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Conformal Ceramic Electrodes That Record Glutamate Release and Corresponding Neural Activity in Primate Prefrontal Cortex*

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Abstract— **Conformal ceramic electrodes utilized in prior recordings of nonhuman primate prefrontal cortical layer 2/3 and layer 5 neurons were used in this study to record tonic glutamate concentration and transient release in layer 2/3 PFC. Tonic glutamate concentration increased in the Match (decision) phase of a visual delayed-match-to-sample (DMS) task, while increased transient glutamate release occurred in the Sample (encoding) phase of the task. Further, spatial vs. object-oriented DMS trials evoked differential changes in glutamate concentration. Thus the same conformal recording electrodes were capable of electrophysiological and electrochemical recording, and revealed similar evidence of neural processing in layers 2/3 and layer 5 during cognitive processing in a behavioral task.**

I. INTRODUCTION

The excitatory inputs from glutamate neurotransmission have been implicated to be intimately involved in learning and memory. The human nonhuman primate NHP model of cognition is associated with not only with working memory, but also executive decision-making in the form of selection of the appropriate motor response[1-3]. In the NHP, glutamate antagonists disrupt the performance of animals in cognitive tasks such as delayed-match-to-sample (DMS) showing the direct involvement of glutamate in working memory[4;5]. Single unit recordings demonstrate alterations in the firing rate in the frontal cortex of nonhuman primates during the performance of the DMS task[2] and that those firing patterns were essential to task performance[1;3]. We therefore hypothesized the DMS task would evoke glutamate release within layer 2/3 of the NHP frontal cortex related to the neurophysiological recordings obtained in the same task.

II. METHODS

A. Animal: Training & Surgery

All procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Wake Forest University, and performed in accordance with established practices as described in the National Institutes of Health and Public Health Service policy on humane care and use of laboratory animals. Five adult male rhesus (Macaca mulatta) NHPs (6-15 kg) housed in stainless steel cages in temperature and humidity controlled colony rooms with lighting maintained on a 0600:1800 day/night cycle and visual contact with conspecifics at all times except during experimental sessions[1;6;7]. Animals were fed a daily diet of NHP chow supplemented by fresh fruit and chewable multiple vitamin tablets.

Behavioral Apparatus and Training: NHPs were trained to sit quietly in a custom designed primate chair (Crist Instruments, Hagerstown, MD) configured for free arm movement across a horizontal table attached to the primate chair and positioned 1.5 meters from a LCD-front-projection[1;7;8]. Animals were trained to move a cursor on the projection screen by positioning their right hand (Tracked via Plexon, Inc CinePlex tracker) within a two dimensional coordinate system on a table surface attached to the chair. Behavioral responses consisted of placing the cursor onto 25 cm clip-art images projected onto the video screen from an experimental control computer. Behavioral responses were reinforced with a 0.5 ml sip of juice deliver to a tube at the animal's lips.

Multi-Item Visual Delayed-Match-to-Sample (DMS) Task: A previously utilized delayed-match-to-sample (DMS) short-term memory task with different degrees of “cognitive load” or task difficulty for NHPs [1;2;6] was employed to test the effects of baclofen on cocaine induced cognitive deficits. Trial events consisted of a Start Signal, Sample phase, Delay and Match phase; each trial was varied as to 1) the number of individual clip-art distracter images (1-6) randomly presented in the Match phase and 2) the duration of the interposed Delay interval (1-30s). All combinations of delay and #

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images were presented randomly in normal daily testing sessions and all images were unique for all trials within the session[1;3;9]. Two types of trials were presented: Object or Spatial, presented randomly as signaled by the trial Start Signal image if the Start Signal was a circle it indicated that the subsequent object in the Sample image (i.e. the image itself) was to be recalled and selected in the Match phase of the trial irrespective of where it appeared on the screen; if the Start Signal image was a square it meant that the spatial position of the Sample image on the screen during the Sample phase was to be recalled and selected in the Match phase irrespective of what Object-based image occupied that location on the screen in the Match phase. Subjects performed 100-200 trials daily, with object/spatial trial type, delay and # of images determined independently and randomly for each trial. Clip art images were not repeated (other than Sample images included in Match phase) within or across daily sessions.

Surgery: Animals were surgically prepared with cranial access cylinder for attachment of microelectrodes over the specified brain regions of interest. During surgery animals were anesthetized with ketamine (10 mg/kg), then intubated and maintained with isoflurane (1-2 % in oxygen 6 l/min). Craniotomy sites were selected to overlie the stereotaxic coordinates for Frontal Cortex (25 mm anterior to Interaural line and 12 mm lateral to midline/vertex) in the caudal region of the Principal Sulcus, the dorsal limb of Arcuate Sulcus in area 8 and the dorsal part of premotor area 6 (Fig. 1C), areas previously shown by PET imaging to become activated during task performance[6]. The scalp was incised and retracted over the frontal and parietal lobes (caudal from the brow ridge) with craniotomies (17 mm diameter) cut into skull over the recording sites. Recording cylinders (Crist Instruments, Hagerstown, MD) were sealed to the skull and cemented over the craniotomies for electrode access[1;8]. Two titanium posts were secured to the skull caudal to the cylinders, for head restraint. Titanium steel screws (n=15-20) were placed in the skull around the cylinders and posts, and embedded in bone cement to secure the fixtures to the skull.

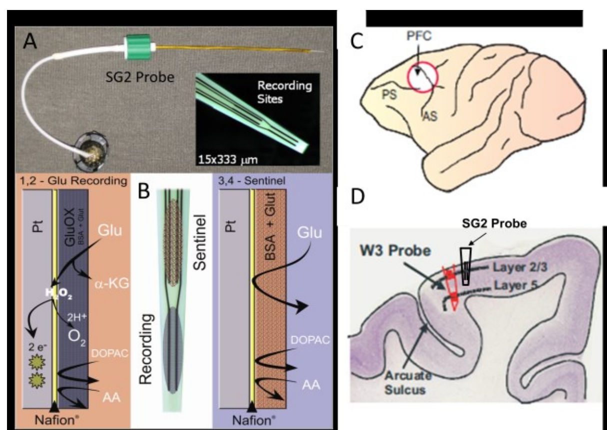

Figure 1: Schematic of ceramic recording probe and recording site. A. Ceramic microelectrode array (MEA) with four recording sites (B). Distal recording site was coated with glutamate oxidase (GluOx) and Nafion anion exclusion layer. Proximal (sentinel) reference sites were coated with Nafion only. C. Placement site for craniotomy access cylinder over prefrontal cortex (PFC) of nonhuman primate. D. SG2 ceramic MEA was inserted perpendicular to PFC Layer 2/3. W3 ceramic MEA used for electrophysiological recording spanned layers 2/3 and layer 5, and is shown for comparison.

B. Neural Activity and Glutamate Recording

Customized ceramic electrodes, designed and manufactured in collaboration with Dr. Greg Gerhardt (Center for Microelectrode Technology – CenMet, Lexington, KY) at the University of Kentucky[10-12], consisted of etched printed circuit platinum recording pads (Fig. 1A&B), were employed for recording of glutamate electrochemistry as well as multiple single neuron activity[8;12]. Fig. 1A shows the CenMet SG2 microelectrode array (MEA) consisting of four recording sites (15 x 333 µM each) with a 7 cm polyimide shank which was inserted stereotaxically with recording sites in cortical layers 2/3. Prior to insertion, MEAs were dip coated with Nafion®, to exclude anions from the recording surface. Dorsal recording sites (Sentinel) were coated with BSA + glutaraldehyde to record current generated from any sources not excluded by the Nafion® coating (Fig. 1B. Ventral recording (active) sites were coated with Glutamate oxidase and BSA + glutaraldehyde to record glutamate concentration via enzymatic production of H_2O_2. For second-by-second glutamate measurement, a +0.7V (50 ms) potential was applied to the MEA once per second and referenced to a Ag/AgCl reference electrode outside the dura. The oxidation of H_2O_2 by the pulse results in a relaxation current proportional to free glutamate concentration at the electrode[13-15].

For electrophysiological recording, extracellular action potentials were isolated and analyzed via Plexon, Inc. Multineuron Acquisition Processor, and synchronized with DMS trial events. The CenMet W3 configuration probe was specially designed such that the top 4 recording pads recorded activity from neurons in the supra-granular layer 2/3 while the lower set of four pads simultaneously recorded neuron activity in the infra-granular layer 5 (Fig. 1D)[12;16-19].

Data Analysis: Glutamate electrodes were prepared fresh each day and calibrated using fixed glutamate concentration solutions prior to each experiment. Basal glutamate current was recorded once per second and converted to glutamate concentration via the calibrations performed prior to the experiment. Tonic glutamate concentration and phasic glutamate release were all analyzed in relation to the phase of the DMS task. Phasic release was assessed in terms of number/frequency of transient glutamate release events (defined as >10% increase in glutamate concentration with <5 sec duration) as well as peak glutamate concentration recorded during the release event.

DMS events were sorted according to type of trial (spatial vs. object, Fig. 2A), delay and # of images in the Match phase. Behavioral performance, tonic and phasic glutamate concentration and neural activity recording from prefrontal electrodes in separate sessions were analyzed according to trial parameters and phase of trial. Statistical comparisons were performed using multifactor ANOVA.

III. Results

Ceramic MEAs indentified as "Spencer-Gerhardt type-2" were inserted into the Prefrontal Cortex near the junction of Arcuate Sulcus and Principal Sulcus (Fig. 1C), an area that encompasses Brodman's Area 6(dorsal premotor), Area 8 (frontal eye fields) and area 46 (dorsolateral prefrontal).

MEAs were configured with four recording sites (15 x 333 µM) and a 7 cm polyimide shank, dip coated on all four sites with Nafion®, an anion exclusion layer (Fig. 1B). Dorsal recording sites (Sentinel) were coated with BSA + glutaraldehyde. Ventral recording sites were coated with Glutamate oxidase and BSA + glutaraldehyde. The GluOx coating allows the ventral pads to be sensitive to glutamate release through the enzymatic production of H_2O_2. A +0.7V potential applied to the MEA vs. a Ag/AgCl reference electrode oxidizes H_2O_2 resulting in a current that is directly related to the glutamate concentration.

Fig. 2 shows the sequence of events in the DMS task: 1) presentation of Start "'Ring" to initiate the trial, 2) "Sample Target" image, followed by cursor movement into the image as the "Sample Response", then 3) a variable 'Delay' period of 1-60 sec with the screen blank, finally 4) the "Match" phase in which the Target (Sample image) was accompanied by 1-6 other Non-match (distracter) images on the same screen. Correct response was determined by the shape of the Start Ring – Object trials required a match to the same image as the Sample, while Spatial trials required a match to the same position as the Sample.

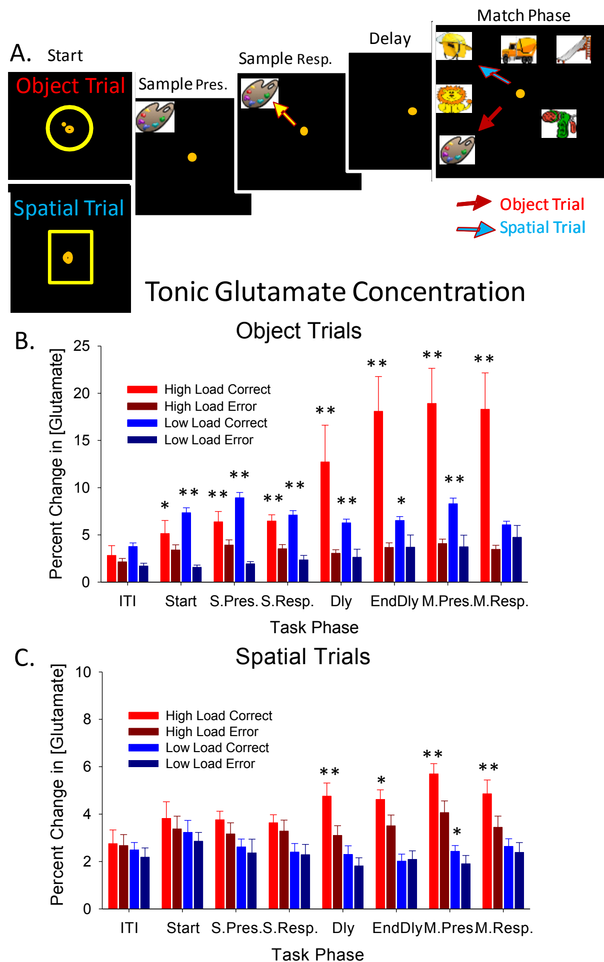

Figure 2: Glutamate concentration during phases of the DMS task. A. Phases of the DMS task, including Object vs. Spatial trial contingency. B. Mean (± S.E.M.) glutamate concentration on Object trials. C. Mean glutamate concentration on Spatial trials. DMS trials were sorted by Correct vs. Error behavioral responses and by cognitive load (difficulty). High load trials included 5-7 Match phase images and 20-40 s delays; Low load trials included 2-4 Match phase images and 1-20 s delays.

Tonic (steady-state) glutamate concentrations in the NHP prefrontal cortex collected over 7 recording sessions in four different animals are shown in Fig. 2B&C. An average basal glutamate concentration of 8.694 ± 0.773 µM was observed over all trials and conditions. Bargraphs in Fig. 2B show that tonic glutamate concentrations were significantly elevated on all phases of Correct vs. Error Object trials, with even greater increase in Glutamate during Delay and Match phase on trials labeled as High Cognitive Load (5-7 distracter images and 20-40 s delays vs. Low load trials with 2-4 images and 1-20 s delays). Glutamate concentration was significantly elevated on Spatial trials only on High load trials during Delay and Match phases. Significance was determined by a one-way ANOVA with Bonferroni's post-hoc tests (Correct vs. Error: *p <0.01, **p<0.001).

The average number of transient glutamate release events was detected and analyzed relative to the phase of the trial, and likewise sorted by behavioral outcome and cognitive load. Fig. 3 shows the cumulative number of glutamate release events per second for Object (A) and Spatial (B) trials. Object trials showed more frequent glutamate release during Sample phase for Correct and Error trials, irrespective of cognitive load, however, only Correct trials exhibited increased glutamate release durign Match phase. Spatial trials exhibited more frequent glutamate release during Start and Match phases (and not Sample), although on Correct trials only for both phases. Asterisks indicate significant difference between Correct and Error trials as determined by a one-way ANOVA with Bonferroni's post-hoc tests (*p <0.01, **p<0.001).

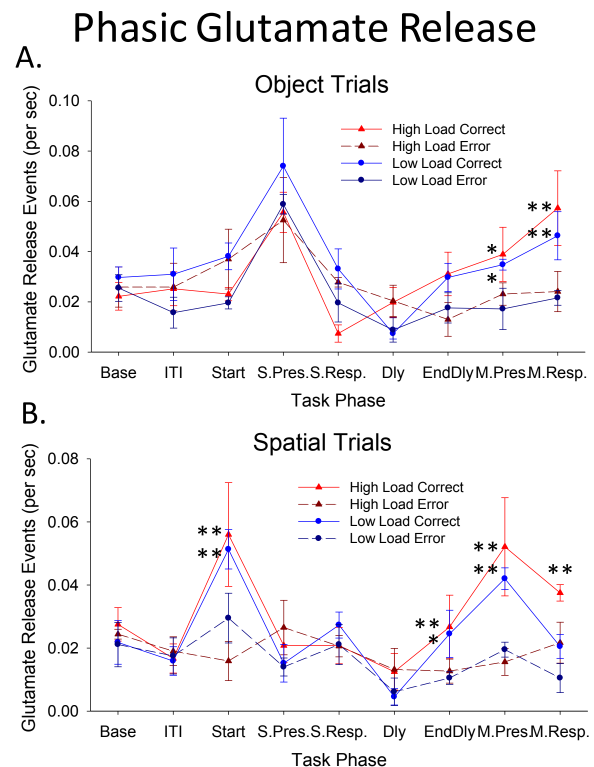

Figure 3: Frequency of phasic glutamate release events for DMS Object and Spatial trials. A. Mean (± S,E,M,) frequency of glutamate release events averaged across animals and sorted by DMS trial phase, behavioral outcome (Correct vs. Error) and cognitive load. B. Mean frequency of glutamate release events for Spatial trials.

IV. CONCLUSIONS

Electrophysiological recordings of single neurons and ensembles from layer 2/3 and layer 5 of PFC demonstrate task-dependent firing during the Match phase that correlates with correct vs. error performance of the task[1]. Furthermore, the firing patterns of these neurons reveal processing within cortical minicolumns[8] that distinguishes Object from Spatial trials[3]. Thus, the same ceramic MEA utilized for neurophysiological recording in the above studies were configured to electrochemically record glutamate concentration and transient release in prefrontal cortical cell layer 2/3.

Glutamate concentration significantly increased during DMS trials on the same task phase(s) that showed the greatest change in neural firing. Glutamate release events also increased during Sample indicating neural activity that may not have been captured by the ensemble recordings.

However, it is important to note the differences in firing on Object vs. Spatial DMS trials. Object trials showed increased glutamate release events during Sample, while Spatial trials did not. Further, although Spatial trials exhibited Start phase release, it was only on correct trials. These differences in glutamatergic activity may help explain the demonstrated lower behavioral success rate on Spatial vs. Object trials[2;3].

These results demonstrate that the same ceramic MEAs can be used for both electrophysiological and electrochemical recording. Further, the combination of both recording modes reveals more information regarding prefrontal processing of cognitive tasks than revealed by either type of recording alone.

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