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TITLE:

Evaluation of Hemisphere Lateralization with Bilateral Local Field Potential Recording in Secondary Motor Cortex of Mice

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KEYWORDS:

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SUMMARY:

We present in vivo electrophysiological recording of the local field potential (LFP) in bilateral secondary motor cortex (M2) of mice, which can be applied to evaluate hemisphere lateralization. The study revealed altered levels of synchronization between the left and right M2 in APP/PS1 mice compared to WT controls.

ABSTRACT:

This article demonstrates complete, detailed procedures for both in vivo bilateral recording and analysis of local field potential (LFP) in the cortical areas of mice, which are useful for evaluating possible laterality deficits, as well as for assessing brain connectivity and coupling of neural network activities in rodents. The pathological mechanisms underlying Alzheimer's disease (AD), a common neurodegenerative disease, remain largely unknown. Altered brain laterality has been demonstrated in aging people, but whether or not abnormal lateralization is one of the early signs of AD has not been determined. To investigate this, we recorded bilateral LFPs in 3–5-month-old AD model mice, APP/PS1, together with littermate wild type (WT) controls. The LFPs of the left and right secondary motor cortex (M2), specifically in the gamma band, were more synchronized in APP/PS1 mice than in WT controls, suggesting a declined hemispheric asymmetry of bilateral M2 in this AD mouse model. Notably, the recording and data analysis processes are flexible and easy to carry out, and can also be applied to other brain pathways when conducting experiments that focus on neuronal circuits.

INTRODUCTION:

Alzheimer's disease (AD) is the most common form of dementia[1,2]. Extracellular beta amyloid protein (β-amyloid protein, Aβ) deposition and intracellular neurofibrillary tangles (NFTs) are the main pathological features of AD[3-5], but the mechanisms underlying AD pathogenesis remain largely unclear. Cerebral cortex, a key structure in cognition and memory, is impaired in AD[6], and motor deficits such as slow walking, difficulty navigating the environment and gait disturbances occur with advancing age[7]. Aβ deposition and neurofibrillary tangles have also been observed in the premotor cortex (PMC) and supplementary motor area (SMA) in AD patients[8] and cognitively impacted older adults[9], indicating the involvement of an impaired motor system in AD pathogenesis.

The brain is formed by two distinct cerebral hemispheres that are divided by a longitudinal fissure. A healthy brain exhibits both structural and functional asymmetries[10], which is called "lateralization", allowing the brain to efficiently deal with multiple tasks and activities. Aging results in a deterioration in cognition and locomotion, together with a reduction in brain laterality[11,12]. The motor abilities of the left hemisphere are readily apparent in the healthy brain[13], but in the AD brain aberrant laterality occurs as a consequence of the failure of left hemisphere dominance associated with left cortical atrophy[14-16]. Therefore, an understanding of a possible alteration of brain lateralization in AD pathogenesis and the underlying mechanisms may provide new insights into AD pathogenesis and lead to identification of potential biomarkers for treatment.

Electrophysiological measurement is a sensitive and effective method of evaluating changes in the neuronal activities of animals. The reduction of hemispheric asymmetry in elders (HAROLD)[17] has been documented by electrophysiological research with synchronized interhemispheric transfer time, which shows weakening or absence of hemispheric asymmetry to monaurally presented speech stimuli in the elderly[18]. Utilizing APP/PS1, one of the most commonly used AD mouse models[19-22], in combination with in vivo bilateral extracellular recording of LFPs in both left and right M2, we evaluated possible laterality deficits in AD. In addition, with simple parameter settings, the built-in function of data analysis software (see the **Table of Materials**) provides a faster and more straightforward way to analyze the synchronization of electrical signals than mathematically complex programming language, which is friendly to beginners with in vivo electrophysiology.

PROTOCOL:

All animals were paired-housed under standard conditions (12 h light/dark, constant temperature environment, free access to food and water) according to the Chinese Ministry of Science and Technology Laboratory Animals Guidelines and experiments were approved by the local ethical committee of Guangzhou University.

NOTE: For data shown in the representative results, APP/PS1 (B6C3-Tg (APPswe, PSEN1dE9) 85Dbo/J) double-transgenic mice and littermate wild-type (WT) controls at 3–5 months of age, were used for recordings (n = 10, per group).

1. Animal anesthesia and surgery

1.1. Weigh and anesthetize the mouse by intraperitoneal (i.p.) injection of urethane (2 g/kg).

1.2. Perform a tail or toe pinch with forceps to confirm deep anesthesia prior to surgery.

NOTE: A supplemental dose of 0.1–0.2 g/kg urethane can be used if necessary.

1.3. Position the mouse in a stereotaxic apparatus and fix its head.

1.4. Apply eye ointment on both eyes before animal surgery.

1.5. Shave the hair using surgical clippers or scissors. Make a small incision (12–15 mm) in the middle of the exposed surgical area using a scalpel. Using forceps, gently pull the scalp away from the midline.

1.6. Separate the skin gently and remove residual tissue using scissors and spatula. Clean the skull using hydrogen peroxide-coated cotton buds.

1.7. Drill two small holes of radii 1.0–1.5 mm on both left and right sides of the skull to allow insertion of the recording microelectrodes into the M2 regions under a stereomicroscope (**Figure 1A**).

NOTE: Stereotaxic locations of bilateral M2: 1.94 mm anterior to the bregma, 1.0 mm lateral to the midline, and 0.8–1.1 mm ventral to the dura.

1.8. Pull glass borosilicate micropipettes (outer diameter: 1.0 mm) as recording microelectrodes with resistance of 1–2 MΩ.

1.9. Remove the dura mater carefully with a tungsten needle.

1.10. Insert two separate recording microelectrodes filled with 0.5 M NaCl at 60° angles into the holes using mechanical micromanipulators (**Figure 1B**).

2. LFP recordings in bilateral M2 of mice

2.1. Adjust the depth of glass electrodes in the left and right M2 by slowly lowering the mechanical micromanipulators until an appropriate coordinate (**Figure 1C**).

2.2. For quality control, test the resistance of each electrode using the differential amplifier before capturing LFPs.

2.3. Set the recording process at 0.1 Hz high-pass and 1,000 Hz low-pass with 1,000x amplification, using the differential amplifier.

2.4. Collect digitized (sampling rate: 2,500 Hz) raw LFP data of at least 60 s spontaneous activities in stable state, with mice breathing evenly at 2 breaths/s respiratory rate under anesthesia.

2.5. After recording, slowly raise the electrodes out of the brain, then euthanize the mice by fast cervical dislocation.

2.6. Save the data and analyze offline with the analysis software.

3. Cross-correlation analysis

3.1. Click **Analysis - Waveform correlation** in the analysis software and import the data.

3.2. Parameter settings

3.2.1. Determine which waveform channel signal is the first channel and which is the reference. Set width as 2 and offset as 1 (**Figure 2A**).

3.2.2. Set the duration of both LFPs for 100 s by selecting the start time and end time. Press the **Process** button to perform cross-correlation analysis (**Figure 2B**).

NOTE: Simultaneous bilateral signals with such durations would be long enough to show neuronal spontaneous activities, thereby revealing the basic properties of synchronization.

3.4. Click **File - Export As**, then save the cross-correlation results corresponding to the resulting pop-up chart in .txt format.

3.5. Open the .txt file (**Figure 2C**), remove the correlation values at time lags ranged 0 ± 0.01 s (since two continuous gamma waves have at least 0.01 s interval), then average the rest of the cross-correlation data in the negative time lag part or average the rest of the cross-correlation data in the positive time lag part.

4. Coherence analysis

4.1. Load the COHER script in analysis software. Import data and run this script.

4.2. Select the two LFP signals as the first and second waveform channels separately. Then set the block size value (**Figure 3A**).

NOTE: Block size means the number of data points used in the FFT. The larger the block size, the better the frequency resolution. Here we recommend setting it as 4096.

4.3. Move the dotted lines manually to ensure the time accuracy for signals in both channels being selected as 100 s durations (**Figure 3B**). Press the **Add Area** button to load the area and perform coherence analysis.

4.4. Click **File - Save As** to save the coherence results corresponding to the resulting pop-up chart in .txt format (**Figure 3B**).

REPRESENTATIVE RESULTS:

To see whether early AD pathology impairs the capacity of hemisphere lateralization, we conducted bilateral extracellular LFP recordings in the left and right M2 of APP/PS1 mice and WT controls (aged 3–5 months), and analyzed the cross-correlation of these left and right LFPs. In WT mice, the results demonstrated that the mean correlation between left and right LFPs at positive time lags differed significantly from that at negative time lags, implicating the existence of hemispheric asymmetries in M2 areas of WT controls (**Figure 4C**; WT-positive, 0.08161 ± 0.01246; WT-negative, 0.0206 ± 0.01218; $p = 4.74531E\text{-}4 < 0.001$ by a two sample *t*-test). In comparison, the left and right LFPs of APP/PS1 mice showed higher synchronized in time domain, suggesting a reduction of asymmetry between the left and right M2 (**Figure 4C**; APP/PS1-positive, 0.13336 ± 0.0105 APP/PS1-negative, 0.12635 ± 0.01066; $p = 0.64157 > 0.05$ by a two sample *t*-test).

We then filtered gamma oscillations from the LFPs (**Figure 5A**) and performed a coherence analysis as described in the protocol to measure the similarity of electrical signals in the gamma frequency range. The result showed that the gamma coherence between left and right M2 in APP/PS1 was significantly higher than that in WT mice (**Figure 5B,C**; WT, 0.13267 ± 0.00598; APP/PS1, 0.17078 ± 0.0072; $p = 0.00550 < 0.01$ by two sample *t*-test), indicating a higher synchronization, and consequently reduced lateralization, between left and right M2 in APP/PS1 mice.

FIGURE AND TABLE LEGENDS:

Figure 1: Diagram of the simultaneous LFP recording procedure. (**A**) Stereotaxic mouse with skull exposed and dura mater removed for in vivo bilateral recording of LFPs in left and right M2. (**B**) Two glass microelectrodes in touch with the cortical surface in the hole drilled simultaneously. (**C**) Recording microelectrodes along with the Ag/AgCl wires as reference electrodes positioned at appropriate sites.

Figure 2: Illustration of cross-correlation analysis. (**A**) Settings for the waveform correlation dialog box. This provides options for choosing which waveform channel is the reference and for analyzing the correlation of two signals. (**B**) The process dialog box. This provides options for setting the time length of the reference waveform and the duration of another waveform will be appended. The analysis is only done for regions of data in which both waveform channels exist. (**C**) Example .txt file with values of cross-correlation at negative and positive time lag ranges separately.

Figure 3: Illustration of coherence analysis. (**A**) Parameter settings for the coherence dialog box. The block size determines the number of data points used in the analysis, and the frequency resolution. (**B**) The dotted lines are adjustable for operator to move manually in order to set the duration of signals for analyzing. (**C**) After the software has created a chart, click **File - Save As** to save the coherence results as a file with a .txt filename extension for statistics.

Figure 4: Cross-correlation indicates the declined hemisphere lateralization between left and right M2 of APP/PS1 mice. (**A**) Representative raw LFP traces of simultaneous bilateral M2 recording in WT and APP/PS1 mice using the extracellular recording method (L: left M2; R: right M2). (**B**) The cross-correlation curve shows correlation of bilateral LFP signals at different time lags. (**C**) Between left and right M2, WT controls showed significantly higher cross-correlation value at positive time lag ranges than negative ones. In contrast, the cross-correlation value of APP/PS1 mice has a similarity, indicating a decline of asymmetry (n = 10, per group). Value represents mean ± standard error of the mean. ***$p < 0.001$; two sample *t*-test.

Figure 5: Coherence of gamma oscillations between left and right M2 of WT and APP/PS1 mice. (**A**) Representative traces of gamma oscillations filtered from LFPs in left and right M2. (**B**) Coherence distribution between LFPs simultaneously recorded in the left and right M2. APP/PS1 mice differ largely from WT controls in gamma frequency range. (**C**) The coherence between gamma oscillations of left and right M2 in APP/PS1 mice are significantly higher than WT controls (n = 10, per group). Value represents mean ± standard error of the mean. **, $p < 0.01$; two sample *t*-test.

DISCUSSION:

We report here the procedure for in vivo bilateral extracellular recording, along with analyzing the synchronization of dual-region LFP signals, which is both flexible and easy to conduct for estimating brain hemisphere lateralization, as well as the connectivity, directionality or coupling between neural activities of two brain areas. This can be widely used to reveal not only group-neuronal activities, but also some basic properties of interregional electrophysiology, especially for labs which are interested in screening oscillatory activities or labs which do not have systems for multi-channel recording in behaving animals[23].

In general, a series of techniques are available to monitor brain activities, including electroencephalography (EEG), magnetoencephalography (MEG), and functional magnetic resonance imaging (fMRI). Such methods have relatively lower temporal and spatial resolution in comparison with our presented recordings. For example, EEG is one of the oldest and most commercially available instruments for investigating extracellular activity of the brain. Although there are studies using “high density” EEG in freely moving rodents to improve the insufficient spatial resolution[24-26], the skull always generates more noise and thus reduces the signal-to-noise ratio of cortical gamma oscillation, especially for small-sized mice. Our method with glass microelectrodes would be a good choice to prevent researchers from that “distorting noise” since microelectrodes could be inserted into the brain structure directly. Moreover, the recording glass pipettes used here are inexpensive, highly maneuverable, and can be applied to explore deeper brain areas not limited to cortical areas.

Close attention should be paid to the following. First, it is mandatory to carry out anesthesia strictly based on the body weight, and to test the depth of anesthesia hourly. This is because the physiological state of the mouse plays an important role in the quality of the LFP recorded, and any movement of the referencing sites caused by, e.g., sudden awakening of the animal, could generate background electrophysiological noise that would depreciate the availability. Second, because microelectrode resistance varies with the shape and diameter of the glass pipette tip, the heating must be carefully adjusted within the range for appropriate impedance when pulling microelectrodes. As described earlier in the protocol section, we found that the electrodes with impedance ranging from 1 to 2 MΩ captured high qualitied cortical oscillatory activities.

Gamma oscillations reflect the neuronal synchronization of different brain regions when animals are engaged in learning or stimulation-cued tasks[27-29]. The synchronization of gamma-band modulates excitation rapidly to activate postsynaptic neurons effectively[30]. It is worth noting that although the gamma oscillation was defined in the present study as oscillatory activity with frequency in the range 25–80 Hz as shown by several groups[28,31,32], there are studies that describe 30–70 Hz as low gamma and 70–100 Hz as high gamma[33-35]. Regardless of the definition, the principles for data analysis remain similar. In signal processing, cross-correlation is used for determining the time delay between electrical signals of two brain regions[36]. For signals under stimulation conditions, the durations selected for cross-correlation analysis could be shorter[37].

Though there are limitations in the use of LFP recording for the evaluation of neural activities; for instance, it can neither distinguish between pre- and post-synaptic activities nor detect resting membrane potentials of the neurons recorded[23], the approach introduced here serves as a useful tool for the measurement of activities of a group of neurons from different brain areas of mice, allowing the investigation of brain-area functional connectivity and the coupling of electrical signals before and after drug infusion.

Several explanations have been proposed for the emergence of hemispheric asymmetry, e.g., asymmetry enhances an individual's ability to perform two different tasks at the same time[38]; or asymmetry increases neural capacity, avoiding unnecessary duplication of neural networks[39]; or two different cognitive processes may be more readily performed simultaneously if they are lateralized to different hemispheres[40]. Hemisphere lateralization is assumed to provide cognitive advantages, but it changes with age[12,41]. Neuroimaging studies have shown consistently that prefrontal activation tends to be less lateralized in older adults than in younger individuals[42,43]. AD patients with early unilateral or bilateral pathological changes develop brain abnormalities, including lateralization associating with forgetfulness, slow responses to sound stimulation and cognitive decline[11,44]. We observed, in the present study, a disrupted level of hemisphere lateralization between left and right M2 of APP/PS1 mice at 3–5 months, which is the period when such mice do not aggregate apparent deposition of beta amyloid plaques[45,46], implying that toxicity induced by soluble beta amyloid oligomers may contribute, at least in part, to aberrant cortical hemisphere lateralization, which could accelerate brain deterioration in AD pathogenesis[16,47].

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DISCLOSURES:
The authors have nothing to disclose.

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Figure 1

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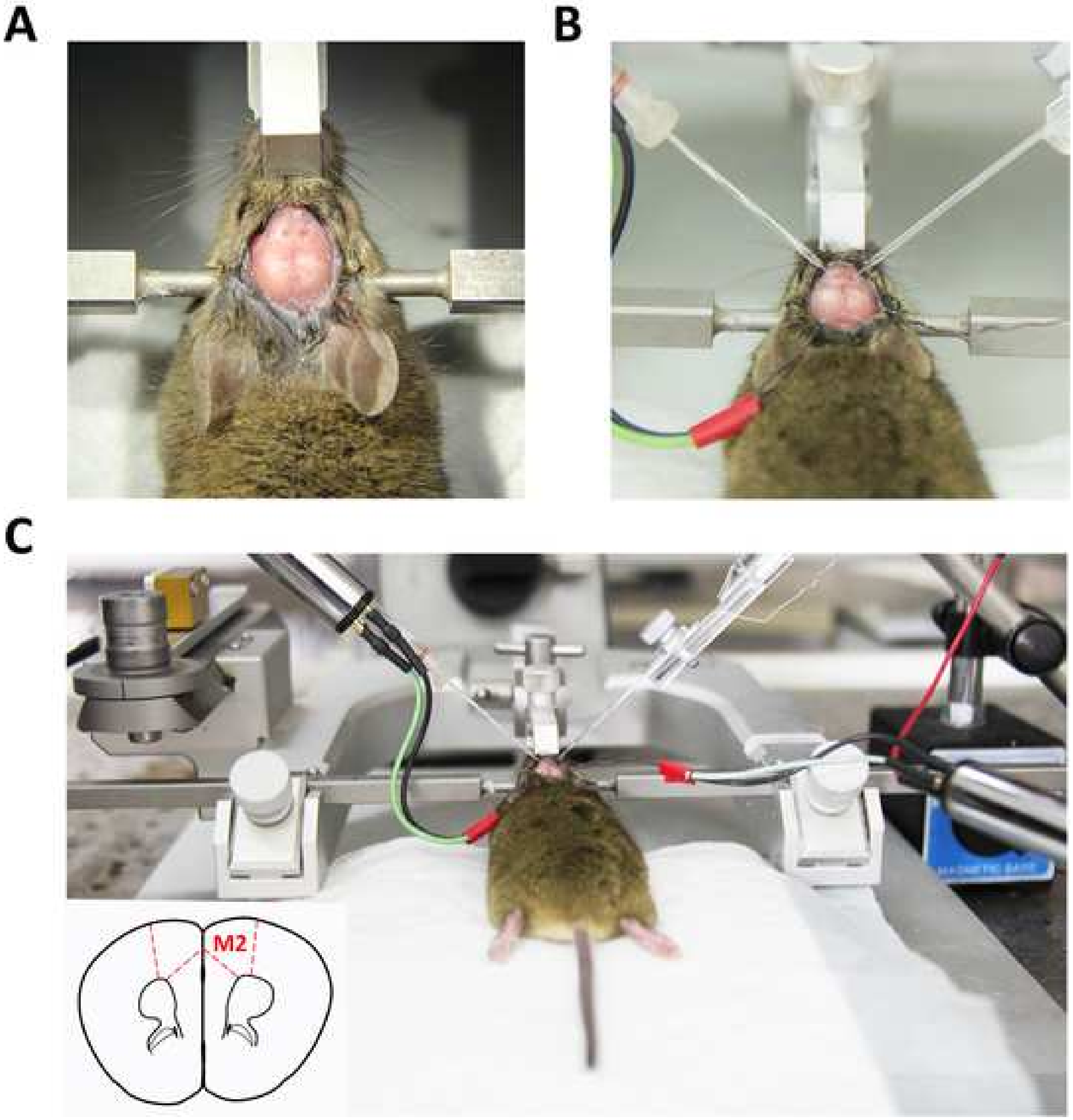
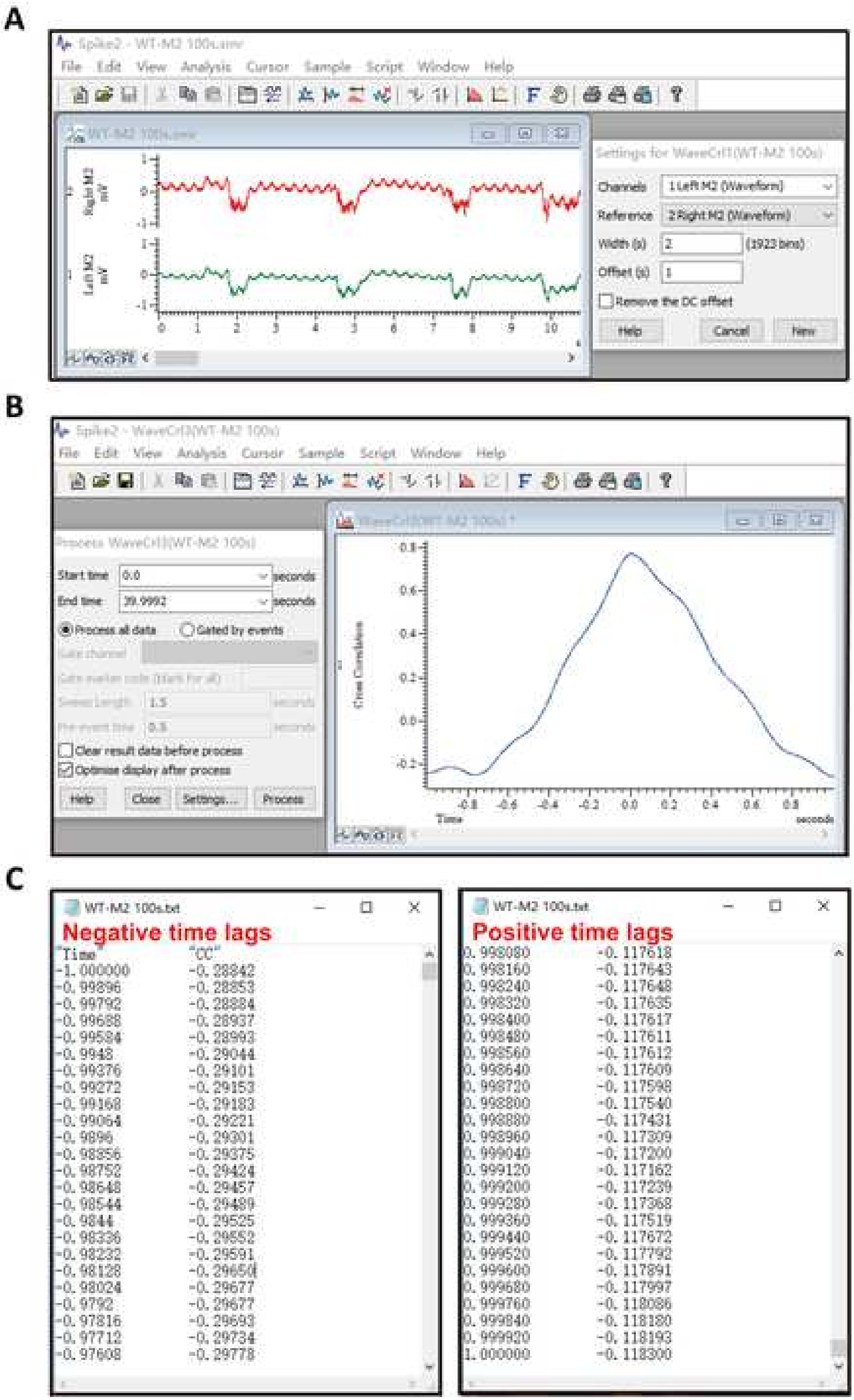


Figure 2


A
Spike2 - WT-M2 100s.smr
File Edit View Analysis Cursor Sample Script Window Help
WT-M2 100s.smr
Right M2 mV
Left M2 mV
Settings for WaveCrl1(WT-M2 100s)
Channels 1 Left M2 (Waveform)
Reference 2 Right M2 (Waveform)
Width (s) 2 (1923 bins)
Offset (s) 1
Remove the DC offset
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B
Spike2 - WaveCrl3(WT-M2 100s)
File Edit View Analysis Cursor Sample Script Window Help
Process WaveCrl3(WT-M2 100s)
Start time 0.0 seconds
End time 39.9992 seconds
Process all data
Gated by events
Clear result data before process
Optimise display after process
Help Close Settings... Process
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WT-M2 100s.txt
Negative time lags
"Time" "CC"
-1.000000 -0.28842
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-0.9792 -0.29677
-0.97816 -0.29693
-0.97712 -0.29734
-0.97608 -0.29778
WT-M2 100s.txt
Positive time lags
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0.998160 -0.117643
0.998240 -0.117648
0.998320 -0.117635
0.998400 -0.117617
0.998480 -0.117611
0.998560 -0.117612
0.998640 -0.117609
0.998720 -0.117598
0.998800 -0.117540
0.998880 -0.117431
0.998960 -0.117309
0.999040 -0.117200
0.999120 -0.117162
0.999200 -0.117239
0.999280 -0.117368
0.999360 -0.117519
0.999440 -0.117672
0.999520 -0.117792
0.999600 -0.117891
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0.999760 -0.118086
0.999840 -0.118180
0.999920 -0.118193
1.000000 -0.118300

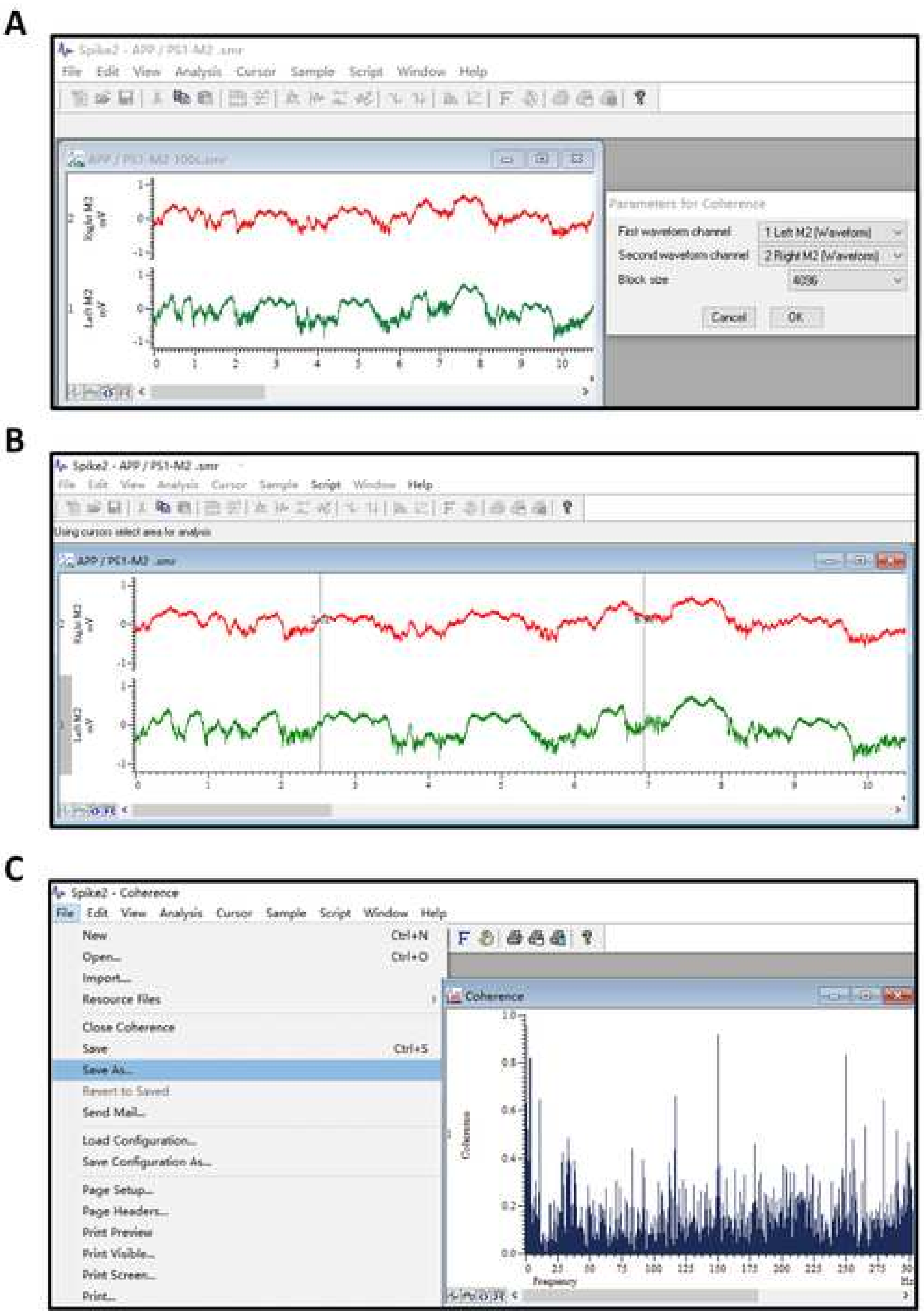

A
Spike2 - APP / PS1-M2 .smr
File Edit View Analysis Cursor Sample Script Window Help
Parameters for Coherence
First waveform channel
1 Left M2 (Waveform)
Second waveform channel
2 Right M2 (Waveform)
Block size
4096
Cancel
OK
Right M2 mV
Left M2 mV
B
Spike2 - APP / PS1-M2 .smr
File Edit View Analysis Cursor Sample Script Window Help
Using cursors select area for analysis
APP / PS1-M2 .smr
Right M2 mV
Left M2 mV
C
Spike2 - Coherence
File Edit View Analysis Cursor Sample Script Window Help
New Ctrl+N
Open... Ctrl+O
Import...
Resource Files
Close Coherence
Save Ctrl+S
Save As...
Revert to Saved
Send Mail...
Load Configuration...
Save Configuration As...
Page Setup...
Page Headers...
Print Preview
Print Visible...
Print Screen...
Print...
Coherence
Coherence
Frequency
Hz

Figure 4

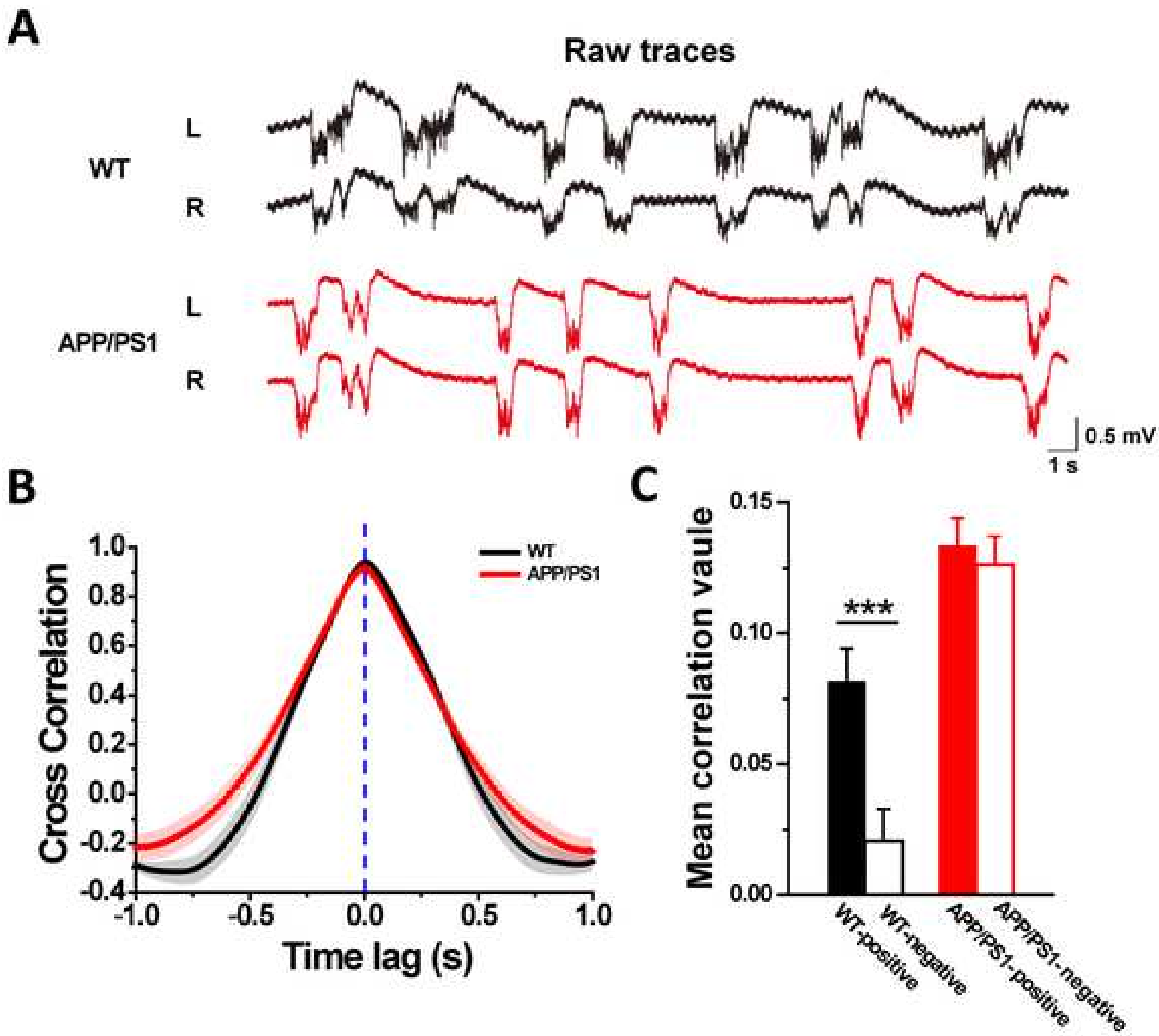
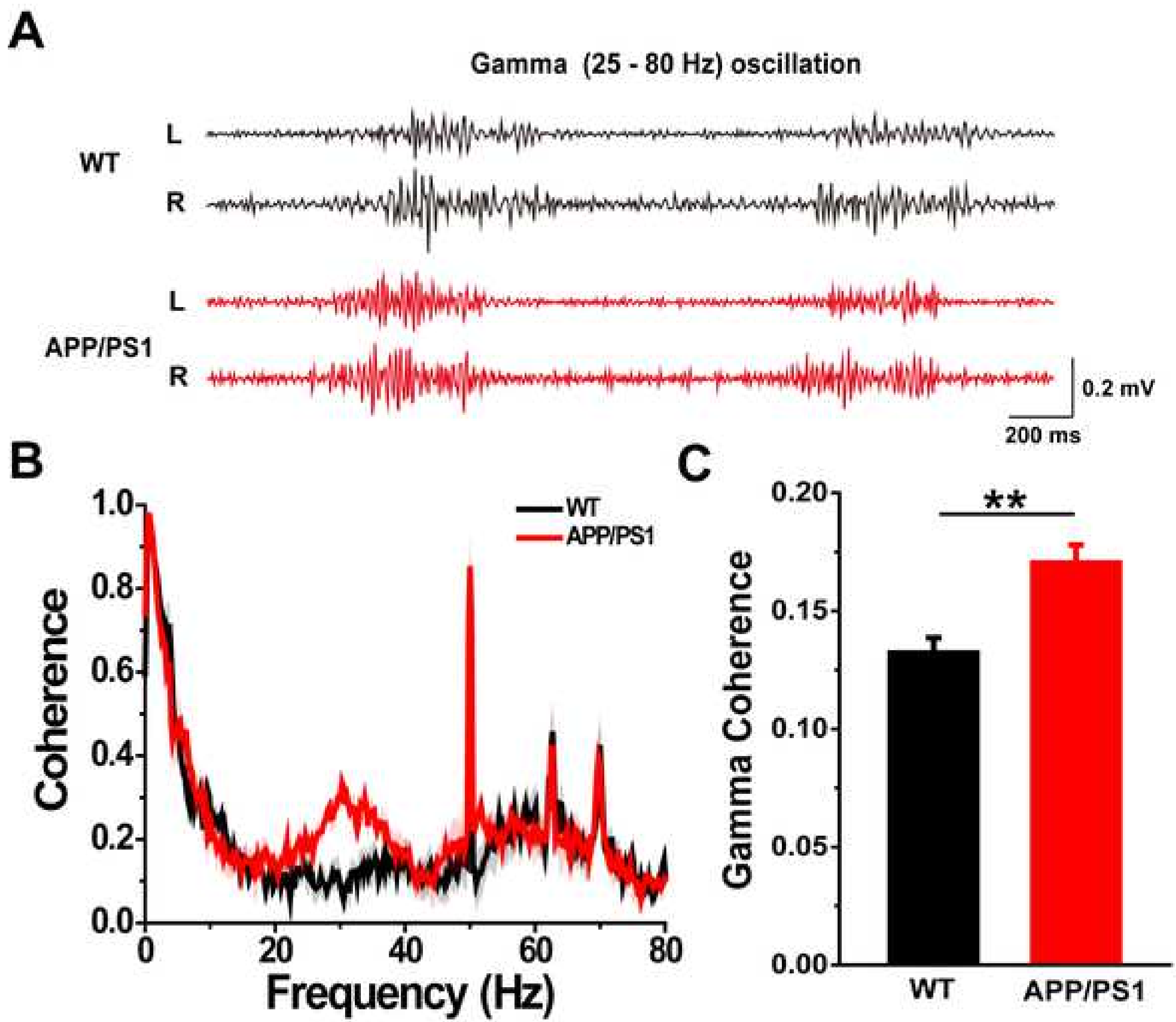

A
Raw traces
WT
L
R
APP/PS1
L
R
0.5 mV
1 s
B
Cross Correlation
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-1.0
-0.5
0.0
0.5
1.0
Time lag (s)
WT
APP/PS1
C
Mean correlation vaule
0.15
0.10
0.05
0.00
***
WT-positive
WT-negative
APP/PS1-positive
APP/PS1-negative

Figure 5

Click here to access/download;Figure;New Figure5.tif



Name of Material/ Equipment	Company	Catalog Number
AC/DC Differential Amplifier	A-M Systems	Model 3000
Analog Digital converter	Cambridge Electronic Design Ltd.	Micro1401
Glass borosilicate micropipettes	Nanjing spring teaching experimental equipment company	161230
Microelectrode puller	Narishige	PC-10
NaCl	Guangzhou Chemical Reagent Factory	7647-14-5
Pin microelectrode holder	World Precision Instruments, INC.	MEH3SW10
Spike2	Cambridge Electronic Design Ltd.	
Stereomicroscope	Zeiss	435064-9020-000
Stereotaxic apparatus	RWD Life Science	68045
Urethane	Sigma-Aldrich	94300

Comments/Description

Outer diameter: 1.0mm

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Author(s): Ming Li, Yunan Chen, Ying Zheng, Li Yang

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Institution: Guangzhou University

Article Title: Evaluation of hemisphere lateralization by dual-channel LFP recording in the secondary motor cortex in mice

Signature: Li Yang Date: 29/10/2018

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Dear Dr. Dsouza,

Once again, thank you very much for recently handling the review for our manuscript numbered JoVE59310 and giving us the opportunity to revise. We greatly appreciate the helpful and insightful comments of all reviewers, and hope we will satisfy the reviewers' concerns. Modifications made are in red in our revised manuscript. Please see below the point-by-point responses to the concerns/suggestions raised by the reviewers.

Reviewer #1:

In Protocol on line 98-9: What is the hardware filter for this sampling frequency?

Now the frequency band of the bandpass filtering is specified, "band pass filtered from 0.1 to 1,000 Hz, using the differential amplifier", and the specified AM system does have a bandpass capability, but for this specific system, you need to set both high pass and low pass setup with 40 dB attenuation. Thus, please make sure to describe the exact procedure so that users can reproduce your experiment exactly and filtering specifications.

Response: We are really appreciated with this concern and have modified the sentence to make it clear. See line 112-113 please.

In Protocol on line 100: What is the definition of "stable"? Describe.

I don't believe that the definition of what authors used here for the stability is not good. So if the animal is about to die and somehow managed to keep "even" breathing, can we use such an animal for experiments? The authors need to work on the wording here to avoid potentially misleading interpretations.

Response: Thanks for the concern. We have now modified the definition accordingly. See line 115 please.

In Protocol on line 108: Even though there are the threshold values, there are no recommendations or any on the actual durations to be used for the cross correlation. Please include the value and support the choice with rational arguments or results to establish as a protocol.

Thank you for the revision, but I still don't see why 100 sec. Please elaborate.

Response: We are thankful for this concern. We accepted the way that several publications used which set the duration for processing as 100 sec. Because we wanted to evaluate the basic properties of electrophysiological signals of left and right M2 in the absence of any stimulations, stable signals from both channels with 100-s-durations would be long enough to reveal reliable neuronal spontaneous activities. But for signals in the conditions of stimulations, e.g., before and after odorant, the durations selected should be shorter (Li et al., 2010; Zitnik et al., 2016). We have added a note following the step. See line 126-127, 244-245 please.

In Protocol on line 120: What kind of manual adjustments are needed for what types of accuracy? Please describe. From this sentence, it is not clear what's been done at all and rational of doing this undescribed manual procedure.

Still there is no explanation as to why. If you are using a system like Spike2, it is very easy to send or encode external events. Also there is no clear criteria for this manual adjustment. Clarify.

Response: Thanks for the concern. We have made changes in both the protocol and Figure 3B (the transparent area). See line 141-142, 184-185 please.

In Protocol on lines 111-2 (Along with Figure 4 results): It is not clear what the following sentence says: "Compute the cross-correlation … ". Since there is no indication of the outputs of the function, "Analysis - Waveform correlation", specify what the output(s) are, then describe what values are averaged, as in terms of the correlations, many things can be computed, and especially the values are removed by some arbitrary threshold (explain the \pm 0.1 s part as it does not make any sense without any explanations), it is not clear what metric is computed, and since the time window is not defined here, what's been computed here does not mean much, based on this write up. Also, without showing any results of correlations with correlations values between L and R, readers have no sense of the difference. Please include the cross correlations from both sides before Figure 4.C.

Also, exactly what type of cross correlations are computed here, and any windowing effect is accounted for? If authors account for windowing effect, can the results shown in Figure 4.C still exhibit significant difference?

First of all, there is no issue about the output file type itself. It is not clear to me what the authors mean by "minimum duration". Please clarify.

Response: We apologize for the confusion caused. As in most of cases the gamma band ranges from 20-100 Hz (Palop et al., 2007; Verret et al., 2012), thus the period (reciprocal of frequency) ranges from 0.01-0.05 sec. That means two continuous gamma waves have at least 0.01 s interval (which we called "minimum duration" in last-time revision). In order to exclude the possible auto-correlation of signals with themselves and the cross-correlation of LFPs at frequencies over 100 Hz, we set 0.01 s as the threshold and removed the values at time lags ranged at 0±0.01 s. We have now added details to the discussion section. See line 130-133 please.

See line 228-237 please.

Authors should familiarize themselves with newer studies and devices where there are many commercially available high density EEG for rodents. Thus, it is not correct to point out the density issue. The real issue here is range of oscillation frequency that such devices can record. Thus, the authors really need to amend the citations to include real limitations of EEG, especially for rodents. Otherwise, the proposed study is very meaningless.

Response: Thanks for the suggestions. We agree that though EEG is useful, it has limitations. We have modified the discussion accordingly. See line 209-212, 217-225 please.

Some new parts need some justifications or to have some consistency throughout the document. It seems that now the authors want to say that using some software like Matlab is complex, but now there is a section to use Matlab functions (lines 150-8 in the revised manuscript). Also with all the available patches for data

acquisition toolbox and compatible hardware, I don't really see a point to use Spike2, then use Matlab. Also the editing of the figures that authors are mentioning using Adobe product is more for the figures for papers as opposed to present experimental data. Thus, I suggest that the authors need to revise the whole section over lines 150-8 to have a consistency.

Response: Thanks for the suggestions. We have now deleted the part in our revised manuscript.

Reviewer # 3:

Major Concerns:

1) A literature link between synchronization of LPS (or synchronization of gamma band) and brain laterality should be provided in the Introduction.

Response: Thanks for your valuable suggestion. We have now added the correlation between synchronization of electric signals and brain laterality to the introduction section with citations. See line 67-70 please.

2) What is the certainty that electrodes' terminations were in the M2 area? Was the histological verification done?

Response: We are thankful for this concern. When lowering an electrode, a stereomicroscope was also used to make sure that the electrode's tip is just in touch with the cortical surface in the hole drilled, then manipulate the depth of the electrode according to Brain Atlases. Thus, electrodes terminations are located in M2. We did histological verification after each experiment. We have added description to the protocol section in our revised manuscript. See line 97, 108-109 please.

3) What was the fate of the animals after experiment? Urethane anesthesia is very toxic and mice should be subjected to euthanasia after experiment. The euthanasia should be described briefly in the Protocol.

Response: Thanks for the concerns. Since the experiment is one-time acute recording, mice were euthanized by fast cervical dislocation as previously described (Carbone et al., 2012). The procedure has been specified now in the protocol section. See line 116-117 please.

Minor Concerns:

1) How many animals were used? Please, provide the n for each group in the Protocol.

Response: We are thankful for this concern. For each genotype group we used 10 mice. The n value has been specified now in the protocol section. See line 83 please.

2) In the discussion there is the statement "Because the data are obtained from 3-5-month-old APP/PS1 and WT mice, an age when APP/PS1 mice do not show apparent deposition of beta amyloid plaques..."

A quantity of amyloid plaques do not correlate with cognitive deficits. Soluble amyloid-beta oligomers are more toxic and involved with synaptic deterioration, so they can contribute to dementia (e.g. Lacor et al. 2007, J Neurosci 27).

Response: We apologize for this. We have modified the discussion section accordingly. See line 263-268 please.

References:

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Palop, J.J., Chin, J., Roberson, E.D., Wang, J., Thwin, M.T., Bien-Ly, N., Yoo, J., Ho, K.O., Yu, G.Q., Kreitzer, A., et al. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55, 697-711.

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Zitnik, G.A., Curtis, A.L., Wood, S.K., Arner, J., and Valentino, R.J. (2016). Adolescent Social Stress Produces an Enduring Activation of the Rat Locus Coeruleus and Alters its Coherence with the Prefrontal Cortex. Neuropsychopharmacology 41, 1376-1385.