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TITLE:

Simultaneous Eye Tracking and Single-Neuron Recordings in Human Epilepsy Patients

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KEYWORDS:

Human single-neuron recording, Eye tracking, Visual search, Attention, Epilepsy patients, Medial temporal lobe, Target detection.

SHORT ABSTRACT:

We describe a method to conduct single-neuron recordings with simultaneous eye tracking in humans. We demonstrate the utility of this method and illustrate how we used this approach to obtain neurons in the human medial temporal lobe that encode targets of a visual search.

LONG ABSTRACT:

Intracranial recordings from patients with intractable epilepsy provide a unique opportunity to study the activity of individual human neurons during active behavior. An important tool for quantifying behavior is eye tracking, which is an indispensable tool for studying visual attention. However, eye tracking is challenging to use concurrently with invasive electrophysiology and this approach has consequently been little used. Here, we present a proven experimental protocol to conduct single-neuron recordings with simultaneous eye tracking in humans. We describe how the systems are connected and the optimal settings to record neurons and eye movements. To illustrate the utility of this method, we summarize results that were made possible by this setup. This data shows how using eye tracking in a memory-guided visual search task allowed us to describe a new class of neurons called target neurons, whose response was reflective of top-down attention to the current search target. Lastly, we discuss the significance and solutions to potential problems of this setup. Together, our protocol and results suggest that single-neuron recordings with simultaneous eye tracking in humans are an effective method to study human brain function. It provides a key missing link between animal neurophysiology and human

cognitive neuroscience.

INTRODUCTION:

Human single-neuron recordings are a unique and powerful tool to explore the function of the human brain with extraordinary spatial and temporal resolution[1]. Recently, single-neuron recordings have gained wide use in the field of cognitive neuroscience because they permit the direct investigation of cognitive processes central to human cognition. These recordings are made possible by the clinical need to determine the position of epileptic foci, for which depth electrodes are temporarily implanted into the brains of patients with suspected focal epilepsy. With this setup, single-neuron recordings can be obtained using microwires protruding from the tip of the hybrid depth electrode (a detailed description of the surgical methodology involved in the insertion of hybrid depth electrodes is provided in the previous protocol[2]). Among others, this method has been used to study human memory[3,4], emotion[5,6], and attention[7,8].

Eye tracking measures gaze position and eye movements (fixations and saccades) during cognitive tasks. Video-based eye trackers typically use the corneal reflection and the center of the pupil as features to track over time[9]. Eye tracking is an important method to study visual attention because the gaze location indicates the focus of attention during most natural behaviors[10-12]. Eye tracking has been used extensively to study visual attention in healthy individuals[13] and neurological populations[14-16].

While both single-neuron recordings and eye tracking are individually used extensively in humans, few studies have used both simultaneously. As a result, it still remains largely unknown how neurons in the human brain respond to eye movements and/or whether they are sensitive to the currently fixated stimulus. This is in contrast to studies with macaques, where eye-tracking with simultaneous single-neuron recordings has become a standard tool. In order to directly investigate the neuronal response to eye movements, we combined human single-neuron recordings and eye tracking. Here we describe the protocol to conduct such experiments and then illustrate the results through a concrete example.

Despite the established role of the human medial temporal lobe (MTL) in both object representation[17,18] and memory[3,19], it remains largely unknown whether MTL neurons are modulated as a function of top-down attention to behaviorally relevant goals. Studying such neurons is important to start to understand how goal-relevant information influences bottom-up visual processes Here, we demonstrate the utility of eye tracking while recording neurons using guided visual search, a well-known paradigm to study goal-directed behavior[20-25]. Using this method, we recently described a class of neurons called target neurons, which signals whether the currently attended stimulus is the goal of an ongoing search[8]. In the below, we present the study protocol needed to reproduce this previous scientific study. Note that along this example, the protocol can easily be adjusted to study an arbitrary visual attention task.

PROTOCOL:

All participants provided written informed consent according to protocols approved by the institutional review boards of the Cedars-Sinai Medical Center and the California Institute of Technology.

1. Participants

1.1. Recruit neurosurgical patients with intractable epilepsy who are undergoing placement of intracranial electrodes to localize their epileptic seizures.

1.2. Insert depth electrodes with embedded microwires into all clinically indicated target locations, which typically include a subset of amygdala, hippocampus, anterior cingulate cortex and pre-supplementary motor area. See details for implantation in our previous protocol[2].

1.3. Once the patient returns to the epilepsy monitoring unit, connect the recording equipment for both macro- and micro- recordings. This includes carefully preparing a head-wrap that includes head stages (see our previous description for details[2]). Then, wait for the patient to recover from the surgery and conduct testing when the patient is fully awake (typically at least 36 to 48 h after surgery).

2. Experimental setup

2.1. Connect the stimulus computer to the electrophysiology system and eye tracker following the diagram in **Figure 1**.

2.2. Use the remote non-invasive infrared eye tracking system (see **Table of Materials**). Place the eye tracking system on a robust mobile cart (**Figure 1A, B**). To the same cart, attach a flexible arm that holds an LCD display. Use the **remote mode** to track the patients head and eyes.

2.3. Place a fully charged uninterrupted power supply (UPS) on the eye tracking cart and connect all devices related to eye tracking (i.e., LCD in front of patient, eye tracker camera and light source, and eye tracker host computer) to the UPS rather than to an external power source.

2.4. Adjust the distance between the patient and the LCD screen to 60-70 cm and adjust the angle of the LCD screen so that the surface of the screen is approximately parallel to the patient's face. Adjust the height of the screen relative to the patient's head such that the camera of the eye tracker is approximately at the height of the patient's nose.

2.5. Provide the patient with the button box or keyboard. Verify that triggers (TTLs) and button press are recorded properly before starting the experiment.

3. Single-neuron recording

3.1. Start the acquisition software. First visually inspect the broadband (0.1 Hz-9 kHz) local field potentials and make sure they are not contaminated by line noise. Otherwise, follow standard procedures to remove noise (see **Discussion**).

3.2. To identify single neurons, band-pass filter the signal (300Hz-9KHz). Select one of the eight microwires as a reference for each microwire bundle. Test different references and adjust the reference so that (1) the other 7 channels show clear neurons, and (2) the reference does not contain neurons. Set the input range to be ±2500 µV.

3.3. Enable saving the data as an NRD file (i.e., the broadband raw data file that will be used for subsequent off-line spike sorting) before recording data. Set the sampling rate to 32 kHz.

4. Eye tracking

4.1. Start the eye tracking software. Because it is a head-fixation free system, place the sticker on the patient's forehead so that the eye tracker can adjust for head movements.

4.2. Adjust the distance and angle between the eye tracker and patient so that the target marker, head distance, pupil, and corneal reflection (CR) are marked as ready (as shown in green in the eye tracking software; **Figure 2** shows a good example camera setup screen). Click on the **eye** to be recorded and set the sampling rate to 500Hz.

4.3. Use the **auto-adjustment** of pupil and CR threshold. For patients wearing glasses, adjust the position and/or angle of the illuminator and camera so that reflections from the glass will not interfere with pupil acquisition.

4.4. Calibrate the eye tracker with the built-in 9-point grid method at the beginning of each block. Confirm that eye positions (shown as "+") register nicely as a 9-point grid. Otherwise, redo calibration.

4.5. Accept the calibration and do validation. Accept the validation if the maximal validation error is < 2° and the average validation error is < 1°. Otherwise, redo validation.

4.6. Do drift correction and proceed to the actual experiment.

5. Task

5.1. In this visual search task, use the stimuli from our previous study[14] and follow the task procedure as described before[8].

5.2. Provide task instructions to participants. Instruct the participants to find the target item in the search array and respond as soon as possible. Instruct the participants to press the left button of a response box (see **Table of Materials**) if they find the target and the right button if they think

the target is absent. Explicitly instruct the participants that there will be target-present and target-absent trials.

5.3. Start stimulus presentation software (see **Table of Materials**) and run the task: Present a target cue for 1 s and present the search array using the stimulus presentation software. Record button presses and provide trial-by-trial feedback (Correct, Incorrect, or Time Out) to participants.

6. Data analysis

6.1. Because the acquisition and eye tracking systems run on different clocks, use the behavioral log file to find the alignment timestamp for electrophysiology recording and eye tracking. Match the triggers from electrophysiology recording and eye tracking before proceeding to further analysis. Extract segments of data according to timestamps and analysis windows separately for electrophysiology recording and eye tracking.

6.2. Use the semi-automatic template matching algorithm Osort[26] and follow the steps described before[2,26] to identify putative single neurons. Assess the quality of the sorting before moving to further analysis[2].

6.3. To analyze eye movement data, first convert the EDF data from the eye tracker into ASCII format. Also, extract fixations and saccades. Then, import the ASCII file and save the following information into a MAT file: (1) time stamps, (2) eye coordinates (x,y), (3) pupil size, and (4) event time stamps. Parse the continuous recording into each trial.

6.4. Follow previously described procedures to analyze the correlation between spikes and behavior[8].

REPRESENTATIVE RESULTS:

To illustrate the usage of the above-mentioned method, we next briefly describe a use-case that we recently published[8]. We recorded 228 single neurons from the human medial temporal lobe (MTL; amygdala and hippocampus) while the patients were performing a visual search task (**Figure 3A, B**). During this task, we investigated whether the activity of neurons differentiated between fixations on targets and distractors.

First, when we aligned responses at the button press, neurons were found that showed differential activity between target-present trials and target-absent trials (**Figure 3C, D**). Importantly, with simultaneous eye tracking, the fixation-based analysis was conducted. To select such target neurons, the mean firing rate in a time window starting 200 ms before fixation onset and ending 200 ms after fixation offset (next saccade onset) was used. A subset of MTL neurons (50/228; 21.9%; binomial $P < 10^{-20}$) showed significantly different activities between fixations on targets vs. distractors (**Figure 3E, F**). Furthermore, one type of such target neuron had a greater response to targets relative to distractors (target-preferring; 27/50 neurons; **Figure 3E**) whereas the other had a greater response to distractors relative to targets (distractor-

preferring; 23/50; **Figure 3F**). Together, this result demonstrates that a subset of MTL neurons encode whether the present fixation landed on a target or not.

The dynamic process of visual search is demonstrated in **Movie 1**.

FIGURE AND TABLE LEGENDS:

Figure 1. Experimental setup. (A) The left panels show a sketch of the connections between the different systems. The stimulus computer serves as the central controller. It connects to the electrophysiology system through the parallel port and sends TTL pulses as triggers. The stimulus computer connects to the eye tracking system through an ethernet cable, over which it sends text messages to the eye tracker and receives the current gaze position online. The stimulus computer also presents stimuli on the stimulus screen (VGA) and receives a response from the patient from a USB button box or keyboard. Blue lines show the connections between devices and the arrows show the direction of communication between devices. The right panel shows the signal flow between systems and data saved in each system. **(B)** An example setup with key parts of the system labeled. **(C)** Electrophysiology system. **(D)** Docking station that has the parallel port and ethernet port. **(E)** UPS for electrophysiology system (left) and eye tracking system (right).

Figure 2. Example eye tracker camera setup screen. Target marker bounding box, eye bounding box, head distance, pupil, and corneal reflection (CR) should be marked as green and/or "OK" before proceeding.

Figure 3. Example results. (A) Task. The search cue was presented for 1s, immediately followed by the search array. Participants were instructed to indicate by button press whether the target is present or absent (timeout 14s). Trial-by-Trial feedback is given immediately after button press ('Correct', 'Incorrect', or 'Time Out'), followed by a blank screen for 1-2 s. **(B)** Example visual search arrays with fixations indicated. Each circle represents a fixation. Green circle: first fixation. Magenta circle: last fixation. Yellow line: saccades. Blue dot: raw gaze position. Red box: target. **(C-F)** Single neuron examples. **(C-D)** Button-press-aligned examples. **(C)** The neuron that increased its firing rate for target-present trials, but not for target-absent trials. **(D)** The neuron euron that decreased its firing rate for target-present trials, but not for target-absent trials. Trials are aligned to the button-press (gray line) and are sorted by reaction time. Black lines represent the onset and offset of the search cue (1 s duration). The inset shows waveforms for each unit. Asterisk indicates a significant difference between target-present and absent trials in that bin ($P < 0.05$, two-tailed t-test, Bonferroni-corrected; bin size = 250 ms). Shaded area denotes ±SEM across trials. **(E-F)** Fixation-aligned examples. t=0 is fixation onset. **(E)** The neuron that increased its firing rate when fixating on targets, but not distractors (the same neuron as **(C)**). **(F)** The neuron that decreased its firing rate when fixating on targets but not distractors (the same neuron as **(D)**). Fixations are sorted by fixation duration (black line shows the start of the next saccade). Asterisk indicates a significant difference between fixations on targets and distractors in that bin ($P < 0.05$, two-tailed t-test, Bonferroni-corrected; bin size = 50 ms). This figure has been modified with permission from[8].

Movie 1. Typical trials of visual search with responses from a single target neuron. In target-present trials, this neuron increased its firing rate regardless of the identity of the cue. Yellow dot denotes eye position. Yellow vertical bars at the bottom are event markers (i.e., cue onset, array onset, and inter-trial-interval onset). Red vertical bars at the bottom show spikes, which are also played as sound. The red dotted box denotes the location of the search target (not shown to participants).

DISCUSSION:

In this protocol, we described how to employ single-neuron recordings with concurrent eye tracking and described how we used this method to identify target neurons in the human MTL.

The setup involves three computers: one executing the task (stimulus computer), one running the eye tracker, and one running the acquisition system. To synchronize between the three systems, the parallel port is used to send TTL triggers from the stimulus computer to the electrophysiology system (**Figure 1C**). At the same time, the stimulus computer sends the same TTLs using an ethernet cable to the eye tracker. The stimulus computer should have a parallel port on its docking station in the example shown (**Figure 1D**), or alternatively, have a PCI Express parallel port card or a similar device.

The mobile cart for the stimulus computer and eye tracker with the flexible arm attached allows flexible positioning of the screen in front of the patient (**Figure 1A, B**). The usage of a UPS to power the devices on the cart is strongly suggested to eliminate line noise introduced into the electrophysiological recordings due to the proximity of the eye tracking devices to the patient's head (**Figure 1E**). Furthermore, laptops running on battery power should be used as stimulus computer and eye tracker computer.

If the recordings are contaminated by noise, the eye tracker should be removed first to assess whether it is the source of the noise. If not, standard procedures should be used denoise before using the eye tracker again[2]. Note that typical sources of line noise include the patient bed, IV devices, devices in the patient room, or ground loops created by using different plugs for different systems. If the eye tracker is the source of the noise, all devices (the camera, light source, and LCD screen) should be powered from the battery and/or UPS. If there is still noise, it is likely that the LCD screen and/or the power supply for the LCD screen of the eye tracker is faulty. A different screen / power supply should then be used. If possible, an LCD screen with an external power supply should be used. It is also important to ensure that the TTL cable does not introduce noise (i.e., use a TTL isolator).

The significance of recording single-neuron data in neurosurgical patients simultaneously with eye tracking is high for several reasons. First, single-neuron recordings have a high spatial and temporal resolution, and, thereby, allow the investigation of fast cognitive processes such as visual search. Second, they provide a much-needed link between human cognitive neuroscience and animal neurophysiology, which relies heavily on eye tracking. Third, because human single-

neuron recordings are often performed simultaneously from multiple brain regions, our approach permits the temporal resolution that will help distinguish between visually driven vs. top-down modulation from frontal cortex. In summary, single-neuron recordings with eye tracking make it possible to isolate specific processes that underlie goal-directed behavior. In addition, our concurrent eye tracking permitted fixation-based analysis, which greatly increased statistical power (e.g., **Figure 3A, B** vs. **Figure 3C, D**).

A challenge of this method is that the eye tracking system may introduce additional noise into the electrophysiological data. However, with the procedures outlined in this protocol, such additional noise can be eliminated, and once these procedures are established, they can be executed routinely. Furthermore, eye tracking lengthens the time needed for a given experiment because additional setup is required, especially when calibration of the eye tracker is challenging for some patients, in particular those with small pupils or glasses. However, the benefits from simultaneous eye tracking are worth this additional effort for several studies, making eye tracking a valuable addition to single-neuron recordings.

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DISCLOSURES:

The authors declare no conflict of interest.

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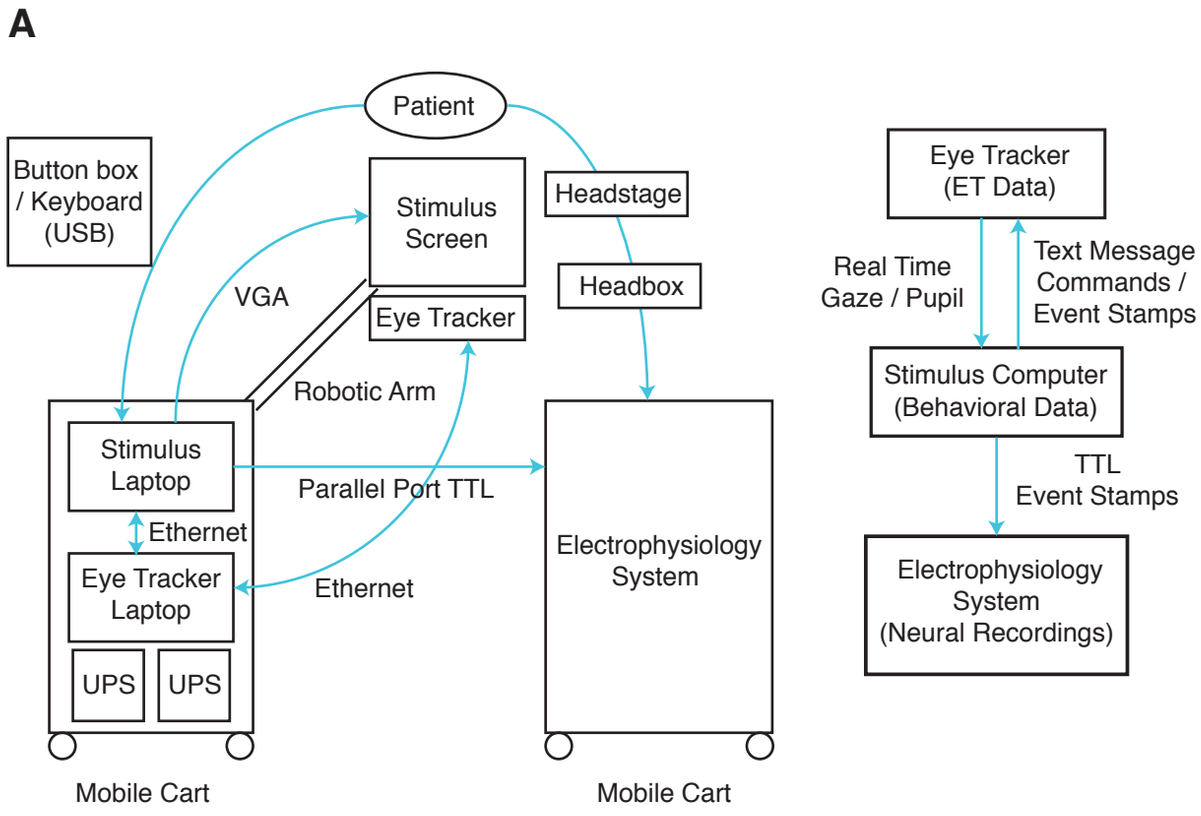
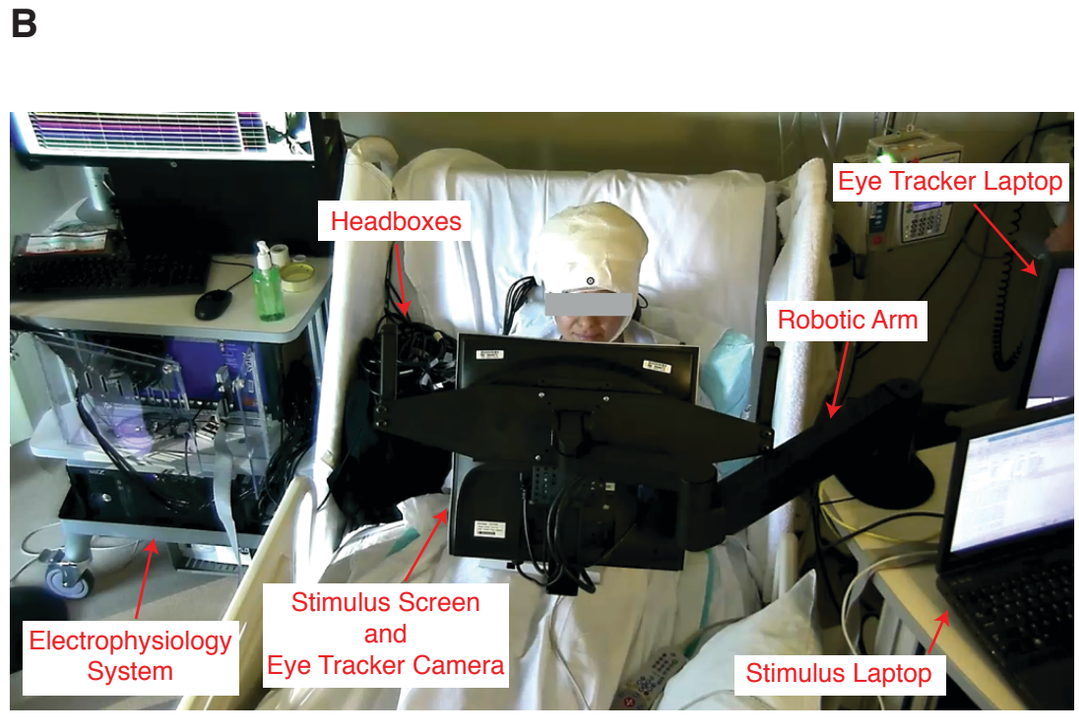
Figure

Figure 1





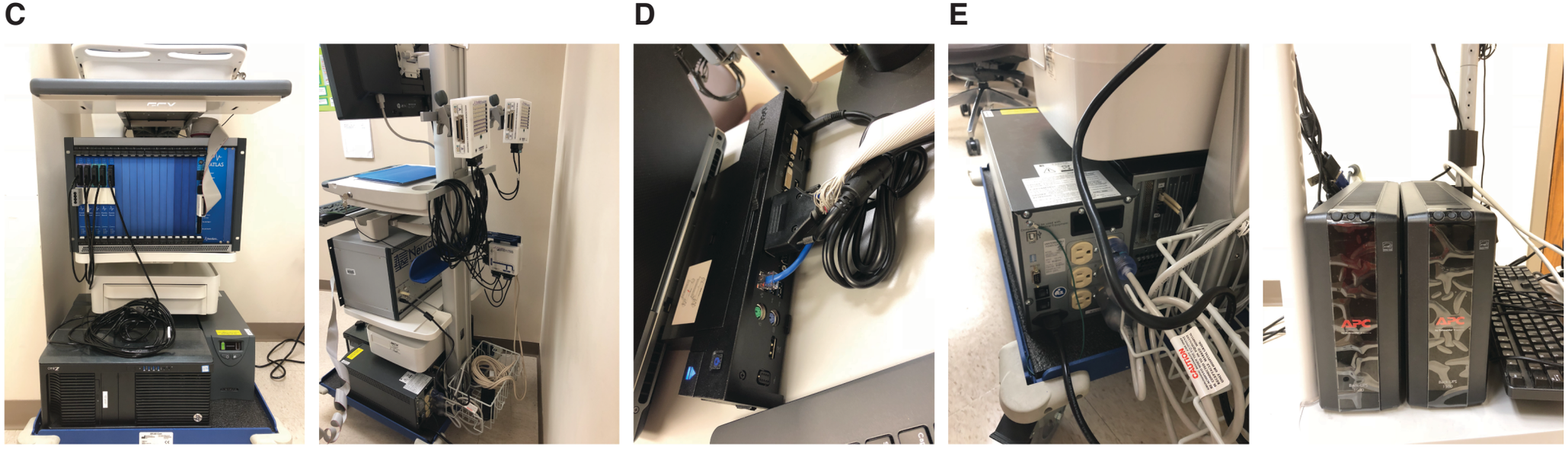



Image Thresholds
Pupil
Corneal
Tracking Mode
Pupil-CR
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Pupil Tracking
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Image Display
Crosshairs
Threshold Coloring
Image->Display PC
Align Eye Window
Illuminator Power
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75%
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DIST OK
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CR : 223 AUTO x1.00
PUPIL OK
CR OK
Eye Tracked:
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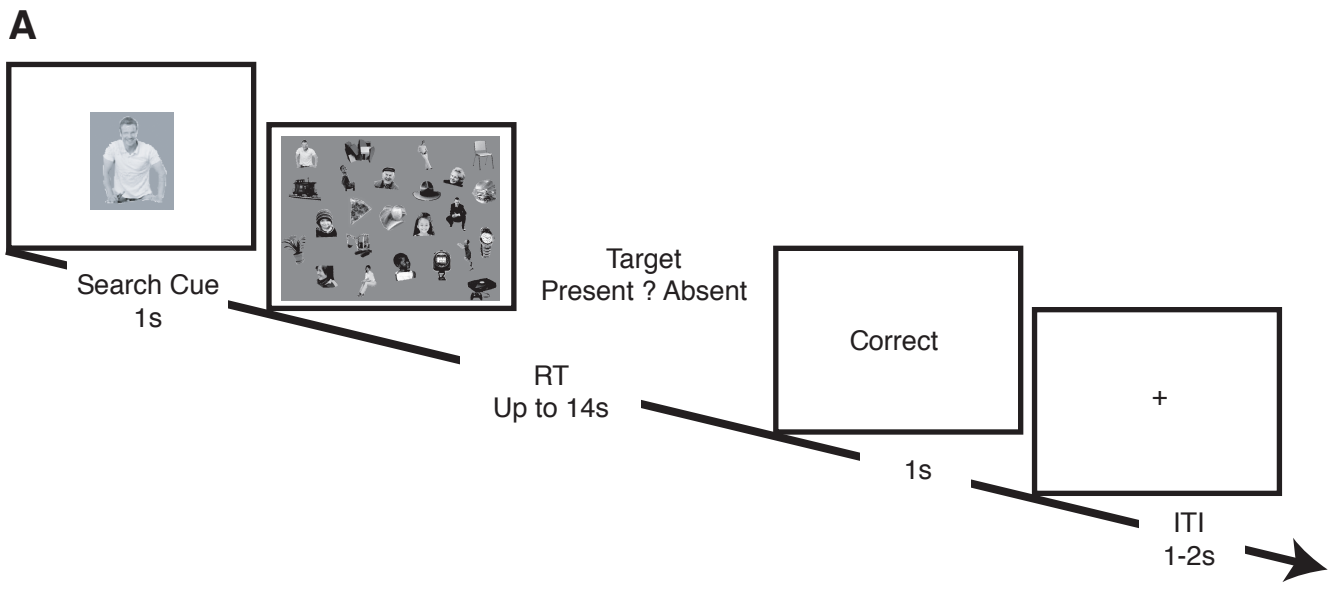
Validate
Drift Correct

Figure 3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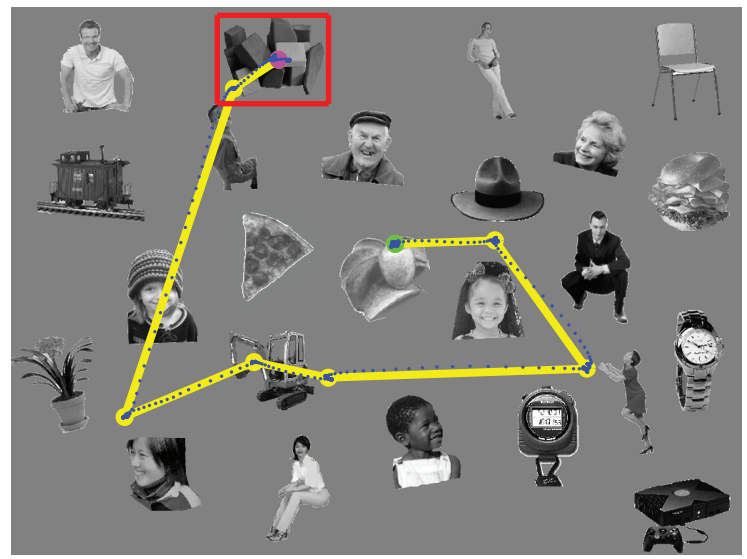
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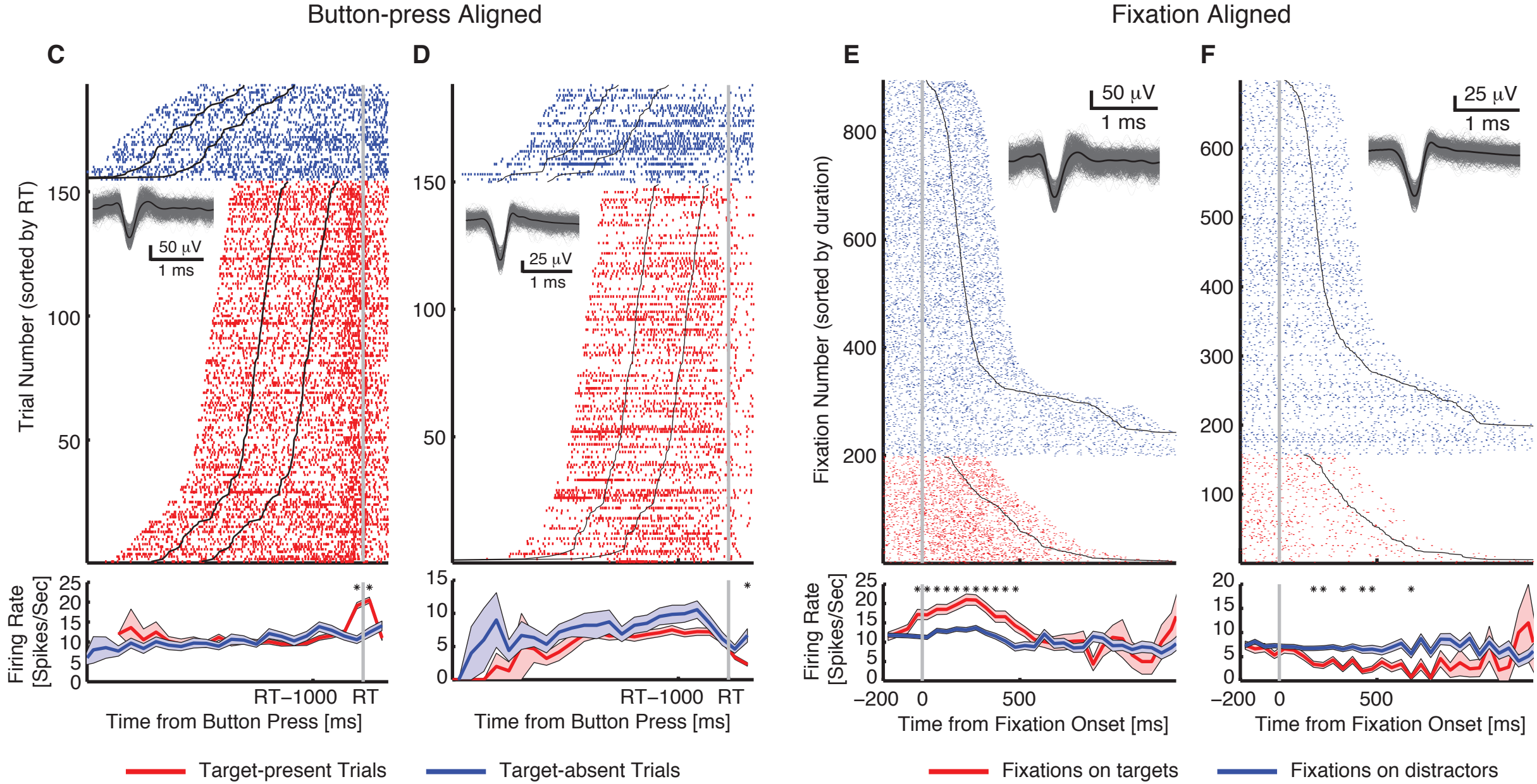
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Fixation Aligned
C
D
E
F
Trial Number (sorted by RT)
Fixation Number (sorted by duration)
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1 ms
Firing Rate [Spikes/Sec]
RT–1000
RT
Time from Button Press [ms]
Time from Fixation Onset [ms]
Target-present Trials
Target-absent Trials
Fixations on targets
Fixations on distractors

Video or Animated Figure

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Table of Materials

Name of Material / Equipment	Company	Catalog Number
Cedrus Response Box	Cedrus (https://cedrus.com/)	RB-844
Dell Laptop	Dell (https://dell.com)	Precision 7520
EyeLink Eye Tracker	SR Research (https://www.sr-research.com)	1000 Plus Remote with laptop host computer and LCD arm mount
MATLAB	MathWorks Inc	R2016a (RRID: SCR_001622)
Neuralynx Neurophysiology System	Neuralynx (https://neuralynx.com)	ATLAS 128
Osort	Open source	v4.1 (RRID: SCR_015869)
Psychophysics Toolbx	Open source	PTB3 (RRID: SCR_002881)

Comments/Description
Button box
Stimulus Computer
Eye tracking
Data analysis
Electrophysiology
Spike sorting algorithm
Matlab toolbox to implement psychophysical experiments

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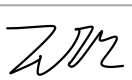
Signature:	 Date: 11/17/18

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Reply to editorial comments

Changes to be made by the author(s) regarding the manuscript:

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3. Figure 1: Please remove commercial language (Eyelink, Neuralynx) and replace them with generic terms.

We have removed these and replaced them with generic terms.

4. Figure 3: Please change the time unit "sec" to "s".

We have changed this in the figure.

5. Table of Equipment and Materials: Please sort the items in alphabetical order according to the name of material/equipment.

We have sorted these items in alphabetical order.

6. Please number the figures in the sequence in which you refer to them in the manuscript text. Currently, Figure 3 is introduced before Figure 1 and Figure 2.

We have modified this in the revised manuscript.

7. Please provide an email address for each author.

We have provided an email address for each author.

8. Keywords: Please provide at least 6 keywords or phrases.

We have provided two additional keywords (i.e., Medial temporal lobe, Target detection).

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We have changed this throughout the manuscript.

10. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

We have included the following ethics statement "All participants provided written informed consent according to protocols approved by the institutional review boards of the Cedars-Sinai Medical Center and the California Institute of Technology."

11. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have revised this throughout the protocol.

12. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

We have revised this throughout the protocol.

13. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

We have revised this throughout the protocol.

14. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

We have revised this accordingly.

15. 2.1-2.3: Are these performed by using a computer program? Please describe how to randomly assign array items and select trials.

We refer this to published material specifying how to create stimuli.

16. 2.4: How is the target cue presented?

We have clarified this in the revised manuscript.

17. Please order the steps properly so that the protocol can be followed in chronological order.

We have adjusted the order of protocol actions.

18. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

We have highlighted the essential steps.

19. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense. Notes cannot usually be filmed and should be excluded from the highlighting.

We have ensured that the the highlighted part of the step includes at least one action that is written in imperative tense and there are no notes.

20. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

We have included all relevant details that are required to perform the step in the highlighting.

21. Discussion: Please describe critical steps within the protocol.

We have included discussion of critical steps.

22. For in-text references, the corresponding reference numbers should appear as superscripts after the appropriate statement(s) in the text (before punctuation but after closed parenthesis). The references should be numbered in order of appearance.

We have ensured that the in-text references follow the suggested format.

23. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then et al. Please do not abbreviate journal titles. See the example below:

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We have ensured that the references follow the suggested format.

Reply to comments from Reviewer 1

In this manuscript, Rutishauser and colleagues present a universal approach to allow simultaneous acquisition of neural data and eye motion tracking in human patients. This is a particularly useful method as it enables direct comparison and cross validation of data between non-human primate animal models and human patient data. Additionally, they validated their method using eye tracking in a memory-guided visual search task and described a new class of neurons called target neurons, whose response was reflective of top-down attention to the current search target.

The manuscript figures are very clean and illustrative. I believe that this method will be interesting to a wide range of researchers in systems neuroscience.

Figure 1a: since the authors provided illustrative figures showing the setup arrangement, it would be more informative if they rearrange the schematic to show the signal flow as opposed to the physical location of the modules.

We thank the reviewer for the constructive comments. We have updated Figure 1A to show signal flow.

Reply to comments from Reviewer 2

Major Concerns:

None.

Minor Concerns:

(1) From looking at Fig. 3 it seems that panels C and E show the same neuron. Wouldn't it make sense to show the same neuron in F that was shown in D?

We thank the reviewer for the suggestion. We have now shown the same neurons for button-press-aligned and fixation-aligned examples.

Typos and other stuff.

Copy-editing by a native speaker would be helpful, particularly for the Introduction.

l.60: "gaze position and eye movements (saccades and fixations)" should be "gaze position and eye movements (fixations and saccades)".

l.106 and others: "array stimulus" should be "stimulus array".

l.123: "can not respond"should be "does not respond".

We have corrected these typos.

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TITLE:
A method for simultaneous eye tracking and single-neuron recordings in human epilepsy patients

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KEYWORDS:
Human single-neuron recording, Eye tracking, Visual search, Attention, Epilepsy patients, Medial temporal lobe, Target detection.

SHORT ABSTRACT:
We describe a method to conduct single-neuron recordings with simultaneous eye tracking in humans. We demonstrate the utility of this method and illustrate how we used this approach to obtain neurons in the human medial temporal lobe that encode targets of a visual search.

LONG ABSTRACT:
Intracranial recordings from patients with intractable epilepsy provide a unique opportunity to study the activity of individual human neurons during active behavior. An important tool for quantifying behavior is eye tracking, which is an indispensable tool for studying visual attention. However, eye tracking is challenging to use concurrently with invasive electrophysiology and this approach has consequently been little used. Here, we present a proven experimental protocol to conduct single-neuron recordings with simultaneous eye tracking in humans. We describe how the systems are connected and the optimal settings to record neurons and eye movements. To illustrate the utility of this method, we summarize results that were made possible by this setup. This data shows how using eye tracking in a memory-guided visual search task allowed us to describe a new class of neurons called target neurons, whose response was reflective of top-down attention to the current search target. Lastly, we discuss the significance and solutions to potential problems of this setup. Together, our protocol and results suggest that single-neuron recordings with simultaneous eye tracking in humans are an effective method to study human

brain function. It provides a key missing link between animal neurophysiology and human cognitive neuroscience.

INTRODUCTION:

Human single-neuron recordings are a unique and powerful tool to explore the function of the human brain with extraordinary spatial and temporal resolution[1]. Recently, single-neuron recordings have in particular gained wide use in the field of cognitive neuroscience because they permit the direct investigation of cognitive processes central to human cognition. These recordings are made possible by the clinical need to determine the position of epileptic foci, for which depth electrodes are temporarily implanted into the brains of patients with suspected focal epilepsy. With this setup, single-neuron recordings can be obtained using microwires protruding from the tip of the hybrid depth electrode (a detailed description of the surgical methodology involved in the insertion of hybrid depth electrodes is provided in previous protocol[2]~~).~~). Among others, this method has been used to study human memory[3,4], emotion[5,6], and attention[7,8].

Eye tracking measures gaze position and eye movements (~~saccades and~~ fixations and saccades) during cognitive tasks. Video-based eye trackers typically use the corneal reflection and the center of the pupil as features to track over time[9]. Eye tracking is an important method to study visual attention because the gaze location indicates the focus of attention during most natural behaviors[10-12]. Eye tracking has been used extensively to study visual attention in healthy individuals[13] and neurological populations[14-16].

While both single-neuron recordings and eye tracking are individually used extensively in humans, few studies have used both simultaneously. As a result, it still remains largely unknown how neurons in the human brain respond to eye movements and/or whether they are sensitive to the currently fixated stimulus. This is in contrast to studies with macaques, where eye-tracking with simultaneous single-neuron recordings has become a standard tool. In order to directly investigate neuronal response to eye movements, we combined human single-neuron recordings and eye tracking. We here describe the protocol to conduct such experiments and then illustrate the results through a concrete example.

Despite the established role of the human medial temporal lobe (MTL) in both object representation[17,18] and memory[3,19], it remains largely unknown whether MTL neurons are modulated as a function of top-down attention to behaviorally relevant goals. Studying such neurons is important to start to understand how goal-relevant information influences bottom-up visual processes Here, we demonstrate the utility of eye tracking while recording neurons using guided visual search, a well-known paradigm to study goal-directed behavior[20-25]. Using this method, we recently described a class of neurons called 'target neurons', which signals whether the currently attended stimulus is the goal of an ongoing search[8]. In the below, we present the study protocol needed to reproduce this previous scientific study. Note that along this example, the protocol can easily be adjusted to study an arbitrary visual attention task.

PROTOCOL:

All participants provided written informed consent according to protocols approved by the institutional review boards of the Cedars-Sinai Medical Center and the California Institute of Technology.

1. Participants

1.1. Recruit neurosurgical patients with intractable epilepsy who are undergoing placement of intracranial electrodes to localize their epileptic seizures.

1.2. Insert depth electrodes with embedded microwires into all clinically indicated target locations~~. These~~, which typically include a subset of amygdala, hippocampus, anterior cingulate cortex and pre-supplementary motor area. See details for implantation ~~are documented~~ in our previous protocol[2]~~.~~.

1.3. Once the patient returns to the epilepsy monitoring unit, connect the recording equipment for both macro- and micro- recordings. This includes carefully preparing a head-wrap that includes headstages (see our previous description for details[2]~~1.3.~~). Then, wait for the patient to recover from the surgery and conduct testing when the patient is fully awake (typically at least 36 to 48 hours after surgery).

2. ~~Stimuli and task~~

~~2.1. Create the stimuli following . Briefly, create 20 distinct visual search arrays with 12 faces (faces and people with different postures, emotions, ages, and genders, etc.) and 12 non-faces (furniture, toys, food, etc.) (**Figure 3B**). Equalize low-level properties of face and non-face items in each array stimulus by the Itti-Koch model .~~

~~2.2. From each array stimulus, randomly assign array items as targets, and counterbalance face targets and non-face targets.~~

~~2.3. In each block of 100 trials, randomly select 80 trials as target-present trials and 20 trials as target-absent trials, and counterbalance the target-present trials and target-absent trials in each array and for each target type (i.e., for each array stimulus, there will be 2 face-target trials, 2 non-face-target trails, and 1 target-absent trial). Completely randomize the array and target orders.~~

~~2.4. Present a target cue for 1 second and present the search array. Instruct the participants to find the target item in the search array and respond as soon as possible. Instruct the participants to press the left button of a response box (we use RB-844 from Cedrus Inc) if they find the target and the right button if they think the target is absent. Explicitly instruct the participants that there will be target-present and target-absent trials.~~

~~2.5. Terminate the search if the participant can not respond within 14 seconds. Provide feedback to participants ('Correct', 'Incorrect', or 'Time Out'). Use a jittered inter-trial-interval (ITI) of 1 to 2 seconds between trials.~~

~~3.~~ Experimental setup

~~3~~2.1. Connect the stimulus computer to the electrophysiology system and eye tracker following the diagram in **Figure 1**.

2.2. ~~3.2. Note that~~ Use the remote non-invasive infrared eye tracking system (see table of materials). Place~~The setup involves three computers: one executing the task (stimulus computer), one running the eye tracker, and one running the acquisition system. To synchronize between the three systems, use parallel port to send TTL triggers from the stimulus computer to the electrophysiology system (Neuralynx; **Figure 1C**). At the same time, the stimulus computer sends the same TTLs using an ethernet cable to the eye tracker (Eyelink). Note that we use a stimulus computer with a parallel port on its docking station in the example shown (**Figure 1D**), but using a PCI Express parallel port card or similar device is also a viable alternative. Check triggers and button press before the actual experiment.~~

~~3.3. Place~~ the eye tracking system on a robust mobile cart (**Figure 1A, B**). To the same cart, attach a flexible arm that holds an LCD display. ~~This setup allows flexible positioning of the screen in front of the patient (**Figure 1A,B**). Here, we~~ Use the 'remote mode' to track the patients head and eyes.

2.3.~~4.~~ Place a fully charged uninterrupted power supply (UPS) on the eye tracking cart and connect all devices related to eye tracking (i.e., LCD in front of patient, eye tracker camera and light source, and eye tracker host computer) to the UPS rather than to an external power source. ~~This is necessary to eliminate line noise introduced into the electrophysiological recordings due to the close proximity of the eye tracking devices to the patient's head (**Figure 1E**). Use laptops running on battery power as stimulus computer and eye tracker computer.~~

~~3.5~~2.4. Adjust the distance between the patient and the LCD screen to 60-70cm and adjust the angle of the LCD screen so that the surface of the screen is approximately parallel to the ~~patients~~patient's face. Adjust the height of the screen relative to the ~~patients~~patient's head such that the camera of the eye tracker is approximately at the height of the patient's nose. ~~Provide the patient with the button box or keyboard.~~

~~3.6. Use MATLAB and Psychtoolbox 3 () to present stimuli.~~

~~4.~~2.5. Provide the patient with the button box or keyboard. Verify that triggers (TTLs) and button press are recorded properly before starting the experiment.

3. Single-neuron recording ~~and analysis~~

43.1. Start ~~Neuralynx Pegasus~~the acquisition software. First ~~check~~visually inspect the broadband (0.1Hz-9kHz) local field potentials and make sure they are not contaminated by line noise. ~~If they are, first remove the eye tracker to assess whether this is the source of noise. If not, use~~Otherwise, follow standard procedures to ~~denoise before using the eye tracker again~~remove noise (see ~~).~~ **Discussion** ~~Note that typical sources of line noise include the patient bed, IV devices, devices in the patient room, or ground loops created by using different plugs for different systems. If the eye tracker is the source of noise, assure that all devices (in particular the camera, light source, and LCD screen) are powered from battery and/or UPS. If there is still noise, it is likely that the LCD screen and/or the power supply for the LCD screen of the eye tracker is fault. Use a different screen / power supply. If at all possible, use an LCD screen with an external power supply. Make sure that the TTL cable does not introduce noise (use a TTL isolator~~).

43.2. To identify single neurons, band-pass filter the signal (300Hz-9KHz). Select one of the eight microwires as reference for each microwire bundle. Test different references and adjust the reference so that (1) the other 7 channels show clear neurons, and (2) the reference does not contain neurons. Set the input range to be ±2500 uV.

~~4.~~3. ~~Before recording data,~~3. Enable saving the data as an NRD file~~, which is the~~ (i.e., broadband raw data file that will be used for subsequent off-line spike sorting~~.~~) before recording data. Set the sampling rate to 32 kHz.

4.~~4. After concluding the recording, use the semi-automatic template matching algorithm Osort and follow the steps described in and to identify putative single neurons. Assess the quality of the sorting before moving to further analysis .~~

~~4.5. Because the acquisition and eye tracking systems run on different clocks, use the behavioral log file to find the alignment timestamp for electrophysiology recording and eye tracking. Match the triggers from electrophysiology recording and eye tracking before proceeding to further analysis. Extract segments of data according to timestamps and analysis windows separately for electrophysiology recording and eye tracking.~~

~~4.6. To analyze the correlation between spikes and behavior, consider only single units with an average firing rate of at least 0.2Hz (entire task). Align trials to stimulus onset or button press for trial analysis, and align fixations to fixation onset for fixation analysis. Compute average firing rates (PSTH) by counting spikes across all trials in consecutive 250 ms bins, and across all fixations in consecutive 50 ms bins. Use two tailed t test at P < 0.05 for pairwise comparisons between conditions. Use Bonferroni correction to account for multiple comparisons when comparing group PSTH.~~

~~4.7. Lastly, to confirm electrode positions, use post implantation structural MRIs to verify target locations (see).~~

~~5.~~ Eye tracking ~~and analysis~~

~~5.1. Use the remote non-invasive infrared Eyelink 1000 system (SR Research, Canada).~~4.1. Start the ~~Eyelink~~eye tracking software. Because it is a head-fixation free system, place the sticker ~~from Eyelink~~ on the ~~patient~~patient's forehead so that the eye tracker can adjust for head ~~movement~~movements.

~~5~~4.2. Adjust the distance and angle between the eye tracker and patient so that the target marker, head distance, pupil, and corneal reflection (CR) are ~~all good~~marked as ready (as shown in green in the ~~Eyelink~~eye tracking software; **Figure 2** shows a good example camera setup screen). Click on the eye to be recorded and set the sampling rate to ~~be~~ 500Hz.

4.3. Use the auto-adjustment of pupil and CR threshold. For patients wearing glasses, adjust the position and/or angle of the illuminator and camera so that reflections from the glass will not interfere with pupil acquisition.

~~5.3~~4.4. Calibrate the eye tracker with the built-in 9-point grid method at the beginning of each block. ~~The~~Confirm that eye positions (shown as "+") ~~should~~ register nicely as a 9-point grid. Otherwise, redo calibration.

4.5.~~4.~~ Accept the calibration and do validation. Accept the validation if the maximal validation error is < 2° and the average validation error is < 1°. Otherwise, redo validation.

~~5.5~~4.6. Do drift correction and proceed to the actual experiment.

5. Task

5.1. In this visual search task, use the stimuli from our previous study[14] and follow the task procedure as described before[8].

5.2. Provide task instructions to participants. Instruct the participants to find the target item in the search array and respond as soon as possible. Instruct the participants to press the left button of a response box (see table of materials) if they find the target and the right button if they think the target is absent. Explicitly instruct the participants that there will be target-present and target-absent trials.

5.3. Start stimulus presentation software (see table of materials) and run the task: Present a target cue for 1 second and present the search array using the stimulus presentation software. Record button presses and provide trial-by-trial feedback ('Correct', 'Incorrect', or 'Time Out') to participants.

6. Data analysis

6.1. Because the acquisition and eye tracking systems run on different clocks, use the behavioral log file to find the alignment timestamp for electrophysiology recording and eye tracking. Match

the triggers from electrophysiology recording and eye tracking before proceeding to further analysis. Extract segments of data according to timestamps and analysis windows separately for electrophysiology recording and eye tracking.

6.2. Use the semi-automatic template matching algorithm Osort[26] and follow the steps described before[2,26] to identify putative single neurons. Assess the quality of the sorting before moving to further analysis[2].

6.3. To analyze eye movement data, first convert the EDF data from ~~Eyelink~~the eye tracker into ASCII format ~~using the Eyelink software.~~. Also, ~~use this software to~~ extract fixations and saccades. Then, ~~use MATLAB to~~ import the ASCII file and save the following information into a MAT file: (1) time stamps, (2) eye coordinates (x,y), (3) pupil size, and (4) event time stamps. Parse the continuous recording into each trial.

6.4. Follow previously described procedures to analyze the correlation between spikes and behavior[8] ~~5.7. Define rectangular regions of interest (ROI) for each array item and compare the firing rate for fixations landing on the target vs. distractors.~~

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REPRESENTATIVE RESULTS:

To illustrate the usage of the above-mentioned method, we next briefly describe a use-case that we recently published[8]. We recorded 228 single neurons from the human medial temporal lobe (MTL; amygdala and hippocampus) while the patients were performing a visual search task (**Figure 3A, B**). During this task, we investigated whether the activity of neurons differentiated between fixations on targets and distractors.

First, when we aligned responses at button press, we found neurons that showed differential activity between target-present trials and target-absent trials (**Figure 3C, D**). Importantly, with simultaneous eye tracking, we were able to conduct fixation-based analysis. To select such target neurons, we used the mean firing rate in a time window starting 200ms before fixation onset and ending 200ms after fixation offset (next saccade onset). ~~Indeed,~~ We found that a subset of MTL neurons (50/228; 21.9%; binomial $P < 10^{-20}$) showed significantly different activities between fixations on targets vs. distractors (**Figure 3E, F**). Furthermore, we found that one type of such target neuron had a greater response to targets relative to distractors (target-preferring; 27/50 neurons; **Figure 3E**) whereas the other had a greater response to distractors relative to targets (distractor-preferring; 23/50; **Figure 3F**). Together, this result demonstrates that a subset of MTL neurons encode whether the present fixation landed on a target or not.

The dynamic process of visual search ~~has been~~is demonstrated in **Movie 1**.

FIGURE AND TABLE LEGENDS:

Figure 1. Experimental setup. (A) The left panels shows a sketch of the connections between the

different systems. The stimulus computer serves as the central controller. It connects to the electrophysiology system ~~(Neuralynx)~~ through the parallel port and sends TTL pulses as triggers. The stimulus computer connects to the eye tracking system ~~(Eyelink)~~ through an ethernet cable~~.~~, over which it sends text messages to the eye tracker and ~~can receive~~receives the current gaze ~~positions~~position online. The stimulus computer also presents ~~stimulus~~stimuli on the stimulus screen ~~through~~ (VGA ~~cable~~) and receives response from ~~patients through~~the patient from a USB ~~Cedrus~~ button box or keyboard. Blue lines show the ~~connection~~connections between devices and the arrows show the direction of communication between devices. The right panel shows the signal flow between systems and data saved in each system. **(B)** An example setup with key parts of the system labeled. **(C)** ~~Neuralynx~~Electrophysiology system. **(D)** Dell docking station that has the parallel port and ethernet port. **(E)** UPS for ~~Neuralynx~~electrophysiology system (left) and ~~Eyelink~~eye tracking system (right).

Figure 2. Example ~~Eyelink~~eye tracker camera setup screen. Target marker bounding box, eye bounding box, head distance, pupil, and corneal reflection (CR) should be ~~all in~~marked as green~~.~~ ~~Figure adapted from Eyelink 1000 Plus manual~~ and/or "OK" before proceeding.

Figure 3. Example result. (A) Task. The search cue is presented for 1s, immediately followed by the search array. Participants are instructed to indicate by button press whether the target is present or absent (timeout 14s). Trial-by-Trial feedback is given immediately after button press ('Correct', 'Incorrect', or 'Time Out'), followed by a blank screen for 1-2s. **(B)** Example visual search arrays with fixations indicated. Each circle represents a fixation. Green circle: first fixation. Magenta circle: last fixation. Yellow line: saccades. Blue dot: raw gaze position. Red box: target. **(C-F)** Single neuron examples. **(C-D)** Button-press-aligned examples. **(C)** Neuron that increased its firing rate for target-present trials, but not for target-absent trials. **(D)** Neuron that decreased its firing rate for target-present trials, but not for target-absent trials. Trials are aligned to the button-press (gray line), and are sorted by reaction time. Black lines represent the onset and offset of the search cue (1s duration). The inset shows waveforms for each unit. Asterisk indicates a significant difference between target-present and absent trials in that bin ($P < 0.05$, two-tailed t-test, Bonferroni-corrected; bin size = 250 ms). Shaded area denotes ±SEM across trials. **(E-F)** Fixation-aligned examples. t=0 is fixation onset. **(E)** Neuron that increased its firing rate when fixating on targets, but not distractors~~.~~ (the same neuron as **(C)**). **(F)** Neuron that decreased its firing rate when fixating on targets but not distractors~~.~~ (the same neuron as **(D)**). Fixations are sorted by fixation duration (black line shows start of the next saccade). Asterisk indicates a significant difference between fixations on targets and distractors in that bin ($P < 0.05$, two-tailed t-test, Bonferroni-corrected; bin size = 50 ms). ~~Figure~~This figure has been modified with permission from[8].

Movie 1. Typical trials of visual search with responses from a single target neuron. In target-present trials, this neuron increased its firing rate regardless of the identity of the cue. Yellow dot denotes eye position. Yellow vertical bars at the bottom are event markers (i.e., cue onset, array onset, and ~~IT~~inter-trial-interval onset). Red vertical bars at the bottom show spikes, which are also played ~~in~~as sound. The red dotted box ~~in~~denotes the location of the search ~~array denotes search~~ target~~, which is~~ (not shown to participants~~.~~).

DISCUSSION:

In this protocol, we described how to employ single-neuron recordings with concurrent eye tracking and described how we used this method to identify target neurons in the human MTL ~~that signaled the goals of the ongoing visual search~~.

The setup involves three computers: one executing the task (stimulus computer), one running the eye tracker, and one running the acquisition system. To synchronize between the three systems, the parallel port is used to send TTL triggers from the stimulus computer to the electrophysiology system (**Figure 1C**). At the same time, the stimulus computer sends the same TTLs using an ethernet cable to the eye tracker. The stimulus computer should have a parallel port on its docking station in the example shown (**Figure 1D**), or alternatively, have a PCI Express parallel port card or a similar device.

The mobile cart for the stimulus computer and eye tracker with the flexible arm attached allows flexible positioning of the screen in front of the patient (**Figure 1A,B**). The usage of a UPS to power the devices on the cart is strongly suggested to eliminate line noise introduced into the electrophysiological recordings due to the close proximity of the eye tracking devices to the patient's head (**Figure 1E**). Furthermore, laptops running on battery power should be used as stimulus computer and eye tracker computer.

If the recordings are contaminated by noise, the eye tracker should be removed first to assess whether it is the source of noise. If not, standard procedures should be used denoise before using the eye tracker again[2]. Note that typical sources of line noise include the patient bed, IV devices, devices in the patient room, or ground loops created by using different plugs for different systems. If the eye tracker is the source of the noise, all devices (in particular the camera, light source, and LCD screen) should be powered from battery and/or UPS. If there is still noise, it is likely that the LCD screen and/or the power supply for the LCD screen of the eye tracker is faulty. A different screen / power supply should then be used. If at all possible, an LCD screen with an external power supply should be used. It is also important to ensure that the TTL cable does not introduce noise (i.e., use a TTL isolator).

The significance of recording single-neuron data in neurosurgical patients simultaneously with eye tracking is high for several reasons. First, single-neuron recordings have high spatial and temporal resolution, and thereby allow the investigation of fast cognitive processes such as visual search. Second, they provide a much needed link between human cognitive neuroscience and animal neurophysiology, which relies heavily on eye tracking. Third, because human single-neuron recordings are often performed simultaneously from multiple brain regions, our approach permits ~~a~~the temporal resolution that will help distinguish between visually driven vs. top-down modulation from frontal cortex. In summary, single-neuron recordings with eye tracking make it possible to isolate specific processes that underlie goal-directed behavior. In addition, our concurrent eye tracking permitted fixation-based analysis, which greatly increased statistical power (e.g., **Figure 3A, B** vs. **Figure 3C, D**).

A challenge of this method is that the eye tracking system may introduce additional noise into the electrophysiological data. However, with the procedures outlined in this protocol, such additional noise can be eliminated, and once these procedures are established, they can be ~~done~~executed routinely. Furthermore, eye tracking lengthens the time needed for a given experiment because additional setup is required, especially when calibration of the eye tracker is challenging for some patients, in particular those with small pupils or glasses. However, the benefits from simultaneous eye tracking are worth this additional effort for a number of studies, making eye tracking a valuable addition to single-neuron recordings.

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DISCLOSURES:
The authors declare no conflict of interest.

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