memory loss and disorientation among the early clinical signs of this disorder. In addition, hippocampal neurons, like those in the cerebral cortex, are arranged in columns, with principal cells arrayed in pyramidal cell layers. Because these layers persist almost throughout this brain region, there is a certain degree of uniformity with respect to cellular function based on recording location. This functional anatomy makes the hippocampus particularly well suited for electrophysiological investigation.

For over 100 years, memory formation was thought to result from changes in the strength of the synaptic connections between neurons (Ramón y Cajal, 1894). However, Bliss and Lomo (1973) described how a brief, strong electrical stimulation induces a gain in

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hippocampal synaptic function that lasts for several hours and possibly days. Detailed in the protocols below are ways to measure this synaptic activity from the amplitude of extracellularly recorded field excitatory post-synaptic potentials with superimposed population spikes evoked by electrical stimulation of the pre-synaptic input. The magnitude of this response, reflecting the aggregate depolarization and excitation of a significant number of neurons, is measured at a low frequency (<0.05 Hz) before and after induction of a long-lasting change in synaptic strength, termed long-term potentiation (LTP), using short-lasting high frequency (≥100 Hz) stimulation. The amount of subsequent synaptic plasticity, or LTP, is a correlate of cognitive function or memory consolidation (Bliss and Collingridge, 1993). Given its importance, this process has been studied extensively for the last 40 years.

The main uses for the neurophysiological study of LTP are for the examination of the augmentation of sub-maximal/threshold increases in synaptic gain in naïve animals, demonstrating enhancement of memory and cognition (Lynch et al., 2011; Lynch and Gall, 2013), and the investigation of deficits in the magnitude of LTP such as those induced in animal disease models (e.g., Nistico et al., 2012). The procedures described below are applicable for investigating changes in the hippocampus in both the threshold and ceiling of synaptic plasticity (Kramar et al., 2004; Hopkins et al., 2013), generating LTP with a pattern of induction that resembles hippocampal pyramidal cell activity in vivo, corresponding to the theta EEG frequency found in exploring rats—theta burst stimulation (TBS; Larson et al., 1986; Larson and Lynch, 1987). Similar stimulation patterns are effective in improving learning in humans (Ackerley et al., 2010; Hsu et al., 2011), supporting the utility of these assays for investigating novel neuropsychiatric treatments that could, for example, reduce the cognitive deficit associated with schizophrenia. Additionally, much work has been performed in examining LTP in disease states. Indeed, there are many animal models of disease, including those used to study neurodegenerative disorders and psychiatric illness, that are associated with changes in hippocampal electrical activity. Procedures used for generating these models range from alterations in their genetic make-up (e.g., Lawlor et al., 2007) to chronic or acute treatment with agents that generate pathological synaptic activity (e.g., Freir et al., 2001). An example is provided in this unit in which acute administration of beta-amyloid oligomers, which are thought to play a role in the development of Alzheimer's disease (Haass and Selkoe, 2007), produces deficits in synaptic function, LTP, and memory formation (e.g., O'Hare et al., 2013). A value of this approach is that it offers a way to identify pathological cellular function in the disease model prior to its use in screening for novel therapeutics to prevent or treat synaptic dysfunction.

Technological Advancement

Although the fundamentals of the electrophysiological approach detailed in this protocol have remained relatively consistent for decades, technological advances are constantly being made in the electronics and equipment used for such studies. Incorporating the latest hardware and software is an important component to maximize data acquisition and optimize analysis. Thus, while the protocols described herein serve as a template for designing and conducting these types of electrophysiological studies, going forward, investigators need to adopt and adapt the latest technologies when executing these protocols.

BASIC PROTOCOL

MEASUREMENT OF POPULATION SPIKE ACTIVITY FROM THE CA1 REGION OF THE HIPPOCAMPUS IN ANESTHETIZED RATS USING EXTRACELLULAR RECORDING TECHNIQUES: SUB-MAXIMAL LTP

Described in this protocol are basic procedures for monitoring the amplitude of population spikes superimposed on field excitatory post-synaptic potentials (fEPSPs) recorded from

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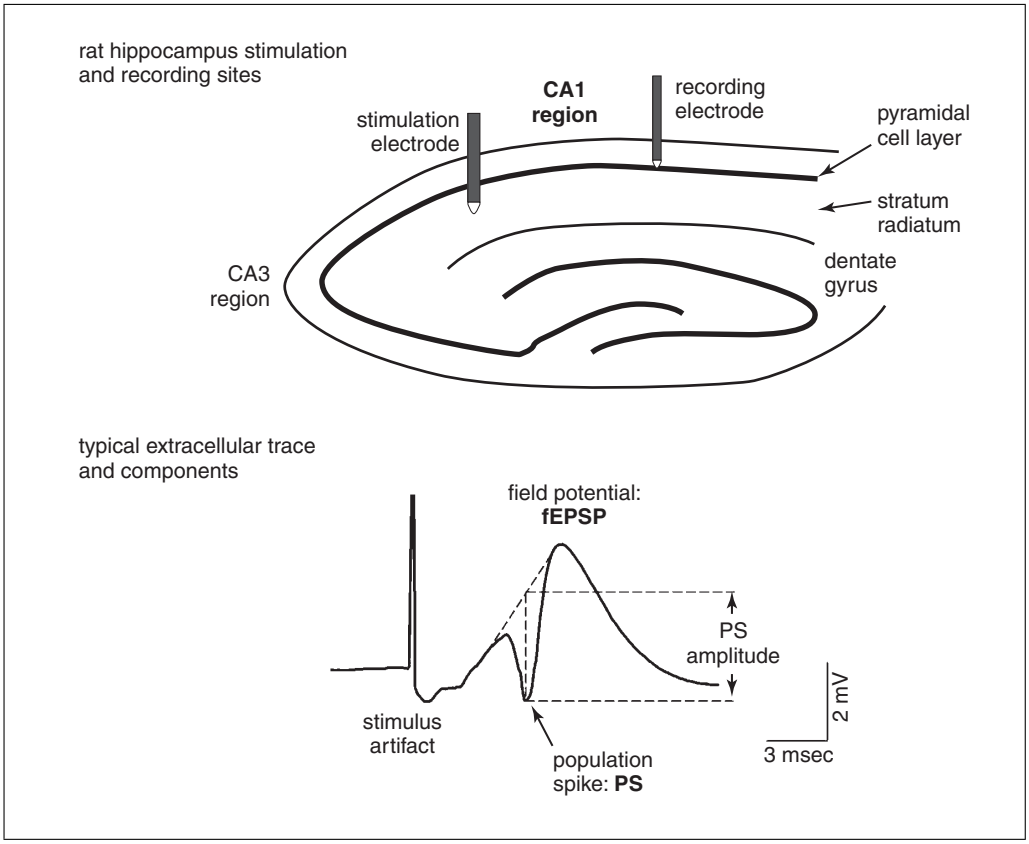


Figure 11.11.1 Top: a schematic of the rat hippocampus highlighting the location of the stimulation electrode within the stratum radiatum, stimulating the Schaffer collateral pathway, and the recording electrode in the pyramidal cell layer, both within the CA1 region. Bottom: an example field excitatory postsynaptic potential (fEPSP) and superimposed population spike (PS), with graphical representation of the amplitude of the PS and the slope of the underlying fEPSP.

the CA1 region of the hippocampus in anesthetized rats (see Fig. 11.11.1). Also included are directions on how to examine the change in synaptic gain, or plasticity, generated using a sub-maximal high frequency-based stimulation paradigm that makes possible an investigation into the ability of treatments to enhance LTP (see Fig. 11.11.2). As these experiments are performed on living animals, blood pressure and body temperature are monitored to ensure adequate anesthesia and optimal physiology. Blood pressure and fEPSP activity are captured electronically and analyzed in real time.

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) and must follow officially approved procedures for the care and use of laboratory animals.

Materials

Sprague Dawley rat (male, 300 to 400 g)
12% urethane (see recipe)
Normal saline (NS, see recipe)
Heparinized normal saline (see recipe)
100% oxygen source
Mineral oil
Paraffin wax
Test substances, e.g., cycloserine
Pentobarbital sodium (see recipe)

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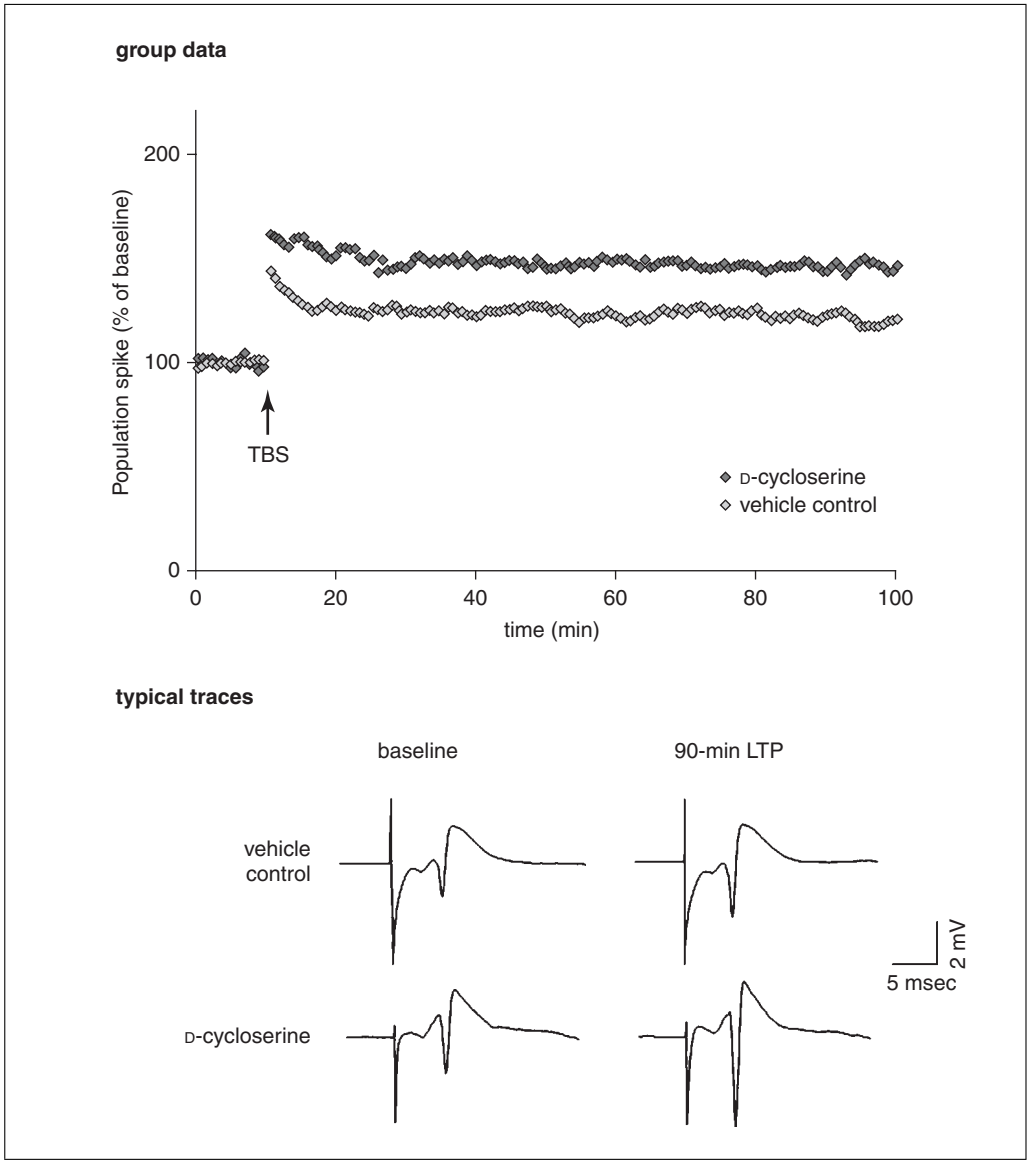


Figure 11.11.2 Top: a scatter plot representing the mean population spike amplitude during the course of a long-term potentiation (LTP) experiment, demonstrating the enhancement in synaptic plasticity induced by intra-peritoneal administration of D-cycloserine (100 mg/kg, 50 min before LTP), compared to vehicle-control experiments performed in parallel. Bottom: typical traces before and 90 min after LTP induction in control animals and animals subject to administration of D-cycloserine.

Homeothermic blanket system and rectal probe (Harvard Apparatus)
Recommended surgical instruments:

- Hemostatic clamp, straight (two are required)
- Hemostatic clamp, curved
- Large scissors
- Medium blunt dissection scissors
- Large blunt dissection forceps, curved
- Small blunt dissection forceps, curved
- Fine forceps (No. 5; two are required)
- Arterial clip
- Fine spring scissors
- Scalpels

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Stereomicroscope
Fiber-optic light source
Polyethylene tubing (external/internal diameter 0.96/0.58 mm) for cannulation of femoral vein/artery
26- and 30-G syringe needles
Three-way tap
Silicon tubing (external/internal diameter 3/2.4 mm) for cannulation of trachea
Stereotaxic frame (ST-7, Narishige)
Vibration isolation table
Stereotaxic atlas for rat (Paxinos and Watson, 1998)
Pressure transducer (Digitimer, cat. no. NL108T2)
A.C. preamplifier, filter and pressure amplifier modules (Digitimer, cat. nos. NL104, NL125, NL108)
Analog/digital converter (Micro1401, Cambridge Electronic Design)
NeuroLog modular acquisition hardware (NL905, NeuroLog, Digitimer)
Computer running Spike2 software (Cambridge Electronic Design)
Micro-cautery device (Gemini, Harvard apparatus)
Q-tips or cotton swabs
Hemostatic gelatin sponge
Surgical bone drill system
Single-barrel carbon-fiber recording electrode (Carbostar-1, Kation Scientific)
Concentric bipolar stimulating electrode (FHC, cat. no. CBBPC75)
Micromanipulators (Narishige)
Headstage (Digitimer, cat. no. NL100AK)
Isolated constant-voltage stimulator (DS2A, Digitimer)

Additional reagents and equipment for injection of rats (Donovan and Brown, 2006)

Prepare for surgery

1. Anesthetize a rat with an intraperitoneal (i.p.) injection of 12% urethane at a dose of 1.2 g/kg (i.e., for a 300 g animal use 3 ml). Administer 0.2 g/kg supplementary anesthetic either i.p. (pre-cannulation) or intravenously (i.v.; post-cannulation) as necessary to ensure adequate anesthesia. Assess the level of anesthesia by monitoring the response to a paw-pinch (withdrawal reflex) and the stability of cardiovascular variables (post-cannulation).

   Frequent anesthetic assessment is imperative for animal welfare and stability of the preparation.

   Urethane is a non-survival anesthetic with a 4- to 8-hr duration of action.

   Animal injection techniques are described in Donovan and Brown (2006).

2. Maintain animal core body temperature by placing the anesthetized subject on a heating blanket connected to a homeothermic system. Monitor body temperature with a rectal probe.

3. Cannulate the left femoral vein and artery for drug administration and arterial blood pressure recording with a 0.96-mm external diameter tubing prefilled with normal saline (NS) and heparinized NS, respectively, and fitted on the free end with a similarly prefilled 26-G needle and three-way tap.

   Ensure a sufficient length of tubing for cannulation (20 to 30 cm) to allow for the administration of drugs and for flushing the arterial cannula during fEPSP recording without disturbing the stereotaxic frame.

4. Cannulate the trachea with silicon tubing, posterior to the larynx, to facilitate spontaneous breathing and to allow for oxygen enrichment once the animal is placed in the stereotaxic frame.

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To enrich the inhaled air with oxygen, pass 100% oxygen (~0.2 liters/min) over the exposed end of the tracheal cannula. This will help optimize blood oxygen levels and maintain vital signs during anesthesia.

Place subject in the stereotaxic frame

5. Place the stereotaxic frame perpendicular to the front of the vibration isolation table with the head end to the left. Situate the heated blanket within the stereotaxic frame and place the animal prone between the ear bars. Grasp the animal's head, neck, and upper body and guide the fixed right ear bar into the auditory meatus, moving it up and down with gentle pressure until accurate placement is confirmed by a popping sound, indicating rupture of the tympanic membrane. While maintaining the rat in position with its head as horizontal as possible between the ears, bring the left ear bar into position using the same procedure. Once both ear bars are secured in position, centralize the animal in the frame, place the upper incisors over the incisor bar, retract the bar until tight, and secure the nose clamp.

   For accurate stereotaxic placement of the stimulating and recording electrodes and i.c.v. cannula, ensure that the longitudinal plane between bregma and lambda (see stereotaxic atlas; Paxinos and Watson, 1998) is level by carefully adjusting the height of the incisor bar and nose clamp.

6. Connect the femoral artery cannula to the pressure transducer prefilled with heparinized NS using the three-way tap and begin monitoring arterial blood pressure via the pressure amplifier, Micro1401 acquisition system, and computer running Spike2 software.

   To optimize the arterial blood pressure signal, it is essential that no tight bends be present in the cannula tubing and no air bubbles be present in the needle, tubing, three-way tap, or pressure transducer.

Brain exposure

7. Using a scalpel, make a midline incision along the top of the skull and reflect the skin and connective tissue laterally, exposing the surface of the skull on the left side just rostral to bregma and caudal to lambda. Use a Q-tip with light pressure to clean any remaining connective tissue overlying the skull and to clearly reveal the skull sutures below, particularly at the intersection of bregma and the midline.

   To minimize bleeding, use a micro-cautery device to seal any bleeding points surrounding the skull exposure site and use light pressure with a Q-tip and/or hemostatic sponge on any remaining bleeds.

8. Using a bone drill with a small burr bit (~2 mm), remove an approximately square section of skull on the left of the midline according to the following stereotaxic coordinates: 3 to 5 mm caudal to bregma and 1.5 to 3.5 mm lateral to the midline.

   Mark out the boundaries of the hole on the skull surface with a marker pen to aid removal. Be sure not to penetrate the dural membrane when removing any remaining skull fragments within the marked boundary with fine forceps.

9. Use fine spring scissors and fine forceps to make a small hole in the dural layer, extend the incision across the full width of the opening, and reflect the dural membrane laterally.

Recording and stimulating electrode placement

10. Mount a single-barrel carbon fiber recording electrode vertically onto a micromanipulator and position it at the intersection of bregma and the midline suture. From this intersect, position the electrode according to the following stereotaxic coordinates: −4.4 mm caudal to bregma, 2.25 mm lateral to the midline (left side). Lower

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the barrel to the dorsal surface of the brain. At the identified site of entry, retract the electrode slightly and, using fine forceps, remove the minimum amount of pia mater to allow unrestricted entry into the brain. Lower the electrode to just above the pyramidal layer of the CA1 region of the hippocampus at a depth of 1.75 mm below the pial surface.

11. Mount a coaxial bipolar stimulating electrode vertically onto a secondary micromanipulator and locate at the intersection of bregma and the midline suture. From this intersect, position the electrode according to the following stereotaxic coordinates: −3.4 mm caudal to bregma, 2.75 mm lateral to the midline (left side). Lower the electrode to the dorsal surface of the brain. At the identified site of entry, retract the electrode slightly and, using fine forceps, remove the minimum amount of pia mater to allow unrestricted entry into the brain. Lower the electrode to just above the Schaffer collateral pathway in the CA1 region of the hippocampus at a depth of 2 mm below the pial surface.

    Once recording and stimulating electrodes are positioned, the exposed surface of the brain surrounding these electrodes should be covered with mineral oil and then paraffin wax to limit drying and to restrict swelling.

Identifying and optimizing fEPSP responses

12. Prepare for recording fEPSP activity by connecting the recording electrode gold pin to the 'A-In' socket of a recording headstage, and the 'B-In' and 'GND' sockets of the headstage to a central earthing point. Connect the headstage to the amplifier unit with the gain set to 500 and the parallel filter low/high frequency pass set to 0.5/5 kHz, respectively.

    To maintain the highest possible signal-to-noise ratio for recorded responses, connect any items that are likely to produce electrical noise (e.g., the fiber optic light source) to the central earthing point, along with any large metallic items (e.g., the stereotaxic frame). A floor- or table-mounted Faraday cage will also help minimize electrical noise.

13. Connect the two poles of the concentric bipolar electrode to the positive and negative poles of a constant voltage stimulator that is externally triggered by Micro1401 hardware and Spike2 software (see Background Information).

14. Use Spike2 software to activate low-frequency stimulation (5 to 50 V, 0.1 Hz, 0.1- to 1-msec pulse width) of the Schaffer collateral pathway to identify and optimize an fEPSP with a superimposed population spike (PS). This is accomplished by alternately descending the recording and stimulating electrodes in small (~25 μm) increments into the CA1 pyramidal cell layer and Schaffer collateral pathway, respectively, until the maximum response is obtained (see Fig. 11.11.3 left panel).

    Measure the amplitude of the PS using Spike2 software. This is defined as the difference between the mean of the two positive peaks directly before and after the negative peak and the maximum deflection of the negative peak itself (see Fig. 11.11.1).

15. Record an input-output curve (Fig. 11.11.3 right panel) to determine maximal PS amplitude and the voltage required to generate a PS with an amplitude of 40% to 50% of maximum. Once this stimulation intensity is identified and selected, maintain stimulation at a lower frequency of 0.033 Hz and allow a period of recovery (~20 to 60 min) until a steady-state PS amplitude of at least 10 min has been achieved. Thereafter, repeat the input-output examination, and, if necessary, reset the voltage to generate a PS with 40% to 50% maximum amplitude. Re-establish the steady-state before continuing with the experimental protocol.

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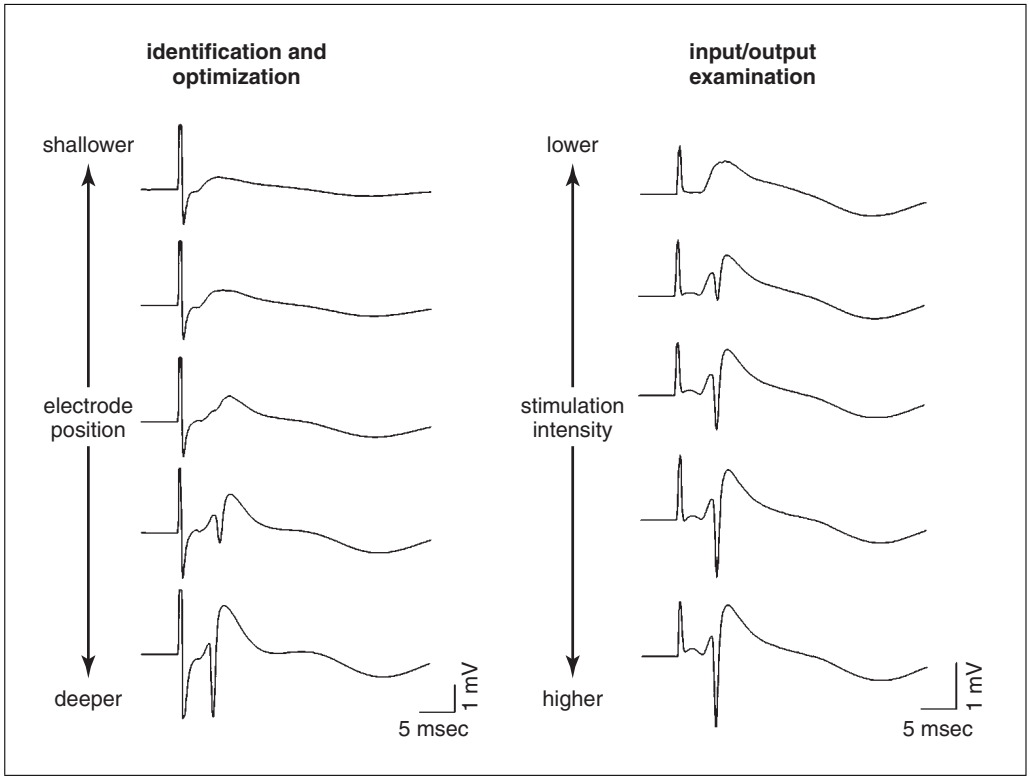


Figure 11.11.3 Left: representative field excitatory post-synaptic potentials (fEPSPs) and superimposed population spikes (PSs) as you descend the stimulating and recording electrodes into position and optimize the response. Right: representative fEPSPs and PSs during examination of input/output responses to determine the appropriate stimulation intensity for baseline generation.

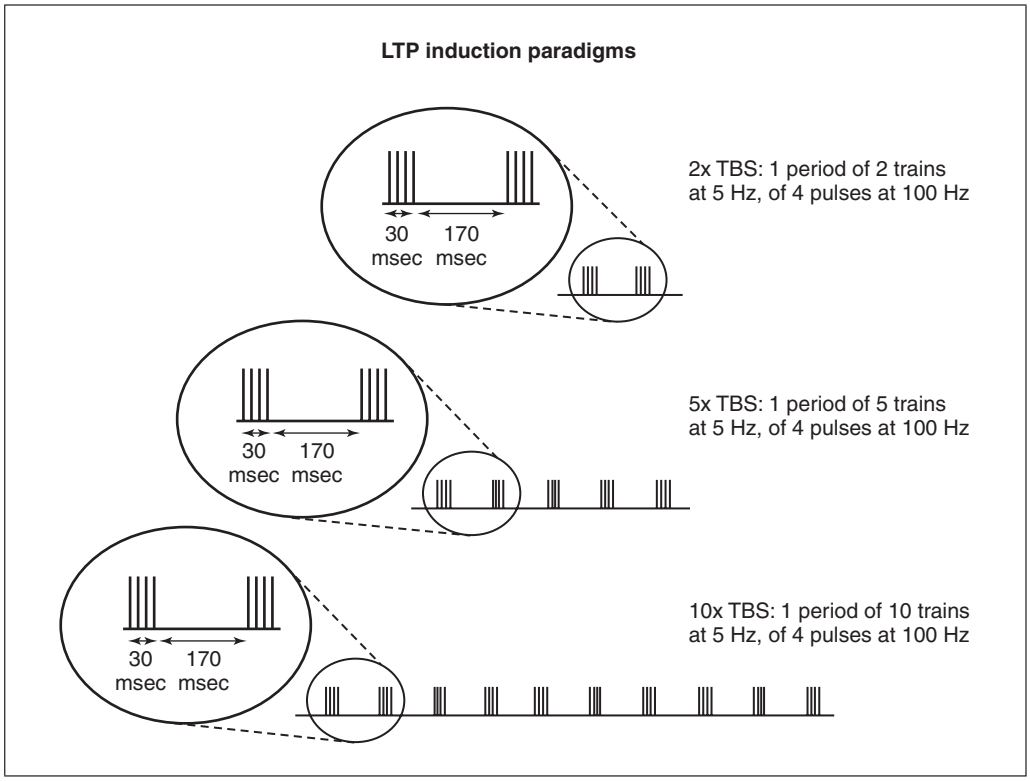


Figure 11.11.4 Graphical illustration of the theta-burst stimulation protocols used to induce varying degrees of long-term potentiation in the CA1 region of the hippocampus of anesthetized rats, ranging from sub-threshold (2× TBS) to close to maximal (10× TBS).

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Induce LTP

16. Monitor fEPSP and PS responses at a frequency of 0.033 Hz to generate a stable 10-min baseline period before the administration of test substances, e.g., D-cycloserine at a dose of 100 mg/kg i.p., at the appropriate pre-treatment time (in this case 50 min) before induction of LTP.

17. Induce LTP with theta-burst stimulation (TBS) of the Schaffer collateral pathway using Spike2 software. Monitor responses for an additional 90 min after LTP induction.

    The TBS paradigm is as follows: 2 to 5 trains, inter-train interval, 200 msec, of 4 pulses; inter-pulse interval 10 msec (Fig. 11.11.4).

18. Terminate the experiment by administering a lethal dose (200 mg/kg, i.v.) of pentobarbital sodium.

    See Donovan and Brown (2006) for injection technique.

19. Save the Spike2 file for later offline analysis of the fEPSP and PS components (see Anticipated Results).

MEASUREMENT OF POPULATION SPIKE ACTIVITY FROM THE CA1 REGION OF THE HIPPOCAMPUS IN ANESTHETIZED RATS USING EXTRACELLULAR RECORDING TECHNIQUES: OLIGOMERIC BETA-AMYLOID-INDUCED DEFICITS

ALTERNATE PROTOCOL

This protocol details some modifications of the procedure to monitor the amplitude of population spikes superimposed on fEPSPs, detailed in the Basic Protocol, to incorporate examination of the deficit in synaptic plasticity induced by pre-treatment with intracerebroventricularly (i.c.v.) administered oligomeric beta-amyloid (Fig. 11.11.5). Execution of this alternate protocol results in a robust induction of LTP, making it possible to examine not only the processes responsible for reduced plasticity, but also to test whether potential therapeutics can either prevent or reverse this deficit.

Additional Materials (also see Basic Protocol)

Disease model agent: (beta-amyloid; see recipe)
2% (w/v) Chicago Sky Blue dye in normal saline (optional; see recipe for normal saline)

Stainless steel tubing for i.c.v. cannulation (Semat)
Syringe pump (Model 100, KD Scientific)
Stereotaxic frame bar-mounted micromanipulator (Narishige)

1. Follow steps 1 to 9 of the Basic Protocol, then make a second small skull opening ipsilateral to the first that is 0 to 1 mm rostral to bregma and 1 to 2 mm lateral to the midline.

2. Use fine spring scissors and fine forceps to make a small hole in the dura, extend the incision across the full width of the opening, and reflect the dural membrane laterally.

Insert lateral ventricle cannula

3. Prefill an i.c.v. stainless steel cannula and tubing with oligomeric beta-amyloid or control vehicle and place the syringe on the syringe pump to ensure that the perfusion system is fully loaded with no air gaps. Set the syringe pump to administer a volume of 10 μl over 4 min, and run one full cycle to confirm patency before placement of the i.c.v. cannula within the brain.

4. Mount the i.c.v. cannula on a stereotaxic frame bar–mounted micromanipulator and lower it to the intersection of bregma and the midline suture at a 15° rostro-caudal

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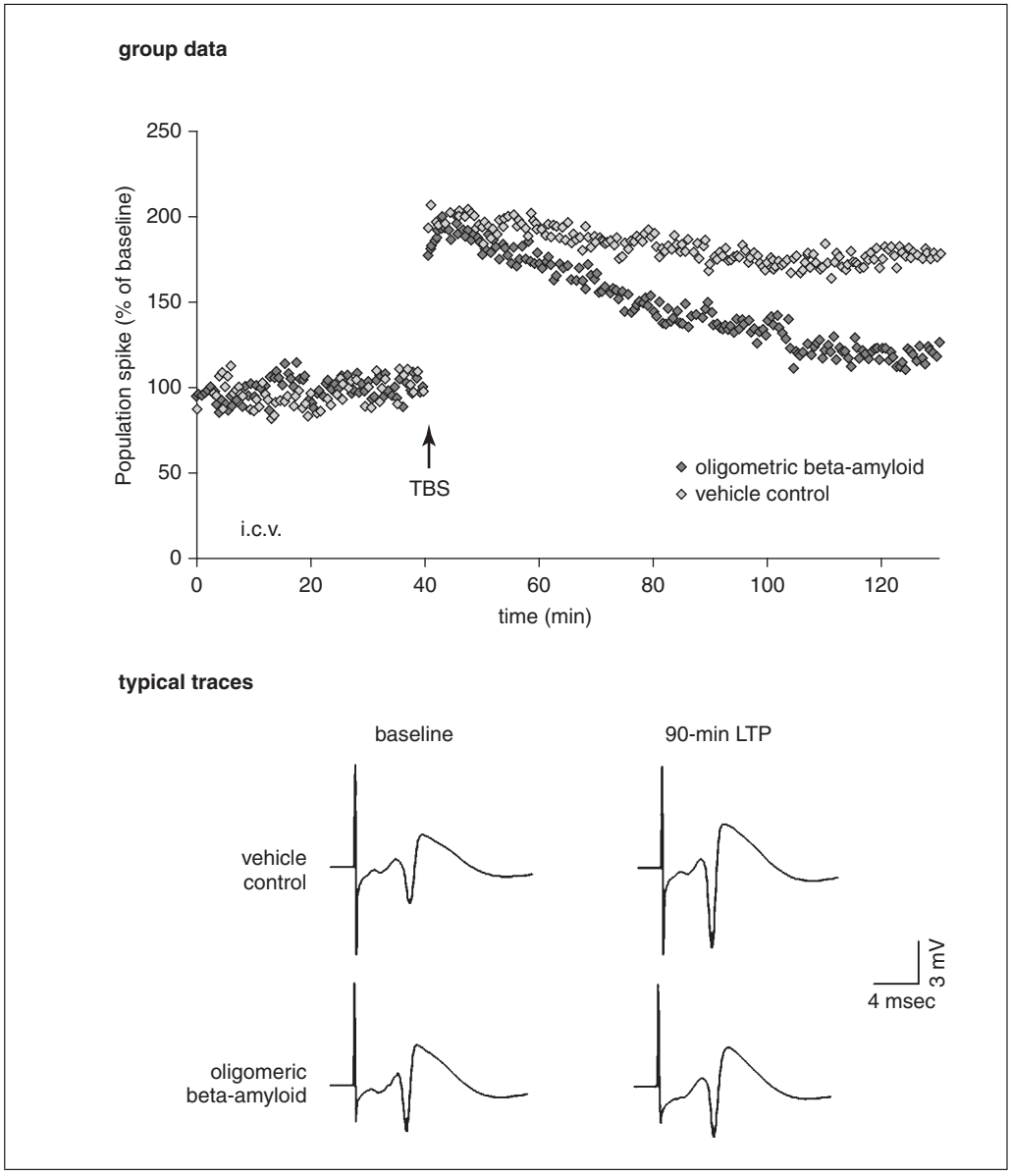


Figure 11.11.5 Top: a scatter plot representing the mean population spike amplitude during the course of a long-term potentiation (LTP) experiment, demonstrating the deficit in synaptic plasticity induced by intracerebroventricular (i.c.v.) administration of oligomeric beta-amyloid, compared to vehicle-control experiments performed in parallel. Bottom: typical traces before and 90 min after LTP induction in control animals and animals subject to a lateral ventricle administration of oligomeric beta-amyloid.

angle. From this intersect, position the cannula according to the following stereotaxic coordinates: +0.5 mm rostral to bregma, 1.5 mm lateral to the midline (left side). Lower the cannula to the dorsal surface of the brain. At the identified site of entry, retract the cannula slightly and, using fine forceps, remove the minimum amount of pia mater necessary to allow unrestricted entry into the brain. Lower the cannula down 3.7 mm from the pial surface into the left lateral ventricle.

It is recommended to confirm the injection site accuracy in pilot studies in which 2% Chicago Sky Blue dye in normal saline is administered i.c.v. instead of oligomeric beta-amyloid. The pilot animal brain is then removed post-mortem and dissected to visualize the circulation of the dye throughout the cerebroventricular system.

5. Perform steps 10 through 15 of the Basic Protocol.

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Apply disease-induction agent

6. Administer 10 μl of 100 nM oligomeric beta-amyloid or vehicle into the lateral ventricle via activation of the syringe pump.

7. Continue monitoring responses for a further 30 to 120 min (pre-treatment time is dependent on oligomeric amyloid preparation method, see Background Information) before inducing LTP with a TBS induction protocol. The TBS paradigm is as follows: 5 to 10 trains, inter-train interval 200 msec, of 4 pulses; inter-pulse interval 10 msec (Fig. 11.11.4). Monitor responses for 90 min after LTP induction.

8. Terminate the experiment by administering a lethal (200 mg/kg, i.v.) dose of pentobarbital sodium.

9. Save the Spike2 file for later offline analysis of the fEPSP and PS components (see Anticipated Results).

REAGENTS AND SOLUTIONS

Use deionized, distilled water in the preparation of all solutions.

D-Cycloserine

Dissolve D-cycloserine (Sigma, cat. no. C7670) at a concentration of 50 mg/ml in normal saline (see recipe) with a short period of sonication (~5 min). Prepare fresh daily.

Heparinized normal saline

Dissolve heparin sodium salt (from porcine intestinal mucosa; Sigma, cat.no. H9399) at a concentration of 50 U/ml in normal saline. Store up to 2 to 3 months at 4°C.

Normal saline

0.9% (w/v) NaCl: dissolve 9 g of NaCl per liter of water. Store up to 2 to 3 months at 4°C.

Oligomeric beta-amyloid

Dissolve $A\beta_{1\text{-}42}$ (e.g., Bachem) in hexafluoroisopropanolol (Sigma, cat. no. 105228) to monomerize the peptide. Once a clear solution is obtained (may take up to 1 week to dissolve completely), lyophilize the solution in appropriately sized vials for administering i.c.v. and freeze at −20°C. Store up to 1 month. Defrost a vial on the day of the experiment and resuspend it in DMSO at a concentration of 2 mM, before diluting it to the final concentration in normal saline (see recipe) and storing at 4°C until use (maximum 2 hr). Ensure that the material has reached room temperature before administration into the brain.

Pentobarbital sodium

Dissolve pentobarbital sodium (Sigma, cat.no. P3761) at a concentration of 200 mg/ml in normal saline. Store up to 2 to 3 months at 4°C.

As pentobarbital sodium is a controlled substance, only licensed investigators may be in possession of this material, and it must be used according to officially approved procedures.

Urethane

Dissolve urethane (Sigma, cat.no. 94300) at a concentration of 12 g/100 ml in normal saline (12% w/v final) at least 1 day prior to use. Store up to 1 month at 4°C.

COMMENTARY

Background Information

Described in this unit are methods for examining a change in synaptic function in whole animals using theta burst stimulation paradigms, which are patterns designed to simulate neuronal firing during learning (Otto

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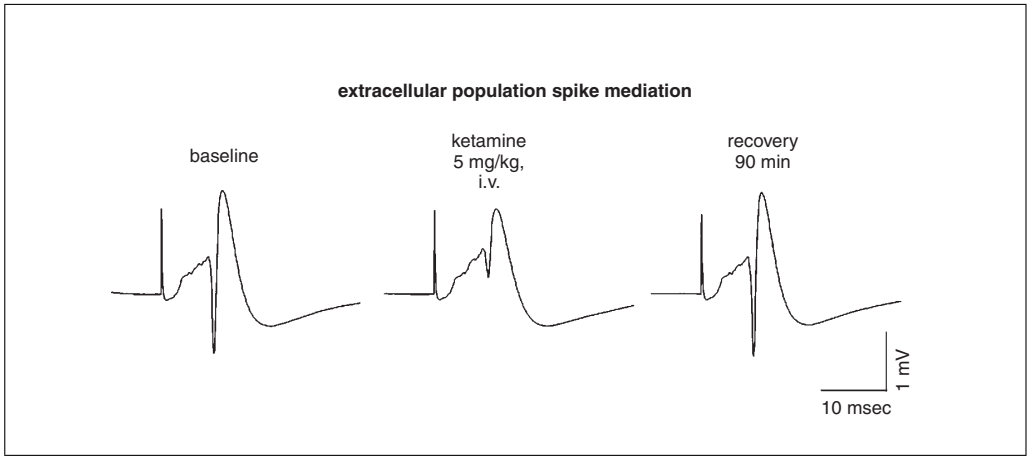


Figure 11.11.6 Representative field excitatory postsynaptic potentials (fEPSPs) and superimposed population spikes (PSs) demonstrating the effect of administration of the NMDA receptor antagonist ketamine on the amplitude of fEPSP and PS components, and the subsequent wash of this response up to 90 min after injection.

et al., 1991; Axmacher et al., 2006). This synaptic plasticity is monitored in the hippocampal CA1 region, an area critical for the consolidation of certain aspects of memory. Two different approaches are described for investigating two aspects of the neurophysiology of this system. The first is through relating changes in the threshold and amplitude of synaptic plasticity to enhancements in memory and cognitive function, and the second is through examination of the perturbation of LTP induced by oligomeric beta-amyloid, an agent used to create an Alzheimer's disease model. With the appropriate modification in stereotaxic positioning of the electrodes, the procedures described in this unit can be applied to other hippocampal regions, such as the dentate gyrus. In this case, evoked population spike activity can be recorded from the granule cell layer after stimulation of the perforant pathway. They can also be applied to the examination of fEPSP activity alone, excluding population spike activity, by descending recording electrodes into the corresponding dendritic layer (e.g., the stratum radiatum in area CA1). This allows greater resolution of effects on the depolarization of the dendritic field separately from effects within the somatic zone, when neurons reach threshold and fire action potentials as represented by the population spike, although more intense stimulation paradigms are necessary to induce plasticity and LTP. Furthermore, while technically more challenging, all of these procedures can be performed in the mouse for the study of transgenic animals.

Baseline examination

Although the aim of the experiments described above is to examine the change in synaptic gain driven by repetitive patterned stimulation of pre-synaptic input, when examining the effects of parenterally administered compounds, it is also possible to monitor the acute effects on baseline fEPSP or population spike amplitude. The extracellular population spike is mediated primarily by glutamate receptors, as evidenced by the ability of the NMDA receptor antagonist ketamine to depress this response (Fig. 11.11.6). If there is a significant and persistent baseline effect when investigating the synaptic plasticity response to a test agent, the investigator must repeat the input/output examination to reestablish the stimulation intensity necessary for LTP induction. In addition, it may be best to perform experiments that do not incorporate an LTP-induction paradigm, e.g., by testing the ability of compounds to enhance baseline synaptic responses using these procedures but without theta-burst stimulation. With small modifications, experiments can be performed to allow investigation of the pre-synaptic contribution to the effects of compounds by examining paired pulse facilitation (typically a pulse-pulse interval of ~20 to 50 msec; Li et al., 2013). This is termed short-term synaptic plasticity, which is thought to be controlled by residual calcium (Zucker and Regehr, 2002).

LTP induction

There is significant variation in the stimulation paradigms used to induce synaptic plasticity in the CA1 region of the hippocampus, with some studies examining the effects of compounds on submaximal/threshold LTP using quite different pulse number(s) and pattern(s) to generate near-threshold changes in synaptic gain (e.g., Ovsepian et al., 2004).

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The range of LTP stimulation paradigms described in this unit (Fig. 11.11.4) is designed to yield varying degrees of synaptic plasticity, from sub-threshold to sub-maximal (Fig. 11.11.2), to close to maximal (Fig. 11.11.5). Although the method of LTP induction described in this unit employs short, four-pulse bursts of high-frequency stimulation (≥ 100 Hz), most investigators interested in examining the effects of test compounds on in vivo disease model–induced deficits in LTP use much longer tetanic stimulation protocols of a second or more at up to 200 Hz (e.g., Frier et al., 2001). In general, however, these studies are aimed at examining changes in the amplitude or slope of an fEPSP recorded from the stratum radiatum, which represents the depolarization of this largely dendritic layer rather than a population spike from the pyramidal cell layer, which includes aggregate somatic action potential firing. Thus, there are differences in the threshold of LTP between these two methods. In contrast, there are other plasticity-induction procedures designed to either reverse the enhancement in synaptic plasticity generated by a conventional LTP-induction pattern or that are used to induce long-term depression. These are typically 15-min stimulations at 1 Hz. These varied approaches for investigating hippocampal synaptic plasticity in vivo highlight the consideration that must be given to experimental design to ensure the working hypothesis is appropriately addressed.

Limitations and alternatives

While the protocols described in this unit allow for investigation into the processes involved in normal and diseased synaptic function, it is important to remember that the responses monitored are representative of population activity from a large number of neurons. This allows for an interpretation of results from a network perspective, with the data providing information on the effects of the treatments on pathway-driven activity through the intact CA1 region of the hippocampus. However, the range of cell phenotypes in the CA1 pyramidal region and the lack of resolution from the perspective of a single neuron limit data interpretation. There are other electrophysiological techniques that can be employed with the hippocampus to characterize the effects of drugs on synaptic function. These include examining changes in spontaneous single-unit firing (Langmead et al., 2008) or neuronal activity induced by glutamate receptor activation (Bursi et al., 2011). Extracellular fEPSP and population spike activity are also routinely recorded from isolated hippocampal tissue in vitro (Bortolotto et al., 2011). This approach has some advantages over the in vivo procedure, such as easier compound penetration to target, as well as disadvantages, such as disrupted systems due to severed pathways.

Oligomeric beta-amyloid

Described in this unit is the use of oligomeric beta-amyloid in the generation of a model of cognitive dysfunction, as evidenced by an impaired LTP. The preparation of stable beta-amyloid oligomers is an emerging, and still somewhat controversial, technique (see Ryan et al., 2010). Therefore, limited information is provided in this unit with regard to preparation of this material. Investigators are advised to follow the supplier instructions and to analyze the product by western blot or size-exclusion chromatography to ensure that the relevant synaptotoxic oligomeric species of beta-amyloid is present. The preparation method and species selected will likely also affect the required pre-treatment time necessary for maximum efficacy. A number of other disease-modeling agents can be administered in a similar manner, with some slight variations. For example, using a sterile technique, aggregated beta-amyloid can be administered into the hippocampus up to 2 weeks before examination of synaptic plasticity (Babri et al., 2012).

Critical Parameters and Troubleshooting

Generally, the condition and stability of the animal preparation are the most important factors for ensuring consistency and reproducibility of results. For this reason, it is critical that investigators exercise extreme care in inducing and maintaining anesthesia and in performing the needed surgical procedures. In addition, as these experiments involve the recording of very small amounts of electrical activity, it is essential to regularly check all electrical connections not only between recording and stimulating contacts, but also all connections to the central earthing point to minimize electrical noise.

Animal variation

Because these procedures are performed in living animals, the results are affected by the biological variation between individual subjects. This must be considered when supplementing anesthesia, which should be driven by changes in the physiological state of the

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animal rather than be uniform for all animals on the basis of weight. There is also considerable variability in the shape and nature of the skull sutures and where the midline and bregma intersect. This must be taken into account when positioning electrodes and cannulae in accord with the stereotaxic coordinates. Because of this, extracellular responses are sometimes atypical, or even absent. This may necessitate repositioning of the recording and stimulating electrodes.

PK/PD considerations

Selection of the mode of administration and pre-treatment time for the test compounds, as well as the disease model agent, is critical for ensuring adequate penetration into the hippocampus. Therefore, it is important that the pharmacokinetic properties of the test agent be known to guide the selection of dose, mode, and timing of administration. Most methods of administration (i.v., i.p., i.m., s.c., p.o.) can be used in the procedures described in this unit. Oral gavage can be difficult, although it can be accomplished when an animal is in the stereotaxic frame. Nonetheless, absorption of compounds through the enteric system can be significantly affected by the reduction in gastric motility that is associated with anesthesia. In addition, some compounds, such as peptides, may not penetrate into the brain, in which case intracerebroventricular administration may be necessary, as was described for beta-amyloid. Furthermore, if pharmacodynamic information is available on the test compound, this can help further tighten the resolution of the study design by directly relating the desired effect of the compound to a known administration route and pre-treatment time. This can be accomplished using autoradiographic or biochemical techniques. However, when only PK data are available and the pre-treatment time is selected on this basis, the experiments described in this unit are valuable for generating PD data. Thus, these assays make it possible to directly relate compound exposure readouts from samples taken during or at the end of recordings to the experimental results. This information can be used to determine the brain concentrations required for target engagement, and for translational PK-PD modeling (Bursi et al., 2011). Moreover, the procedures described herein can be used not only for examining the effects of test agents on LTP, but also for investigating differences in synaptic plasticity and function resulting from acute or chronic behavioral modifications, and between genetically modified populations.

Electrodes

The recommended stimulation and recording electrodes for these protocols can be used multiple times. To this end, they must be maintained in an optimal condition for as long as possible by keeping them in a dry, dust-free container between experiments. As the electrodes are embedded in brain tissue during the experiment, they should be cleaned by submerging the tip in contact lens solution overnight at least once per week. In addition, the central pole of the coaxial bipolar stimulation electrode recommended in the Basic Protocol (from FHC) sometimes becomes offset from the center. Before first using each stimulation electrode, check the alignment of this pole and mark the orientation with a marker pen on the barrel of the electrode. When positioning the stimulation electrode, be sure to orient this mark in the direction of the previously positioned recording electrode for optimal stimulation. In addition, there is often significant variation in the amount of voltage required for identifying and optimizing extracellular responses. While this is difficult to quantify, it is important to consider when using a new stimulation electrode.

Recording instability and drift

Brain inflammation is another factor that affects the stability of the population spike. To monitor brain swelling, it is useful to note the amplitude of the first and second positive peaks of the fEPSP. These provide an indication of the position of the population spike within the fEPSP, which is sensitive to the precise location of the electrode within the hippocampal pyramidal cell layer. The location of the electrode relative to this cell layer changes as the brain swells. This can be assessed electrophysiologically by a gradual but consistent drift in the amplitude of these two peaks, which reflects a change in the position of the population spike. One way to adjust for this inflammation, which may settle over time, is to reposition the recording and stimulating electrodes by making very small (5- to 10-μm) adjustments in their depth, usually by retracting them in the direction of the swelling. Careful removal of the skull bone, exposure of the brain, and insertion of the electrodes will minimize swelling. However, if it becomes a significant problem, it may be necessary to use dental fixative or cement instead of mineral oil and paraffin wax to fix the electrodes. This must be done after fEPSP responses are optimized, as it will be impossible to move the electrodes after they have been cemented. Use of dental fixative or

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cement will almost completely prevent brain swelling. When using fixative or cement, the investigator must be careful in removing and separating the recording and stimulating electrodes at the end of experiments to prevent damage to them and to ensure that they can be used again.

Anticipated Results

Software considerations

The acquisition software Spike2 is recommended for capturing both blood pressure and extracellular signals. This requires setting up a sampling configuration to import the information from the Micro1401 hardware. In addition, a series of stimulation output signals will need to be programmed within the sequencer's graphical editor to trigger the isolated constant voltage stimulator during fEPSP optimization and baseline generation, and for LTP induction. To monitor responses online, it is also necessary to program active cursors to measure the variables described below in real time and to perform the subsequent analysis. Spike2 software has extensive help functions to assist in performing these tasks.

Illustrated in Figure 11.11.1 are typical extracellular responses recorded from the hippocampal CA1 region highlighting the variable to be calculated and monitored for the duration of the experiment. The most straightforward way to calculate the amplitude of the population spike is to measure the mean of the positive peak directly before and directly after the negative peak and to subtract this from the amplitude of the negative peak. Other methods can also be considered, e.g., by measuring the area under the curve or modulus of the population spike, or by measurement of the slope of the underlying fEPSP before the population spike. This method could also be used in experiments in which solely fEPSP activity is induced. Once the selected measurement has been taken, each response is then normalized to a selected period of 20 sweeps, or 10 min, directly before either the administration of a test compound or the induction of LTP, allowing the generation of a scatter plot (Figs. 11.11.2 and 11.11.5). Shown in Figure 11.11.2 is the enhancement in submaximal LTP evoked by a 50-min pretreatment with D-cycloserine, a positive allosteric modulator of NMDA receptors, while illustrated in Figure 11.11.5 are the deficits in LTP induced by a 30-min pre-treatment with oligomeric beta-amyloid. Once the separation between treatment groups is examined in scatter plots, specific time points are selected for comparison between treatment groups. For LTP analysis, these typically include a short-term time point between 0 and 5 min (10 sweeps) after induction and a later time point selected on the basis of the greatest separation between treatment groups but incorporating a minimum of 10 sweeps to account for inter-sweep variability. Often this is the last 10 min, or 20 sweeps, of recording. In the case of the sample data shown in Figures 11.11.2 and 11.11.5, this is between 80 and 90 min after LTP induction. When examining the effects of a compound on the amplitude of population spikes before or without LTP induction (Fig. 11.11.6), the maximal response over a 5-min, 10-sweep period is usually selected for paired comparison of the raw, rather than normalized, amplitude with baseline. In addition, when a compound almost totally blocks the population spike, as seen after ketamine administration (Fig. 11.11.6), it may be necessary to calculate the amplitude of each sweep manually using the cursor functions within Spike2 software, because the method described above can produce inaccurate readouts.

As for group size, the absolute numbers required to provide the necessary power to determine significant differences between treatment regimens will vary with the protocol. It is recommended that group sizes of at least six to eight animals be used to generate the most reliable data.

Time Considerations

As discussed above, the timeline for completing experiments utilizing these protocols will vary depending on the duration between the treatment and the time when LTP is induced. In addition, although 90 min are needed for monitoring PS amplitude after induction, there may be cases when it is worthwhile to monitor responses for longer periods, either to capture a test compound's exposure at a later time point through blood or tissue sampling, or for examining the effects of either a disease-modeling agent or potential therapeutic agent that may have only a small effect with slow onset. However, there are limits to the duration of the test procedure, especially when considering the complications associated with anesthesia. It is recommended that the experiments be completed within 8 hr after induction of anesthesia, keeping in mind that it is often difficult to maintain the stability necessary for monitoring steady-state PS activity for any longer than 5 hr. With practice, the cannulation surgery, placement in the stereotaxic frame,

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brain exposure, and initial electrode positioning can be accomplished within 60 min. There should be a 30-min delay after animal preparation before initiating the search for extracellular responses. The subsequent optimization and input/output processes require approximately 10 min, and the responses need another 30 to 60 min to stabilize before commencing the 10-min baseline recording period in advance of either test agent administration or LTP induction. Assuming a 30-min pre-LTP-induction incubation and a 90-min post-LTP-induction period, a total experiment time of 5 hr should be anticipated. Because these experiments involve living animals, the amount of time needed to identify and optimize responses, and the time required for responses to stabilize, can vary significantly among subjects, affecting the overall time needed to complete an experiment.

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