Journal of Visualized Experiments

Investigation of synaptic tagging/capture and cross-capture using acute hippocampal slices from rodents

--Manuscript Draft--

Manuscript Number:	JoVE53008R4
Full Title:	Investigation of synaptic tagging/capture and cross-capture using acute hippocampal slices from rodents
Article Type:	Methods Article - JoVE Produced Video
Keywords:	Long-term potentiation; Long-term depression; Synaptic tagging; Synaptic tagging and Capture; Cross-capture; Cross-tagging
Manuscript Classifications:	8.1: Natural Science Disciplines; 8.1.158: Biological Science Disciplines; 8.1.158.610: Neurosciences
Corresponding Author:	Saji Kumar Sreedharan National University of Singapore Singapore, Singapore SINGAPORE
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	phssks@nus.edu.sg
Corresponding Author's Institution:	National University of Singapore
Corresponding Author's Secondary Institution:	
First Author:	Mahesh Shivarama Shetty
First Author Secondary Information:	
Other Authors:	Mahesh Shivarama Shetty
	Mahima Sharma
	Sin Hui Neo
	Ananya Dasgupta
	Suma Gopinadhan
Order of Authors Secondary Information:	
Abstract:	Synaptic tagging and capture (STC) and cross-tagging are two important mechanisms at cellular level that explain how synapse-specificity and associativity is achieved in neurons within a specific time frame. These long-term plasticity-related processes are the leading candidate models to study the basis of memory formation and persistence at the cellular level. Both STC and cross-tagging involve two serial processes: (1) setting of the synaptic tag as triggered by a specific pattern of stimulation, and (2) synaptic capture, whereby the synaptic tag interacts with newly synthesized plasticity-related proteins (PRPs). Much of the understanding about the concepts of STC and cross-tagging arises from the studies done in CA1 region of the hippocampus and because of the technical complexity many of the laboratories are still unable to study these processes. Experimental conditions for the preparation of hippocampal slices and the recording of stable late-LTP/LTD are extremely important to study synaptic tagging/cross-tagging. This video article describes the experimental procedures to study long-term plasticity processes such as STC and cross-tagging in the CA1 pyramidal neurons using stable, long-term field-potential recordings from acute hippocampal slices of rats.
Author Comments:	The open access of this video article is sponsored by Cerebos Pacific Limited thus they should be acknowledged in the beginning of the video article.

Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date to satisfy grant requirements, please indicate the date below and explain in your cover letter.	
If this article needs to be filmed by a certain date to due to author/equipment/lab availability, please indicate the date below and explain in your cover letter.	

Yong Loo Lin School of Medicine
Department of Physiology

NUS National University of Singapore

From

Sajikumar Sreedharan. Ph.D
Assistant Professor
Department of Physiology
Centre for Life Sciences (CeLS)
28 Medical Drive
National University of Singapore
Singapore 117456
Tel: (65)65165886 Fax: (65)67773271
E.mail: phssks@nus.edu.sg
Web: http://medicine.nus.edu.sg/phys/lab/Saji_Lab/index.html

13 April 2015
Singapore

To

The Editor
JoVE

Dear Editor

Thank you very much for the helpful suggestions on our video manuscript entitled "***Investigation of synaptic tagging/capture and cross-capture using acute hippocampal slices from rodents" (JoVE53008R3).***

We have now carefully revised the manuscript based on both the editorial and reviewers' suggestions. The point by point responses to reviewers are included as separate file and the changes in the manuscript are highlighted in green. The text for video script is highlighted in yellow. We thank the editors and reviewers for the insightful comments on our manuscript that substantially increased the quality and we hope our manuscript is now suitable for publication in JoVE.

Response to Editorial comments:

1. The length is >2.75 (~3.5) pages highlighted and must be reduced to fit within filming limits.

Response: Done

2. Replace Anesthetize with "Euthanize" in step 3.1.4

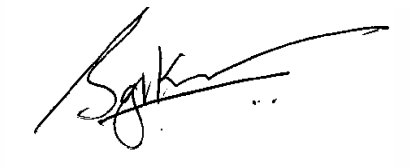
Response: Done

3. What level of ACSF is ideal in 3.3.5?

Response: we have now rephrased the sentence to make it clear.

Thanking you

Sincerely



Dr. Sreedharan Sajikumar

Block MD9, 2 Medical Drive, Singapore 117597 Tel: (65) 6516 3223 Fax: (65) 6778 8161
Website: www.med.nus.edu.sg/phys
Company Registration No: 200604346E

TITLE:

Investigation of synaptic tagging/capture and cross-capture using acute hippocampal slices from rodents.

AUTHORS:

Mahesh Shivarama Shetty, Mahima Sharma, Neo Sin Hui, Ananya Dasgupta, Suma Gopinadhan and Sreedharan Sajikumar [1,2], *

AUTHOR AFFILIATION:

Mahesh Shivarama Shetty,
Department of Physiology,
Yong Loo Lin School of Medicine,
National University of Singapore
Singapore, Singapore
mahesh.shetty@nus.edu.sg

Mahima Sharma, Neo Sin Hui,
Neurobiology/Ageing Programme,
Life Sciences Institute,
National University of Singapore,
Singapore, Singapore
90109385@nus.edu.sg

Neo Sin Hui
Neurobiology/Ageing Programme,
Life Sciences Institute,
National University of Singapore,
Singapore, Singapore
phsnsh@nus.edu.sg

Ananya Dasgupta,
Neurobiology/Ageing Programme,
Life Sciences Institute,
National University of Singapore,
Singapore, Singapore
a0109390@nus.edu.sg

Suma Gopinadhan
Neurobiology/Ageing Programme,
Life Sciences Institute,
National University of Singapore,
Singapore, Singapore
phssg@nus.edu.sg

Sreedharan Sajikumar
Department of Physiology,
Yong Loo Lin School of Medicine,

National University of Singapore
Singapore, Singapore

Neurobiology/Ageing Programme,
Life Sciences Institute,
National University of Singapore,
Singapore, Singapore
phssks@nus.edu.sg

CORRESPONDING AUTHOR:
Dr. Sreedharan Sajikumar
Department of Physiology
Yong Loo Lin School of Medicine
National University of Singapore
117597, Singapore.
Phone: +65- 65165886
Fax: + 65-67773271
Email: phssks@nus.edu.sg

KEY WORDS:
Long-term potentiation, Long-term depression, Synaptic tagging, Synaptic tagging and Capture, Cross-capture

SHORT ABSTRACT:
This video article describes experimental procedures to study long-term plasticity and its associative processes such as synaptic tagging, capture and cross-tagging in the CA1 pyramidal neurons using acute hippocampal slices from rodents.

LONG ABSTRACT:
Synaptic tagging and capture (STC) and cross-tagging are two important mechanisms at cellular level that explain how synapse-specificity and associativity is achieved in neurons within a specific time frame. These long-term plasticity-related processes are the leading candidate models to study the basis of memory formation and persistence at the cellular level. Both STC and cross-tagging involve two serial processes: (1) setting of the synaptic tag as triggered by a specific pattern of stimulation, and (2) synaptic capture, whereby the synaptic tag interacts with newly synthesized plasticity-related proteins (PRPs). Much of the understanding about the concepts of STC and cross-tagging arises from the studies done in CA1 region of the hippocampus and because of the technical complexity many of the laboratories are still unable to study these processes. Experimental conditions for the preparation of hippocampal slices and the recording of stable late-LTP/LTD are extremely important to study synaptic tagging/cross-tagging. This video article describes the experimental procedures to study long-term plasticity processes such as STC and cross-tagging in the CA1 pyramidal neurons using stable, long-term field-potential recordings from acute hippocampal slices of rats.

INTRODUCTION:

The encoding and storage of information in the brain still remains the most significant and keenly pursued challenge in neuroscience. Over the years, long-term potentiation (LTP) and long-term depression (LTD) have emerged as the leading cellular correlates of memory[1,2]. These activity dependent changes, which exhibit input specificity and associativity, result in the stabilization of memory traces in the neuronal networks [1,3,4]. The maintenance of the two forms of synaptic plasticity requires the synthesis of plasticity-related products (PRPs)[5-10]. Synapse specificity that involves the interaction of newly synthesized protein only with specific activated synapses expressing LTP or LTD, is critical to memory. This specificity is explained by the concept of 'Synaptic Tagging and Capture' (STC), where the PRPs interact with recently active, 'tagged' synapses[11,12]. The STC process offers a framework for associative properties of memories at the cellular level. It provides us with a conceptual basis of how short-term forms of plasticity are transformed into long-lasting forms of plasticity in an associative and time-dependent manner[13].

During the process of STC, a strong tetanization in one input that leads to protein synthesis dependent late-LTP, results in the reinforcement of a protein synthesis independent early-LTP induced in another independent input on to the same population of neurons into a persistent one[13]. The setting of a local synaptic tag by a transient neural activity and the synthesis of the diffusible PRPs by the strong neural activity are the two key events during STC[13,14]. The capture of the PRPs by the recently potentiated 'tagged' synapses is fundamental to the maintenance of long-term potentiation. Many studies have been done to confirm the existence of STC phenomenon[15-17] and identify the candidate 'tags'[18] and 'PRPs'[19]. Calcium/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal-regulated kinase1/2 (ERK1/2); CaMKIV, Protein Kinase Mζ (PKMζ) and brain-derived neurotrophic factor (BDNF) are some of the candidate molecules for 'tag' and 'PRP' respectively[19-21]. The synaptic tagging model has further been expanded to include the positive associative interactions between LTP and LTD - the "synaptic cross-tagging"[22]. In synaptic cross-tagging, a late LTP/ LTD in one synaptic input transforms the opposite protein synthesis-independent early-LTD/LTP in an independent input into its long-lasting form or vice versa[22].

The hippocampal slice preparation is the most widely used model in the studies of long-term synaptic plasticity[23,24]. Much of the understanding about the concepts of synaptic tagging and cross- tagging arises from the studies done in CA1 region of the hippocampus and because of the technical complexity many of the labs are still unable to study these processes. Experimental conditions for the preparation of rat hippocampal slices and the recording of stable late-LTP/LTD for extended hours are extremely important to study synaptic tagging/cross-tagging[23,25,26]. This article describes the detailed experimental procedures for studying long-term plasticity processes such as STC and cross-tagging in the CA1 pyramidal neurons using stable, long-term field-potential recordings from acute hippocampal slices of rats.

PROTOCOL:

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of National University of Singapore.

1. Preparation of Artificial Cerebrospinal Fluid (ACSF)

1.1. Prepare the ACSF consisting of (in mM) 124 NaCl, 3.7 KCl, 1.0 $MgSO_4.7H_2O$, 2.5 $CaCl_2.2H_2O$, 1.2 KH_2PO_4, 24.6 $NaHCO_3$, and 10 D-glucose. Ensure the pH of the ACSF is between 7.2-7.4 when bubbled to saturation with 95% O_2 and 5% CO_2 mixture (carbogen).[21] Use this ACSF for both the dissection, slice preparation and for perfusion during the electrophysiological recordings.

NOTE: Use clean apparatus for measuring and holding the ACSF. Using unclean apparatus may lead to cloudy solutions or formation of precipitates. Use deionized water for all the preparations.

1.2. Prepare a 2L 10x ACSF stock excluding $NaHCO_3$ and D-Glucose in a volumetric flask. Add reagents to deionized water in the following order: NaCl (144.96g), KCl (5.52g), $MgSO_4.7H_20$ (4.92g), $CaCl_2.2H_20$ (7.56g), KH_2PO_4 (3.28g) and top up to a volume of 2L. Stir continuously for at least 30 minutes using a magnetic stirrer to ensure all reagents are dissolved. Store the stock in 4°C and use within 2 weeks.

1.3. Prior to the dissection and the experiments, dilute the ACSF stock in a volumetric flask along with the addition of required amounts of $NaHCO_3$ and D-glucose. For 1L solution, dilute 100 mL of the stock to 1L after adding 2.07g $NaHCO_3$ and 1.802g D-glucose. The ACSF should be a clear solution free of any precipitate or undissolved particles.

1.4. Cool about 200-300ml of ACSF on ice, to be used during dissection. Ensure that the ACSF used for dissection is between 2-4°C. Use the remaining ACSF for electrophysiological experiments. Bubble all ACSF solutions to saturation with carbogen (5% CO_2, 95% O_2) continuously. While waiting for the ACSF to cool, prepare the dissection area and the slice chamber.

2. Preparation of Interface Chamber

NOTE: An interface brain slice chamber, used for incubating the slices and maintaining them during electrophysiological recordings (Figure 2B), consists of two compartments. The lower chamber contains distilled water maintained at 32 °C by a temperature controller and continuously bubbled with carbogen.

2.1. Switch on the temperature controller and preset it at 32 °C. Wash the upper chamber for 10 to 15 minutes by running distilled water through the inflow tubing. Ensure that the upper chamber is clean before placing the net. Check that water level in the bottom chamber is about 70% filled with distilled water.

2.2. Place the net in the upper chamber to provide a resting surface for slices (Figure 2C). Adjust the outflow tubing to ensure that the solution level is sufficiently wetting the whole area of the net. Place the lid above the net to maintain a humidified carbogen atmosphere within the upper chamber.

2.3. Adjust the flow rate to 1 ml/min. Maintain this flow rate throughout the slice incubation period and the experiment. Start carbogenating the freshly prepared 1x ACSF

and immerse the inflow tubing into the ACSF. Allow 20 minutes for the ACSF to be saturated with carbogen and for the upper chamber to be filled with it.

3. Preparation of acute hippocampal slices

NOTE: The dissection protocol consists of (1) Removal of brain from the animal into cold ACSF and (2) Isolation and slicing of the hippocampus. In order for neurons to remain viable, isolate and place the brain in cold ACSF quickly and complete the whole process including slicing within 3-5 minutes.

3.1 Removal of the brain into cold ACSF

3.1.1. Lay out the dissection tools in the manner shown in Figure 1A. Arrange the tools according to the order of use to facilitate the dissection process. Before starting, ensure all the dissection tools are ready.

3.1.2. Mount a razor blade, cleaned with ethyl acetate, absolute ethanol and distilled water, onto the manual tissue chopper (Figure 1B), secure it firmly and ensure that the cutting edge is evenly aligned. Test chopping a piece of filter paper to ensure that the blade is firmly secured. Set the sliding Vernier micrometer to its starting position.

3.1.3. Euthanize the animal using carbon dioxide (CO_2) in an induction chamber and decapitate with bandage scissors or Guillotine. Using an Iris scissors, remove the skin and fur above the skull. Make a cut through the posterior to remove the brainstem. Make a small incision along the right side of the skull and a longer incision on the left.

CAUTION! Make only a small incision to the side that is used for the experiments to avoid damaging it. When inserting the scissors, make sure that the force applied is upwards to avoid damage to the brain.

3.1.4. Carefully remove the skull with a bone rongeur starting from the left to the right side of skull to reveal the cortex. A thin layer of dura can also be seen. Carefully remove the frontal plates with the rongeur. Remove most of the dura along with the frontal plates.

CAUTION! Be careful that the dura does not slice through the brain tissue.

3.1.5. Remove the remaining dura, if any, specifically in the junction between cortex and cerebellum with the flat end of a spatula. For steps 3.1.5 and 3.1.6, maintain pressure upwards i.e. away from the brain to avoid damaging it. Using the spatula, gently scoop the brain into a petri dish filled with cold and carbogenated ACSF (2-4°C), placed on an aluminum cooling block.

3.2 Isolation of the hippocampus

3.2.1. Using a scalpel, make a straight cut to remove the cerebellum and another cut to remove the anterior portion of the brain (approximately one quarter). Make a shallow cut along the midline.

3.2.2. Carefully remove the cortex with a sickle scaler, starting from the midline to reveal the dorsal hippocampus. Remove the layer of cortex above the hippocampus. Use fingers or

angled forceps to support the brain. Make a small cut to the hippocampal commissure. Gently remove the hippocampus with the sickle scaler starting from the dorsal hippocampus using rolling motions.

CAUTION! Be gentle to avoid stretching and tearing the hippocampus.

3.2.3. Remove any cortex and connective tissues around the isolated hippocampus with the sickle scaler.

3.3 Slicing the hippocampal tissue and transferring the slices onto the interface chamber

3.3.1. Place a piece of ACSF-soaked filter paper (Grade 1, 30mm) on the slicing stage of the manual slicer. Scoop and place the hippocampal tissue onto the filter paper. Move the filter paper to align the hippocampus at a proper orientation in relation to the blade of the slicer so that the hippocampus is sliced at an angle of about 70^{o} to the fimbria.

3.3.2. Blot the excess solution surrounding the hippocampal tissue with a folded filter paper (Grade 1, 85mm) leaving the hippocampus slightly wet. Start slicing the hippocampus transversely. Slice and discard tissue from the extreme end of the hippocampus where the slice morphology is not clear.

3.3.3. Slice the remaining tissue into 400 µm-thick slices. Pick up hippocampal slices gently from the blade with a brush with soft bristles using gentle swiping movements and place the slices into a small beaker filled with cold carbogenated ACSF. Perform the steps 3.3.1-3.3.4 as quickly as possible since the hippocampal tissue is exposed to air.

NOTE: Generally two-thirds of the hippocampus is sliced, and 4-6 slices with clear morphology can be prepared.

3.3.4. Transfer the slices gently onto the net in the slice chamber using a clean plastic Pasteur pipette with a broad tip (made by cutting away 2-3cm of the tip). Carefully adjust the position of the slices on the net using a small syringe with a bent tip. Position the slices in a manner that facilitates electrode location and recording. Check to ensure that the slices are sufficiently surrounded by ACSF but are not submerged or floating (Figure 2C-D). Cover the chamber and incubate the slices for 2-3 hours.

NOTE: The pyramidal cell layer in the healthy slices should show some transparency.

4. Recording of CA3-CA1 synaptic responses

NOTE: The electrophysiology set-up used for field potential recording is shown in Figure 2A. A Faraday cage is strongly recommended if the electrical interference is beyond the control after the proper grounding of the electrical settings. Many different types of submerged and interface chambers are commercially available. However, interface chambers are preferred as slices exhibit more robust synaptic responses in them.

4.1. Positioning of electrodes

4.1.1. Turn on the electrical apparatus (stimulators and amplifiers) to be used. Mount and secure the stimulating and recording electrodes in the plexiglass holders of the micromanipulators.

NOTE: We use monopolar, lacquer-coated, stainless steel electrodes of 5 MΩ resistance for both stimulating and recording purposes.

4.1.2. Before use, insert these electrodes inside the pulled glass capillaries and secure with epoxy glue exposing only small portion of the electrode tip (Figure 2E). This gives strength to the otherwise slender electrodes and helps to secure them firmly in the electrode holders.

4.1.3. Guided under the microscope, position the stimulating electrode(s) in the stratum radiatum of the CA1 region to stimulate the Schaffer collateral fibers and the recording electrode in the apical dendritic region of CA1 to record field-EPSP (fEPSP) responses.

NOTE: Approaching the liquid surface above the slice with the electrodes gives a sound that helps to locate quickly the surface of the slice (provided, the amplifier is connected to a loudspeaker).

4.1.4. In synaptic tagging and capture experiments, according to the need of the experiment, position two or three stimulating electrodes (S1, S2 or S3) on either side of the recording electrode to stimulate two or more independent but overlapping inputs. Position the stimulating and recording electrodes about 200 µm apart.

4.1.5. Test the pathway independence with a paired-pulse facilitation protocol[27,28]. If necessary, locate another recording electrode in the stratum pyramidale layer for recording population spike (Figure 3A).When both the electrodes have touched the slice, using the acquisition software, give a test stimulation to ensure a proper fEPSP signal.

NOTE: We use biphasic, constant-current pulses (impulse duration 0.1 ms/half-wave) for test stimulation.

4.1.6. Once a proper fEPSP signal is obtained, carefully lower the electrodes about 200 µm deep using fine movement knobs of the manipulators. Allow 20 minutes for the slices to recover.

4.2. Input-output relation

4.2.1. Determine the input-output relation (afferent stimulation vs fEPSP slope) for each input by measuring the slope value at a range of current intensities. Perform this between 20 µA to 100 µA. Then set the stimulation intensity for each input to obtain 40% of the maximum fEPSP slope. Keep this constant throughout the experiment.

4.2.2. After 15-20 minutes, start recording the baseline. Monitor the fEPSP slope closely during this period and reset the stimulus intensity if the slope fluctuates more than 10% from the set value and start a new baseline. Record at least 30 minutes or 1 hour stable baseline before proceeding.

NOTE: For the test or the baseline stimulation, we use four sweeps of 0.2Hz biphasic, constant current pulses (0.1ms per polarity) given every five minutes. An average slope of these four responses is then considered as one repeat. The signals are filtered and amplified by a differential amplifier, digitized using an analog-to-digital converter and monitored online with custom-made software.

4.3. Induction of LTP/LTD using tetanic stimulation

NOTE: Both LTP and LTD have been classified as early and late-LTP/LTD based on the requirements of protein synthesis; the latter requiring translation and/or transcription for its late maintenance [for review see[4]]. A variety of electrical stimulation paradigms can specifically induce the different forms of LTP and LTD.

4.3.1. For early-LTP induction, use a weak tetanization protocol (WTET) consisting of a single high frequency stimulation (100 Hz, 21 biphasic constant current pulses, single burst, 0.2 ms pulse duration).

4.3.2. For late-LTP induction, use a strong tetanization protocol (STET) involving repeated high-frequency stimulation (three trains of 100 Hz, 100 pulses, single burst, 0.2 ms pulse duration) with an inter-train interval of ten minutes[11].

4.3.3. For late-LTD induction, apply a strong low-frequency protocol (SLFS) consisting of 900 bursts over a 15-min duration (one burst consisting of 3 stimuli at 20 Hz, inter-burst interval of 1 s, stimulus duration 0.2 ms per half-wave; total number of stimuli-2700)[29].

5. Cleaning of slice chamber and perfusion system

5.1. After the recording is over, collect the hippocampal slices for further biochemical analysis or else discard appropriately. Turn off the carbogen supply and temperature controller. Wash the carbogen bubbler in distilled water.

5.2. Clean the net thoroughly with a brush and distilled water. Wash the rig for 15-20 minutes with distilled water at a higher flow rate. Once in 3-4 days, change the distilled water in the lower compartment of the chamber and also clean the chamber regularly with 3% hydrogen peroxide solution to avoid fungal growth.

REPRESENTATIVE RESULTS:

The described methodology has been used to study long-lasting forms of LTP/LTD and its associative interactions such as synaptic tagging and cross-capture from acute hippocampal slices of adult rats.[23] This technique has proven effective for experiments with both rats (Wistar) and a variety of mouse strains[30,31]. The methodology has been used successfully for stable LTP recordings of up to 8-12 hours.[32]

The 'tag' set by the weak tetanization of one input (S1) captures the 'PRPs' induced by the strong tetanization of another independent but overlapping input **(S2; Figure 3B; filled circles)** thereby transforming the otherwise decaying form of LTP (early-LTP) in S1 into a long-lasting one **(Figure 3B; open circles)** (For comparison of early-LTP induced by WTET see[20,33]). The PRPs captured by the weak tetanization set tag need not necessarily come from the STET-induced late-LTP but can also be provided by the SLFS-induced late-LTD. This

type of positive associative interaction between LTP and LTD is referred to as 'cross-tagging/capture'. The WTET-induced early-LTP in S1 gets reinforced to late-LTP **(Figure 3C; open circles)** by capturing the PRPs provided by the SLFS-induced late-LTD in S2 **(Figure 3C; filled circles)**. Statistically significant potentiation or depression was maintained in S1 and S2 in both cases when compared to its own baseline (Wilcoxon test; $P < 0.05$).

For the tag-PRP interaction to occur, the temporal order of the two events (weak-before-strong/strong-before-weak) is not crucial as long as the time window between the two events remains within the range of 30-60 minutes. It would be wise to include a third, independent but overlapping synaptic input and use it as a baseline control to monitor the stability of recordings. The electrical stimulation protocols used to induce early- and late forms of LTP/LTD must be validated in single-input experiments for consistency and reliability before using them in STC experiments. We would also like to emphasize the importance of slice preparation methodology described in the protocol since the success of these experiments relies heavily on the quality of the slices.

FIGURE LEGENDS:

Figure 1: Tools used in the dissection of hippocampus: (a) Bandage Scissors (b) Iris scissors (c) Bone rongeur (d) Thin spatula, (e) Scalpel number 11 (f) Sickle scaler (g) Soft-bristle paint brush (h) Plastic Pasteur pipette (i) filter paper (85mm) (j) filter paper (30mm) (k) Glass beakers (l) Aluminum cooling blocks to fit petri dish and beakers (m) Petri dish. **B**. Manual tissue chopper. (a) Platform (b) Cutting arm with blade-holder (c) Vernier micrometer, resolution 10 microns.

Figure 2: Electrophysiology set-up for field-potential recordings consisting of (a) stimulators (b) a differential amplifier (c) an analog-to-digital converter (d) Oscilloscope (e) computer with acquisition software (f) Vibration-resistant table-top (g) microscope with >4x magnification (h) interface brain-slice chamber (i) a perfusion system for ACSF and carbogen supply (j) temperature controller (k) an illumination source (l) manipulators with electrode holders **B**. Interface brain-slice chamber. **C** & **D**. Hippocampal slices in the interface chamber **E**. Stainless steel electrode sealed in a glass capillary.

Figure 3: **A**. Schematic representation of a transverse hippocampal slice and electrode location for field-potential recording: In this representation, two stimulating electrodes (S1 and S2) are positioned in the stratum radiatum of the CA1 region to stimulate two independent but overlapping synaptic inputs onto CA1 pyramidal neurons. Two extracellular recording electrodes, one to record field-EPSP (excitatory post-synaptic potential) from the apical dendritic compartment and another to record somatic population spike from the pyramidal cell bodies, are located in the stratum radiatum and stratum pyramidale respectively. CA1- cornu ammonis region 1, CA3- cornu ammonis region 3, DG- dentate gyrus, SC- Schaffer collateral fibers, S1- stimulating electrode 1, S2-stimulating electrode 2.
B. Weak before strong paradigm to study STC: Weak tetanization (WTET) is applied to S1 (open circles) for inducing early-LTP followed by strong tetanization (STET) of S2 (filled circles) at 30 minutes to induce late-LTP. The early-LTP in S1 gets reinforced to late-LTP showing tagging and capture interaction (n = 6). **C**. Weak before strong paradigm to study cross-tagging: Early-LTP is induced by WTET in S1 (open circles) followed by the induction of late-LTD in S2 (filled circles) using SLFS after 30 minutes. In S1, the early-LTP is transformed

to late-LTP lasting 6 hours showing cross-tagging and capture (n = 6). Single arrow represents weak tetanization applied for inducing early-LTP. Triplet of arrows represents strong tetanization for inducing late-LTP. The broken arrow represents the time point at which SLFS was applied to the representative synaptic input to induce late-LTD. Error bars indicate SEM.

DISCUSSION:

Acute hippocampal slice is an excellent model system for the study of LTP and other functional plasticity processes such as STC and cross-capture. It preserves much of the laminar structural network of the hippocampal circuits, allows precise electrode locations and offers alongside, an open platform for rapid neuropharmacological manipulation without a blood-brain barrier.

This article describes the methodology for the preparation of viable acute hippocampal slices from young adult rats and using them to investigate STC and cross-tagging. Previous research has emphasized that gender and age of the animals are important factors to consider for use in electrophysiology studies.[27,28] Therefore young adult animals with fully expressed adult receptor functions (Male Wistar rats aged 5-7week) are used.[23] Asymmetries in the connections between the left and right hippocampus have been noted in rodents[29]and major differences in NMDA receptor expression have been reported as well [34]. We have used the right hippocampus in order to be consistent with our previous LTP studies.[23,32] However, either of the hippocampi can be used as long as consistency is maintained.

As in any protocol, it is very crucial to perform the isolation and slicing procedures quickly but taking care that the tissue is not stretched, damaged, rendered dry or hypoxic. The variations in pH, temperature and ionic composition of the solutions can have profound effect on the viability of the slices and the results. Hence such variations should be avoided. It has been observed that glutamate receptor-dependent calcium release occurring during the preparation steps can irreversibly affect protein synthesis in nervous tissue [35,36,37]. Using manual tissue slicers can help to minimize this by allowing the process to be completed very quickly as compared to vibraslicers. However, many laboratories also effectively use vibraslicers with necessary precautions to preserve slice viability. Another important factor to consider is the long incubation period before starting the experiments. This has been noted to be really crucial to achieve stability in metabolic state and kinase activation levels in the slices after the disturbance caused during preparation [23]. Such stability is necessary for consistency in long-term recordings. We re-emphasize on this observation and suggest the long incubation hours of about 3 hours.

A variety of stimulation parameters are known to induce LTP, but the molecular mechanisms elicited in each case may not be the same (for review see [38]). This can influence the durability and other characteristics of the LTP which, in turn, can affect the results of synaptic tagging and capture experiments. Hence it is important to validate the stimulation paradigms and characteristics of the elicited LTP under the conditions of the performing laboratory and maintain consistency.

We generally do not consider experiments with very large presynaptic fiber volleys and with maximal fEPSPs less than 0.5 mV and the experiments involving substantial changes in the fiber volley during the recordings are also rejected. Further, while performing two-pathway or three-pathway experiments, it is important to ensure the pathway independence. This can be carried out with a paired-pulse facilitation protocol[28].

One downside of the interface recording systems is the formation of condensation droplets on the electrodes during the long recording hours due to the temperature and humidity differences between the chamber and the surroundings. These droplets need to be carefully blotted from time to time. Otherwise the droplets can drip onto the slices and cause disturbance or even loss of signals. We usually tackle this by skilfully blotting the droplets guided under the microscope using a slender filter paper wick, without touching the electrodes. However, the best solution would be to use a centralized heating system, such as the ETC system developed by University of Edinburgh researchers (www.etcsystem.com), which can completely obviate the need to catch the condensation drops.

On a concluding note, a variety of methodologies exist in the laboratories worldwide that are used for the preparation of hippocampal slices for different experimental purposes. Each of the procedure offers some advantages over the other. One needs to carefully optimize the minute details of the protocol to suit the purpose of the experiment. We hope that this article helps in improving some aspects of the methodology for studying late-associative processes such as STC and cross-capture.

ACKNOWLEDGEMENT:
This video article is sponsored by Cerebos Pacific Limited. This work is supported by National Medical Research Council Collaborative Research Grant (NMRC-CBRG-0041/2013) and Ministry of Education Academic Research Funding (MOE AcRF- Tier 1 - T1-2012 Oct -02).

DISCLOSURES:
None declared.

REFERENCES:

1 Bliss, T. V. P. & Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39, doi:10.1038/361031a0 (1993).
2 Siegelbaum, S. A. & Kandel, E. R. Learning-related synaptic plasticity: LTP and LTD. *Current Opinion in Neurobiology* **1**, 113-120, , doi:10.1016/0959-4388(91)90018-3 (1991).
3 Martin, S. J., Grimwood, P. D. & Morris, R. G. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annual review of neuroscience* **23**, 649-711, doi:10.1146/annurev.neuro.23.1.649 (2000).
4 Malenka, R. C. & Bear, M. F. LTP and LTD: An Embarrassment of Riches. *Neuron* **44**, 5-21, doi:10.1016/j.neuron.2004.09.012 (2004).
5 Krug, M., Lossner, B. & Ott, T. Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain research bulletin* **13**, 39-42 doi:10.1016/0361-9230(84)90005-4 (1984).

6 Frey, U., Krug, M., Reymann, K. G. & Matthies, H. Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. *Brain research* **452**, 57-65, doi:10.1016/0006-8993(88)90008 (1988).

7 Otani, S., Marshall, C. J., Tate, W. P., Goddard, G. V. & Abraham, W. C. Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. *Neuroscience* **28**, 519-526, doi: 10.1016/0306-4522(89)90001-8 (1989).

8 Frey, U., Frey, S., Schollmeier, F. & Krug, M. Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. *The Journal of physiology* **490 (Pt 3)**, 703-711, doi:10.1113/jphysiolo.1996.sp021179 (1996).

9 Nguyen, P. V. & Kandel, E. R. A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **16**, 3189-3198 (1996).

10 Manahan-Vaughan, D., Kulla, A. & Frey, J. U. Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **20**, 8572-8576, (2000).

11 Frey, U. & Morris, R. G. M. Synaptic tagging and long-term potentiation. *Nature* **385**, 533-536, doi:10.1038/385533a0 (1997).

12 Frey, U. & Morris, R. G. Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. *Neuropharmacology* **37**, 545-552, doi:10.1016/s0028-3908(98)00040-9 (1998).

13 Redondo, R. L. & Morris, R. G. Making memories last: the synaptic tagging and capture hypothesis. *Nature reviews. Neuroscience* **12**, 17-30, doi:10.1038/nrn2963 (2011).

14 Martin, K. C. & Kosik, K. S. Synaptic tagging -- who's it? *Nature reviews. Neuroscience* **3**, 813-820, doi:10.1038/nrn942 (2002).

15 Frey, U. & Morris, R. G. Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. *Trends in neurosciences* **21**, 181-188, doi:10.1016/s0166-2236(97)01189-2 (1998).

16 Martin, K. C. Synaptic tagging during synapse-specific long-term facilitation of Aplysia sensory-motor neurons. *Neurobiology of learning and memory* **78**, 489-497, doi:10.1006/nlme.2002.4088 (2002).

17 Shires, K. L., Da Silva, B. M., Hawthorne, J. P., Morris, R. G. & Martin, S. J. Synaptic tagging and capture in the living rat. *Nature communications* **3**, 1246, doi:10.1038/ncomms2250 (2012).

18 Ramachandran, B. & Frey, J. U. Interfering with the actin network and its effect on long-term potentiation and synaptic tagging in hippocampal CA1 neurons in slices in vitro. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **29**, 12167-12173, doi:10.1523/JNEUROSCI.2045-09.2009 (2009).

19 Sajikumar, S., Navakkode, S. & Frey, J. U. Identification of compartment- and process-specific molecules required for "synaptic tagging" during long-term potentiation and long-term depression in hippocampal CA1. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 5068-5080, doi:10.1523/JNEUROSCI.4940-06.2007 (2007).

20 Sajikumar, S., Navakkode, S., Sacktor, T. C. & Frey, J. U. Synaptic tagging and cross-tagging: the role of protein kinase Mzeta in maintaining long-term potentiation but not long-term depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 5750-5756, doi:10.1523/JNEUROSCI.1104-05.2005 (2005).

21 Redondo, R. L. *et al.* Synaptic Tagging and Capture: Differential Role of Distinct Calcium/Calmodulin Kinases in Protein Synthesis-Dependent Long-Term Potentiation. *The Journal of Neuroscience* **30**, 4981-4989, doi:10.1523/jneurosci.3140-09.2010 (2010).

22 Sajikumar, S. & Frey, J. U. Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiology of learning and memory* **82**, 12-25, doi:10.1016/j.nlm.2004.03.003 (2004).

23 Sajikumar, S., Navakkode, S. & Frey, J. U. Protein synthesis-dependent long-term functional plasticity: methods and techniques. *Current Opinion in Neurobiology* **15**, 607-613, doi:10.1016/j.conb.2005.08.009 (2005).

24 Villers, A. & Ris, L. Improved preparation and preservation of hippocampal mouse slices for a very stable and reproducible recording of long-term potentiation. *Journal of visualized experiments : JoVE*, doi:10.3791/50483 (2013).

25 Pohle, W., Reymann, K., Jork, R. & Malisch, R. The influence of experimental conditions on the morphological preservation of hippocampal slices in vitro. *Biomedica biochimica acta* **45**, 1145-1152 (1985).

26 Reymann, K. G. *et al.* The duration of long-term potentiation in the CA1 region of the hippocampal slice preparation. *Brain research bulletin* **15**, 249-255, doi:10.1016/0361-9230(86)90147-9 (1985).

27 Sajikumar, S. & Korte, M. Different compartments of apical CA1 dendrites have different plasticity thresholds for expressing synaptic tagging and capture. *Learning & Memory* **18**, 327-331, doi:10.1101/lm.2095811 (2011).

28 Li, Q. *et al.* Making Synapses Strong: Metaplasticity Prolongs Associativity of Long-Term Memory by Switching Synaptic Tag Mechanisms. *Cerebral Cortex* **24**, 353-363, doi:10.1093/cercor/bhs315 (2014).

29 Sajikumar, S. & Frey, J. U. Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. *Neuroscience Letters* **338**, 147-150, doi:10.1016/S0304-3940(02)01400-3 (2003).

30 Ishikawa, Y., Tamura, H. & Shiosaka, S. Diversity of neuropsin (KLK8)-dependent synaptic associativity in the hippocampal pyramidal neuron. *The Journal of physiology* **589**, 3559-3573, doi:10.1113/jphysiol.2011.206169 (2011).

31 Ishikawa, Y., Horii, Y., Tamura, H. & Shiosaka, S. Neuropsin (KLK8)-dependent and -independent synaptic tagging in the Schaffer-collateral pathway of mouse hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 843-849, doi:10.1523/JNEUROSCI.4397-07.2008 (2008).

32 Sajikumar, S., Morris, R. G. M. & Korte, M. Competition between recently potentiated synaptic inputs reveals a winner-take-all phase of synaptic tagging and capture. *Proceedings of the National Academy of Sciences* **111**, 12217-12221, doi:10.1073/pnas.1403643111 (2014).

33 Navakkode, S., Sajikumar, S. & Frey, J. U. The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic

tagging. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 7740-7744, doi:10.1523/JNEUROSCI.1796-04.2004 (2004).

34 Kohl, M. M. *et al.* Hemisphere-specific optogenetic stimulation reveals left-right asymmetry of hippocampal plasticity. *Nature neuroscience* **14**, 1413-1415, doi:10.1038/nn.2915 (2011).

35 Frey, U., Huang, Y. Y. & Kandel, E. R. Effects of cAMP Simulate a Late Stage of LTP in Hippocampal CA1 Neurons. *Science* **260**, 1661-1664, doi:10.2307/2881723 (1993).

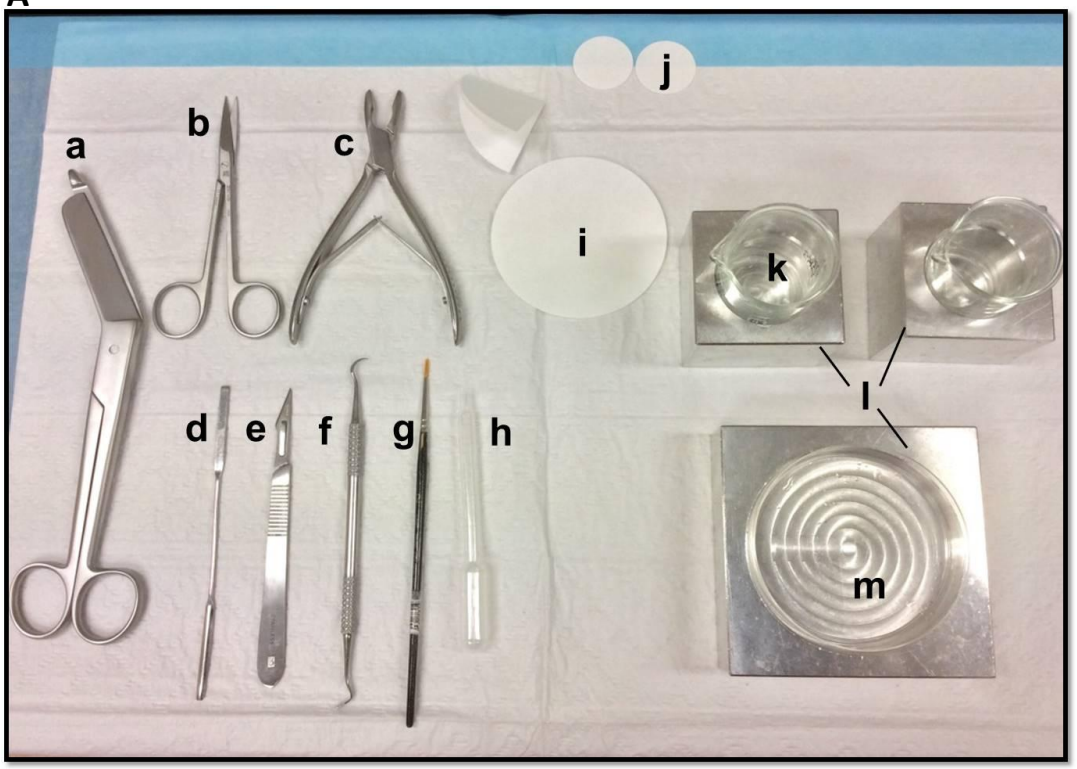
36 Erdogdu, G., Uto, A. & Hossmann, K.-A. The effect of global ischemia and recirculation of rat brain on protein synthesisin vitro. *Metab Brain Dis* **8**, 199-206, doi:10.1007/BF01001061 (1993).

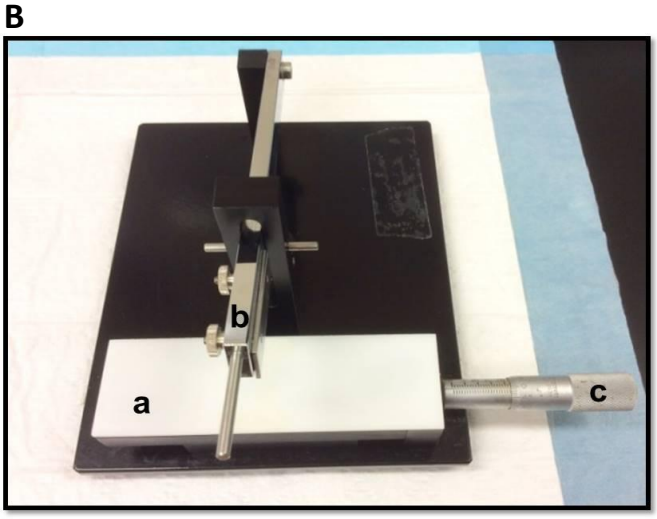
37 Djuricic, B., Berger, R. & Paschen, W. Protein synthesis and energy metabolism in hippocampal slices during extended (24 hours) recovery following different periods of ischemia. *Metab Brain Dis* **9**, 377-389, doi:10.1007/BF02098884 (1994).

38 Park, P. *et al.* NMDA receptor-dependent long-term potentiation comprises a family of temporally overlapping forms of synaptic plasticity that are induced by different patterns of stimulation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **369**, 20130131, doi:10.1098/rstb.2013.0131 (2014).

Figure

Click here to download Figure: JoVE-Figure-1.pptx

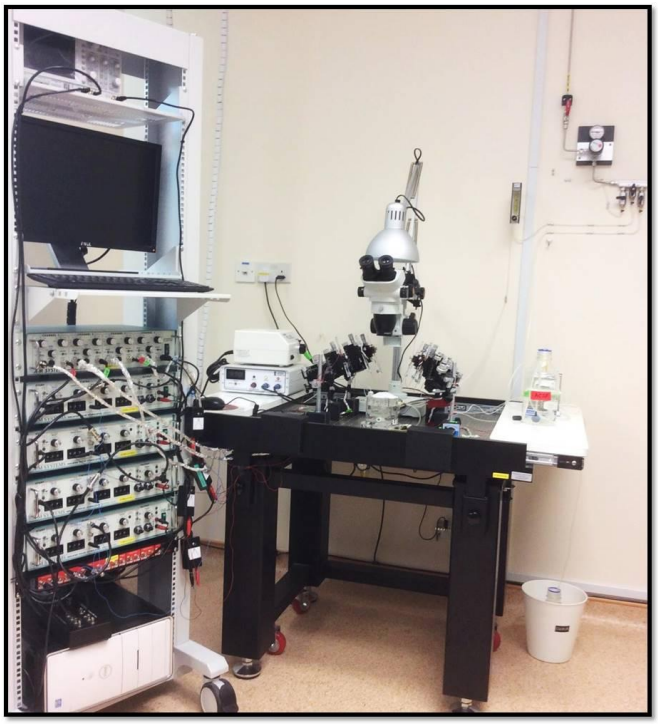




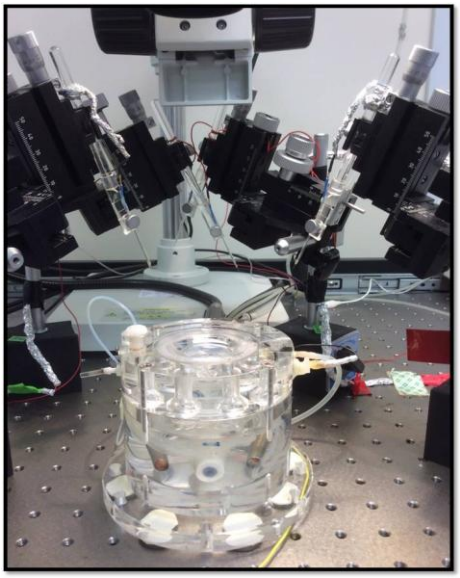
Shetty et al., 2014
Figure. 1

Figure
Click here to download Figure: JoVE-Figure-2.pptx

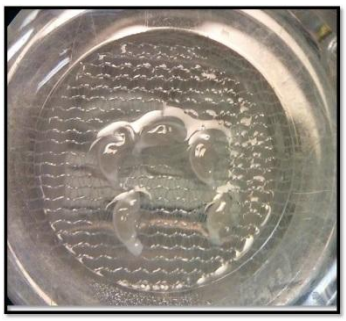
A



B



C



D

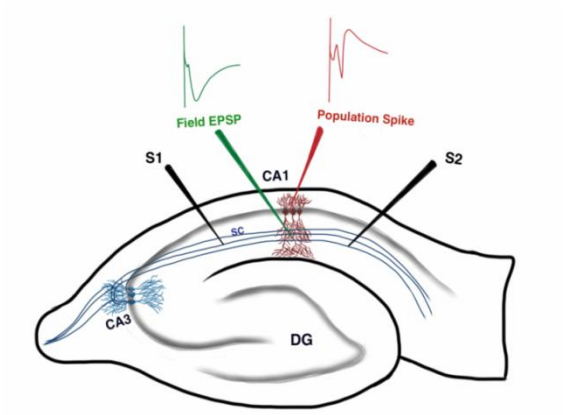
E

Shetty et al., 2014
Figure. 2

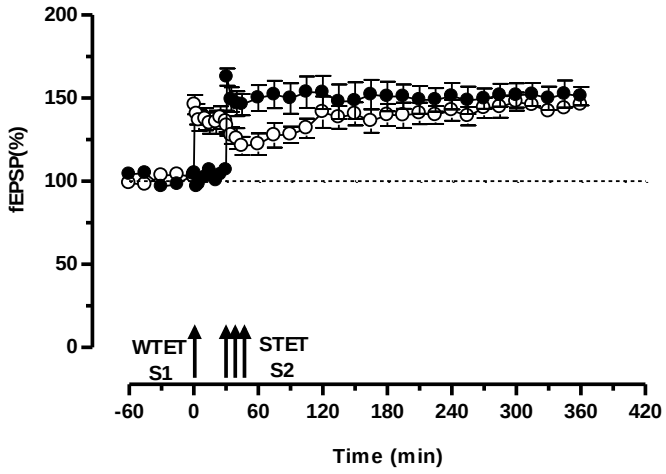
Figure

Click here to download Figure: JoVE-Figure-3.pptx

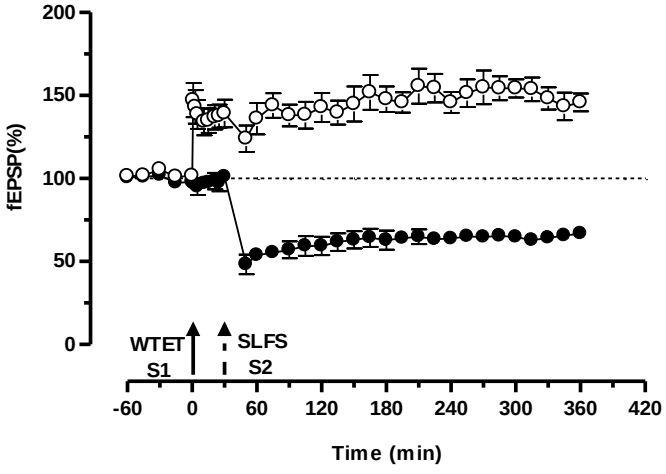
A



B



C



Shetty et al., 2014
Figure. 3

Excel Spreadsheet- Table of Materials/Equipment
Click here to download Excel Spreadsheet- Table of Materials/Equipment: 1.xlsx

Materials and Chemicals	Supplier
I. Dissection Tools	
1. Bandage scissors	KLS Martin, Germany B-Braun/Aesculap, Germany
2. Iris scissors	B-Braun/Aesculap, Germany
3. Bone rongeur	World Precision Instruments (WPI), Germany
4. Scalpel	World Precision Instruments (WPI), Germany; B-Braun/Aesculap, Germany
5. Scalpel blade#11	B-Braun/Aesculap, Germany
6. Sickle scaler	KLS Martin, Germany
7. Angled forceps	B-Braun/Aesculap, Germany
8. Anesthetizing/Induction chamber	MIP Anesthesia Technologies (Now, Patterson
II. ACSF component chemicals	
1. Sodium chloride (NaCl)	Sigma-Aldrich
2. Potassium chloride (KCl)	Sigma-Aldrich
3. Magnesium sulphate heptahydrat	Sigma-Aldrich
4. Calcium chloride dihydrate (CaCl2.	Sigma-Aldrich
5. Potassium phosphate monobasic (	Sigma-Aldrich
6. Sodium bicarbonate (NaHCO3)	Sigma-Aldrich
7. D-Glucose anhydrous (C6H12O6)	Sigma-Aldrich
III. Electrophysiology Instruments	
1. Microscope	Olympus, Japan
2. Temperature Controller	Scientific Systems Design Inc. Canada
3. Differential AC Amplifier	AM Systems, USA
4. Isolated Pulse Stimulator	AM Systems, USA
5. Oscilloscope	Rhode & Schwarz
6. Digital-Analog Converter	Cambridge Electronic Design Ltd. Cambridge,
7. Interface Brain Slice Chamber	Scientific Systems Design Inc. Canada
8. Tubing Pump	Ismatec, Idex Health & Science, Germany
9. Carbogen Flowmeter	Cole-Parmer
10. Fiber Light Illuminator	Dolan-Jenner Industries
11. Micromanipulators	Marzhauser Wetzlar, Germany
12. Electrodes	AM Systems, USA

Catalogue Number / Model

21-195-23-07
LX553R
BC140R,
BC100R
14089-G
500236-G

BB73
BB511

38-685-00
BD321R

AS-01-0530-LG

S5886
P9541
M1880
C3881
P9791
S5761
G7021

Model SZ61
PTC03
Model 1700
Model 2100
HM0722
CED-Power 1401-3
BSC01
REGLO-Analog
03220-44
Fiber Lite MI-150
00-42-101-0000 (MM-33)
00-42-102-0000 (MM-32)
571000 (Stainless steel; 0.010, 5MΩ, 8 degree)

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Investigation of synaptic tagging and capture, cross-capture using acute hippocampal slices from rodents.

Author(s): Mahesh SS, Mahima S, Neo SH, Ananya D, Suma G & Saradharan. S

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via: ☐ Standard Access ☑ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name: Sreedharan Sajikumar

Department: Physiology

Institution: National University of Singapore

Article Title:

Signature: 

Date: 16/01/2015

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Point by point responses to Reviewers

Reviewer #1:

Question
Long abstract:
Would the same protocol work also in mice? Please comment if your or another lab has experience.

Response:

The same protocols have been successfully employed in studies using mice from other labs. For example see Ishikawa et al., 2011 (PMID: 21646406), Ishikawa et al., 2008 (PMID: 18216192). In addition our own studies in which we have used the protocols in APP/PS1 mice and their wild type littermates and these papers will be published soon.

Minor Concerns:

Question:
Chapter 3.3.5.: pyramidal cell layer in healthy slices should show some transparency

Response:

We thank the reviewer for this suggestion. We have incorporated this point as a note under this section.

Question:
Chapter 4.1. One could add, that approaching the liquid surface above the slice gives a sound that helps to locate quickly the surface of the slice (provided the amplifier is connected to a loudspeaker)

Response:

This is a very helpful suggestion. Especially for a beginner, this sound helps to know when the electrode has touched the surface of the slice. Though we are aware of this technique, we had not included this in the manuscript as we do not use this in our laboratory. We have now included it as a note in the section.

Question:
Role of stimulation parameters can be discussed (see Collingridge lab: Park et al., Phil. Trans. R. Soc. B, 369 (2013)20130131)

Response:

We thank the reviewer for this useful suggetion. We have now briefly incorporated the role of stimulation parameters in the discussion section.

Question:

Page 10, line 7: why is fibre volley increase a "representation" of presynaptic release (wrong wording?)

Response:

We acknowledge this insightful comment from the reviewer and apologize for the wrong wording. We have now removed the sentence to avoid confusion.

Questions:
Additional Comments to Authors:
Would be good to cite the original description of this method:
Reymann, K.G., Malisch, R., Schulzeck, K., Brödemann, R., Ott, and Matthies H.: The duration of long-term potentiation in the CA1 region of the hippocampal slice preparation. Brain Res. Bull., 1985, 15, 249-255
Pohle, W., Reymann, K.G., Jork, R., and Malisch, R.: The influence of experimental conditions on the morphological preservation of hippocampal slices in vitro. Biomed. Biochim. Acta, 1986, 45, 1145-1152

Response:

We apologize for not including these important papers in the previous version. We have now incorporated these original references in the revised manuscript.

Reviewer #2:

The manuscript describes a technique to maintain stable long-term potentiation in acute rat hippocampal slices. This manipulation is extremely difficult and only some labs are able to do it.

Major Concerns: No major concerns

We thank the reviewer for this appreciation.

Minor Concerns:

Question:

I would modify the title: "Investigation of synaptic tagging, capture and cross-capture using acute hippocampal slices from rats".

Response:

We thank the reviewer for this suggestion. Since the protocols have been successfully used in rats and mice (see responses to reviewer 1) we would like to keep the title as of earlier.

Question:

Is it possible to show a short-term LTP recorded during 6 hours to compare with fig. 3B?

Response:

We thank the reviewer for the insight with this comment. Since this is a technical paper and as a part of minimizing the number of animals for research work we would like not to include new experiments in this figure. Considering the reviewer's suggestion we have now included the appropriate references in the text in which we have reported early-LTP up to 6 or 8 hrs (Navakkode et al., 2004, PMID: 15342741, Sajikumar et al., 2005, PMID:15958741)

Question

Can the authors explain why they used 21 stimulations to induce weak-LTP?

Response:

We appreciate this query from the reviewer. The 21 stimuli given as a single burst in this protocol are strong enough to set a tag without initiating the protein synthesis (Sajikumar et al., 2005. PMID: 16150586). A much weaker protocol may not be able to set a tag (Sajikumar et al., 2009, PMID:19223601) and a stronger protocol (100 Hz, 100 pulses) may lead to late-LTP. This protocol to induce weak-LTP (WTET) has been used extensively by us and others (Redondo et al 2010, Ishikawa et al., 2011, PMID: 20371818).

Reviewer #3:

Manuscript Summary:

The article is well thought out and the experimental procedures described in great detail. The information may not be novel but it could prove useful nevertheless.

We thank the reviewer for the appreciation.

Questions

Major Concerns:

1. Regarding the experimental setup, a Faraday cage is very strongly recommended if electrical interference is to be eliminated. Figure A shows electrical wiring running next to the electrophysiology table whereas cell-phone signals are also know to cause issues, especially if there are relay towers nearby.

Response:

We thank the reviewer for this insightful comment. We agree that a Faraday cage is recommended to prevent electrical interference. However, we have taken other measures to avoid such major interference problems. The complete field-potential recording setup is secluded in an isolated room. The electrical grounding of the room is very well taken care and all the noise-generating sources are individually grounded to the main ground line. Usage of cell-phones and wireless routers is restricted in the room.

The reviewer's suggestion is important and we have included a sentence in the text (section 4) as follows:

'A *Faraday cage is strongly recommended if the electrical interference is beyond the control after the proper grounding of the electrical settings'*

Question:

The strains of mice use (p.8) need to be specified, as does the age of all animals: synaptic plasticity is known to be more prevalent in young animals whereas slice preparation is harder on older ones.

Response:

We appreciate this suggestion from the reviewer. As the manuscript describes the protocol using rats, we have mentioned the strain and age of these (in 'Discussion' section). We have

now incorporated the relevant references concerning mice strains. Our own data from mice are yet to be published.

Question

Minor Concerns:

1. The authors should consider examining their findings under the Unified Model for Synaptic Plasticity, as proposed in 2011 by Michmizos et al. which attributes a specific mechanism to synaptic tagging (http://www.ncbi.nlm.nih.gov/pubmed/21348800)

Response:

We thank the reviewer for this comment. We agree that the suggested model does offer explanations to unify many observations from the synaptic plasticity experiments. However, we have not gone on to explain the underlying mechanisms of the synaptic tagging and capture considering that the main focus of this video article is on the methodology but not on the research findings and mechanisms.

Question:
Minor vocabulary corrections (use laboratory instead of the more informal "labs")

Response:

We thank the reviewer for this suggestion. We have now revised the manuscript to incorporate the suggested corrections.

Question:

The Note under step 1 of the Protocol, as do most Caution paragraphs and the final cleaning-up instructions could be omitted as it is well known to any neuroscientist. On the same note, the level of details and the language of the article is more gear towards a laboratory manual than a research paper.

Response:

We agree with the reviewer but we would like to keep the part as such as we feel that it will be helpful for the beginners.

Reviewer #4:

Manuscript summary:

If we understand things correctly, the manuscript we are looking over is a third revision of the original although the first time we have seen it. In our view, it is in good shape and well deserves to be published. Our only puzzle is that this is the first time we have reviewed for JoVE and wondered if we should also be looking at a video - or do we just look over the manuscript?

The manuscript describes standard solutions, apparatus, procedures and desirable temperature for doing long-time course LTP experiments. The authors describe methods that have worked for them in laboratories in Magdeburg, Braunschweig and now Singapore - and with different experimenters. We have every confidence that these protocols work well - whereas some others do not.

Question
Very minor comments (not obviating publication):

1. The authors mention a specific tissue slicer, but we believe others, including vibratomes, can work just as well. We absolutely share the author's view that brain slice preparation and slice health is vital.

Response:

We thank the reviewer for this comment. We have mentioned a specific tissue slicer that we have been using and have a good confidence that it works very well for the preparation of the slices within 3-5 min. We definitely do not deny the use of other slicers and vibratomes. In fact, some of the previous video articles in JoVE describing similar methodology make use of vibratomes or other tissue slicers (PMID: 23851639, PMID: 21490565)

Question
The authors mention a marketed system (ETCSYSTEM) for maintaining temperature in hippocampal slice experiments. We wonder if the text should make it a bit clearer that this system obviates the need to catch condensation drops. This is stated, but the impression remains that condensation droplets are an inevitable feature of long time-course interface chamber experiments. This is not correct and a protocol that involves regular "blotting" of condensation droplets feels like a protocol that could yet be improved!

Response:

We appreciate this suggestion from the reviewer. We have revised the phrasing to convey this more clearly.

Question:

It is stated that the experiments must be done with the right-hemisphere. We do understand the desirability of controlling every aspect of variance, but the recent work of Paulsen (University of Cambridge) suggests major differences in NMDA receptor expression in left and right hippocampus that can be investigated using optogenetic stimulation. Perhaps the 'dictat' to use right hemisphere could be changed to merely recommendation about consistent laterality to reduce variability?

Response:

We thank the reviewer for this insightful suggestion. We agree with the reviewer‘s view and aimed to convey that the use of right hippocampus is to maintain consistency with our previous studies. We had briefly mentioned about the hemispheric differences in the discussion. We have now revised the section to make it clearer including the reference suggested by the referee.

Question

An increasing number of researchers are convinced of the need of a third control pathway for such long-term recordings. Figure 3 does not have a control pathway although the text is as follows: "One can also include a third, independent but overlapping synaptic input and use it as a baseline control to monitor the stability of recordings." Perhaps this text should read "It would be wise to include a third ... in some experiments ... and use it…."

Response:

We agree with the reviewer and appreciate the suggestion. We have now revised the section to include the suggested modification.