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A NOVEL TETRODE MICRODRIVE FOR SIMULTANEOUS MULTI-NEURON RECORDING FROM DIFFERENT REGIONS OF PRIMATE BRAIN

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Abstract

A unique custom-made tetrode microdrive for recording from large numbers of neurons in several areas of primate brain is described as a means of assessing simultaneous neural activity in cortical and subcortical structures in nonhuman primates (NHPs) performing behavioral tasks. The microdrive device utilizes tetrode technology with up to six ultra-thin microprobe guide tubes (0.1 mm) that can be independently positioned, each containing reduced diameter tetrode and/or hexatetrode microwires (0.02 mm) for recording and isolating single neuron activity. The microdrive device is mounted within the standard NHP cranial well and allows traversal of brain depths up to 40.0 mm. The advantages of this technology are demonstrated via simultaneously recorded large populations of neurons with tetrode type probes during task performance from a) primary motor cortex and deep brain structures (caudate-putamen and hippocampus) and b) multiple layers within the prefrontal cortex. The means to characterize interactions of well-isolated ensembles of neurons recorded simultaneously from different regions, as shown with this device, has not been previously available for application in primate brain. The device has extensive application to primate models for the detection and study of inoperative or maladaptive neural circuits related to human neurological disorders.

Keywords

Tetrode for monkey; new technologies & innovations; simultaneous recordings; multi brain areas; motor cortex and striatum

INTRODUCTION

In order to record single neuron activity in the brain of nonhuman primates (NHPs) during behavior specially designed probes must be inserted over relatively large distances to isolate and record functional activity from individual cells in related brain areas. Such procedures have been employed successfully in the past (Evarts, 1960) (Andersen et al., 2010; Lebedev et al., 2008) (Crist and Lebedev, 2008), however, probe size and placement utility has been a

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major factor limiting reliable multiple single unit recording from primate brain. Current technology utilizing small (~0.02mm) probes that allow recording of up to 3 neurons per wire, as utilized in rodents, has been utilized in NHPs (Pezeris, 1998); (Aronov et al., 2003); (Sakurai and Takahashi, 2006) (Sakurai et al., 2004); (Skaggs et al., 2007); (Aronov et al., 2003); (Feingold et al., 2011). However, to date no device with the capability to record multiple isolated single neuron activity simultaneously from two or more structures in NHPs has emerged. This paper describes a technique for inserting several independent recording probes (tetrodes and hexatodes) in primate brain allowing simultaneous access to multiple (e.g., cortical and subcortical) brain areas to assess single neuron firing while maintaining minimal tissue damage and allowing repeated access.

Although tetrode recording technology of has been successfully used in rodents for nearly twenty years (Gray et al., 1995; Nguyen et al., 2009), application to the NHP has not been as successful due to several factors that must be overcome to implement localized placement of multiple probes in the larger primate brain. As demonstrated here, a new uniquely designed tetrode-microdrive device was successfully constructed and tested (Santos et al., 2010) in well established neurobehavioral contexts (Hampson et al., 2004; Hampson et al., 2011; Opris et al., 2009) to accomplish these objectives. The device provides the means to access neural firing in specific regions identified in imaging procedures (Porrino et al., 2005) without constraints due to positioning, depth of structure or number of loci within specific brain areas. We show here data obtained with the device in single sessions consisting of relatively large numbers of well-isolated single neurons (n=20–50) recorded simultaneously from multiple cortical as well as multiple subcortical regions while NHPs performed a visuomotor short-term memory task (Hampson et al 2004, Hampson et al 2011). This innovative technology provides a basis for advanced understanding of neural systems in the human brain in which task-related activity of multiple neuron populations in cortical regions can now be related to previously unobtainable *simultaneous* multiple subcortical recordings of the same type. Moreover, since there are well established functional links between cortical cell layers and different subcortical areas in primate brain, another capability of the device described here is to record simultaneous neural activity across layers within the same cortical regions.

MATERIALS AND METHODS

NHP tetrode recording microdrive

Figure 1A–E illustrates the major components of the NHP tetrode microdrive. In Fig 1A the entire device is shown with details of individual components of the device described in Figures 1B, 1C and 1D. Brain regions recorded with the microdrive device can be reached by electrodes of different lengths mounted in the same drive (Fig.1A) as determined by vertical depth and dimensions of the standard size primate skull chamber (Fig.1C). Figure 1 shows the maximum number of individual probes of different lengths (n=6), that can be arranged in the skull chamber in a customized manner (Fig 1B), with tetrodes and guide tubes (Fig 1C) arranged for insertion as in prior recording sessions where multiple placements were performed in the same and different brain regions (Fig 1D). Breakthrough advantages of this technology compared to prior descriptions of tetrode applications in NHPs include: (1) recordings from multiple brain regions at various depths simultaneously because every tetrode can be positioned independently (Fig 1A, b: 1–7), (2) use of smaller (0.3 mm) guide tubes (Fig 1B, inset z) does less brain damage; (3) the smaller recording probes (0.01–0.02 mm) can record smaller cells and lower amplitude signals like in rodents (Fig 2 A–D). Finally the tetrode microdrive does not utilize large guide tubes to puncture the dura mater (Fig 1A.d) as reported in the past (Pezeris, 1998); (Onken et al., 2009; Skaggs et al., 2007) providing for less brain damage with repeated use in the same animals.

Impedance of tetrode and hexatrode wires

Hexatrades and tetrodes are used in the microdrive device described here (Figure 1E). Tetrode technology provides a density of low impedance wires at the same recording location to increase the signal-to-noise ratio and allow isolation of relatively small-amplitude extracellular spikes from more cells than other types of recording probes (Ferguson et al., 2009); (Loeb et al., 1995); (Gray et al., 1995); (Feingold et al., 2011). Two types of VG bond coated wires (Stablohm 675, (Nichrome), Annealed, HML, and Platinum 10% Iridium), with diameters ranging between 17 and 25 μm (mean impedance $\sim 100\text{ K}\Omega$, Supplementary information) were utilized for tetrode and hexatrode recording. The tetrode wires used here (17–25 μm) have an impedance of 1.0–1.5 $\text{M}\Omega$ before plating, and after plating with an automatic electroplate device (NanoZ, Neuralynx, Bozeman, MT) impedances can be decreased to approximately 0.1 $\text{M}\Omega$ (Supplementary information). Electrodes of the same diameter employed for signal reference can also be placed in separate brain regions with the same drive. A standard 20 pin Neuralynx connector was utilized in which 16 pins were attached to amplifier channels. A 64-multichannel acquisition processor (MAP Spike Sorter by Plexon, Inc. Dallas, TX) was employed for tetrode and hexatrode wire recordings with statistical cluster dissection principal component analysis (PCA) in 2D/3D via standard parametric multivariate analyses of variance (MANOVA) procedures (Nicolelis et al., 2003). Perievent histogram (PEHs) and cross-correlation analyses, utilized NeuroExplorer (Nex Technologies, Littleton, MA) software and MATLAB (The Mathworks, Inc, Natick, MA) routine codes.

Implantation of probes with microdrive device

Skull attached recording chambers (20mm outer diameter) were all previously implanted for over at least one year in NHPs used to test the tetrode microdrive device. Surgical procedures followed IACUC rules and NIH guidelines as reported in prior publications (Hampson et al., 2004)(Opris et al., 2010). Skull chambers (Crist Instruments) were previously positioned for recording with other types of recording devices (Opris et al 2009) from multiple brain areas including prefrontal cortex (PFC) in one hemisphere and hippocampus in the other, with the center at Bregma coordinates +03 and –09 mm (Paxinos et al., 2008) respectively. On the day of recording, the entire microdrive with arranged probes was sterilized according to a standard protocol (Nguyen et al., 2009) (Santos et al., 2010) (Opris et al., 2009) and the exposed dura micro-pierced for insertion of guide tubes. The microdrive with guide tubes extending (Fig. 1A.d) was positioned in the well and the tetrodes lowered via standard tetrode screw drives (Fig. 1A.d3). The tetrodes used for signal reference were inserted above cortical cell layer 2/3 overlaying regions from which depth recordings were made. All surgical and recording procedures were approved by Wake Forest University and Institutional Animal Care and Use committee (IACUC).

Ensemble Recordings and Analysis

A 64-multichannel acquisition processor (MAP Spike Sorter by Plexon, Inc. Dallas, TX) with a 16-channel headstage (Neuralynx ®) was used to provide the first level of amplification of the brain neural signals. The MAP (and MAP cluster) provided further amplification and band pass filtering (500 Hz to 5 KHz) to the recordings. Filtered analog signals were routed to digital signal processor (DSP) boards each of which contained four 40 KHz DSPs (Motorola 56002). A multivariable statistical analysis (MANOVA) was used for calculating cluster separation as shown in Fig 2 A–D for cells recorded in four different NHP brain regions. Both two and three dimensional (2D & 3D) principal components (PC) space (Davies and Holdsworth, 1979) are shown where waveform peak-to-peak voltage amplitudes are displayed as clusters in 2D and 3D space (Wheeler, 1999). For cluster separation validation a distance-based algorithm used the Davies Bouldin (BD) index (Davies and Bouldin 1979, Nicolelis et al., 2003)

$$DB = \frac{1}{2} \sum_{i=1}^n \max_{i \neq j} \left\{ \frac{S_n(Q_i) + S_n(Q_j)}{S(Q_i, Q_j)} \right\}$$

where n is the number of clusters, S_n is the average distance of all objects from the cluster to their cluster centre, $S(Q_i, Q_j)$ is the distance between cluster centers. Consequently, if the ratio is small the clusters are compact and far from each other, so that the DB index will have a small value for good clustering

(http://machaon.karanagai.com/validation_algorithms.html). The average DB of those four group of cells recorded in Figure 2A–D (M1, CPu, Hip, PFC) was 0.2 ± 0.1 indicating good segregation of cells recorded with the same tetrode or hexatrode. Figure 2 (right) shows a comparison of isolated spike occurrences from different neurons recorded in the same session, with either a tetrode (upper) or hexatrode (lower) with tips placed in layer 5 in the same region of PFC.

Animals and Behavioral Task

Testing with the microdrive device was performed on 4 adult (rhesus, *Macaca mulatta*) NHPs used in other studies (Opris et al 2009, Hampson et al, 2011). As shown in Figure 3 (middle) prior placement of bilateral skull recording chambers in each animal provided access to motor cortex, hippocampus, and striatum in one hemisphere or different layers of prefrontal cortex (PFC) in the other hemisphere (Hampson et al., 2004; Opris et al., 2010). All recordings were conducted while NHPs performed a well-characterized visuomotor delayed match to sample (DMS) task (Porrino et al., 2005, Deadwyler et al., 2007) in which animals sat in a chair in front of a display screen and moved a cursor with their right arm to perform visually guided selection of items required by the DMS task (Figures 3C). Hand coordinates (Y, X and velocity) of movement position associated with the events (Figure 3D) were recorded simultaneously with neuron firing and synchronized using previously established methodology for cell and behavioral recordings (Fetz, 2007; Moritz et al., 2008, Nicolelis, 2003, Nicolelis et al., 2003), (Hampson et al., 2004). All animals were trained to a stable baseline performance level of 70–80% correct over all trials with different numbers of images and delay duration reflecting task difficulty (Hampson et al., 2009); (Opris et al., 2010). Mean firing rate for each neuron was analyzed in perievent histograms (PEHs) consisting of 25ms time bins for ± 2.0 sec surrounding each task event (e.g., image presentations and behavioral responses). To show that neurons were independently recorded cross-correlation histograms were examined for each tetrode pair (Figure 4). Statistical assessments utilized analysis of variance (ANOVA) and principal component analysis (PCA) for cluster separation.

RESULTS

A detailed description of the microdrive device designed for multi-neuron depth recording in NHPs is presented in Figure 1. Two configurations of the microdrive were implemented in the experiments described here; in Figure 1A–C the drive was set up to record in 3 different brain regions simultaneously, primary motor cortex (M1), dorsal caudate-putamen (CPU) and hippocampus (Hipp) as diagramed in Figure 1D. A second configuration of the microdrive was employed in the same animals for higher resolution positioning of probes to record from different cell layers in the same region of prefrontal cortex (PFC) accessed through a separate cranial well (Figure 1D). Separate skull cylinders allow implementation of the same microdrive configured in different electrode formats to access multiple regions of interest at different times in the same animal. The microdrive guide tubes for the recording probes (Figure 1Ei, ii) contained tetrode or hexatrode wires arranged to fit

compatibly into the skull cylinders implanted over targeted brain areas (Figure 1B&D). Figures 1B&C show the extension of the tetrodes from the guide tubes that pass the tetrodes through the dura (dotted line). The inset (z) in Figure 1C shows microwires extended at different lengths to illustrate the size of individual wires. All electrode wires were plated, which produced final average impedances of $0.110 \pm 0.03 \text{ M}\Omega$ (*Supplementary Information*).

An average ($\pm \text{SEM}$) of 52.2 ± 1.2 simultaneously recorded single neurons (836 total, 350 PFC, 82 CPU, 56 M1 and 348 Hipp) were collected over 16 sessions from four different animals during performance of the visuomotor DMS task. Figure 2 shows representative examples of the quality of single neuron waveforms recorded from individual wires of a tetrode bundle placed in each brain region (Figures 2 A–D). The classification of single units was made based on cell waveforms with at least 99.9% of the refractory period greater than 1.6 ms (Figure 2 A, A1; B, B1; C, C1; D, D1) in addition to shape and waveform-cluster differentiation (Nicolelis et al., 2003), (Hatsopoulos et al., 2004). Classification was also validated by statistical tests (MANOVA) which confirmed the cluster separations of cells in each area: motor cortex M1: $F(2,39) = 34.8$, $P < 0.001$; caudate-putamen CPU: $F(4,20) = 16.0$, $P = 0.002$; hippocampus Hip: $F(8,65) = 20.1$, $P = 0.01$; prefrontal cortex PFC: $F(4,43) = 21.7$, $P = 0.001$. In addition overlap of waveforms (Figure 2 A, B, C, D) and the cluster separation of cells are shown (Figure 2 A.1, B.1, C.1, D.2) for individual/particular firing characteristics as indicated by the Davies Bouldin (BD) index (mean of all regions: 0.25 ± 0.08). Spontaneous action potentials from the same neurons (Figure 2 A = M1; B = CPU; C = Hip; D = PFC) are shown below each quadrant. *Supplementary Information* provides videos of cell firings used for cell identification. The stripcharts in Figure 2 (right) show simultaneous recordings of identified neurons in the same PFC layer 5 recorded in the same session with a tetrode (upper) and a hexatrode (lower) probe.

To highlight the versatility of this technology perievent histograms (PEHs) of cells are shown that were recorded simultaneously, in motor cortex, putamen and hippocampus (Figure 3A), or in prefrontal cortical layers 2/3 and 5 (Figure 4B) in different NHPs respectively, during performance of the DMS task. PEHs depict firing in the match phase of the task (Figure 3C&D), the period of the trial in which selection of the image presented in the prior Sample phase is chosen from 1–7 simultaneous distracter images (Hampson et al., 2010). The locations of the recordings in the brain were determined based on prior studies (Opris et al., 2005) in terms of stereotactic coordinates, cortical landmarks observed during surgery, PET scanned metabolic and high-resolution digital RX (MRIs) imagery. Simultaneous recordings were obtained from motor cortex (MI: $F(1,19) = 17.78$; $p < 0.001$, ANOVA), caudate-putamen during pre-Match phase (CPU: $F(1,19) = 37.52$; $p < 0.001$) and hippocampus (HIP: $F(1,19) = 40.67$; $p < 0.001$) during the same Match phase of the task (Figure 3A). In other recording sessions from dorsolateral prefrontal cortex (PFC), primarily the frontal eye fields (FEF), showed significant differences (FEF: $F(1,19) = 6.044$; $p < 0.05$, ANOVA) across regions during the pre-Match phase epoch (Figure 3B).

Another novel feature of the tetrode microdrive device is the capability to record functionally different cells simultaneously within the same cortical and/or subcortical structures. Figure 4A displays multiple cells ($n=8$) recorded simultaneously in “motor” areas during each phase of the DMS task with the same microdrive configured for tetrodes placed in primary motor cortex (M1, $n=4$) and putamen (CPU, $n=4$) of NHPs (Santos et al., 2011). Cell firing was modulated differentially within the same structures, but across structures firing was characteristically different and modulated in different ways during execution of the behavioral responses (Fig 4A, 0.0s, vertical line). The opposite unique feature of the microdrive is illustrated in a different animal and DMS session in Figure 4B in which 8 cells were recorded simultaneously by two separate tetrode probes positioned adjacent to each other, one in layer 2/3 (upper) and the other in layer 5 (lower) within the same AP/DL

segment of PFC. It is also clear from Figures 4A&B that there were differences between cells with respect to modulations by behavioral events in the various task phases ($p < 0.001$; ANOVA), likely indicating differential operation of the neural circuits that could reflect 1) columnar processing across different cell layers within the PFC as shown previously (Opris et al., 2010; Takeuchi et al., 2011), or 2) in the case of M1 and CPu reciprocal firing consistent with the role of the basal ganglia and motor cortex in the initiation and execution of target related movements (DeLong and Wichmann, 2007) (Graybiel, 1996). To validate the fact that the recordings were from different cells, cross-correlation histograms (CCHs) between all possible pairings of the same 4 cells recorded with the same probe, over the same indicated interval of the task phase (Figure 4A&B ± 2.0 s) are shown for the indicated PEHs. Firing patterns within the events that appeared different between individual cells were verified by significant differences in CCHs between of the same cells ($p < 0.001$) even though all cells in each case were recorded by the same probe.

DISCUSSION

A new microdevice for simultaneously recording from cortical and subcortical regions of the primate brain is reported. This new technology not only allows access to a large number of separate structures simultaneously but also subareas within those structures as shown in Figure 4 for PFC. Although tetrode technology has been developed for application to NHPs in other laboratories (Pezeris, 1998) (Ohiorhenuan et al., 2010; Santos et al., 2010; Skaggs et al., 2007) (Jog et al., 2002) this is the first report that such technology can be implemented to record simultaneously from both cortical and subcortical structures in primate brain. Prior attempts to apply this technology encountered the following difficulties and barriers: (i) toughened dura mater of primates (ii) long distances required for probe travel, each of which has been overcome by the design of the microdrive device reported here. More recently, another technique has been reported (Feingold et al., 2011) for simultaneous extracellular recording has shown excellent maneuverable for depth recordings. However, the leads (0.4–0.6 mm) used for guiding the recording probe, and the size of the probes (~ 0.12 mm) whose impedance ranges between 1–2 M Ω , limit its capacity for recording number of cells (~ 0.3) per wire. In addition, as indicated above, we apply this technology to NHPs performing a visuomotor task where the average number of well-isolated neurons recorded per microwire ($n = 2.1$, SEM ± 0.5 , Figure 2) is slightly more than that reported in other NHP studies (Pezeris, 1998) (Ohiorhenuan et al., 2010; Onken et al., 2009; Skaggs et al., 2007); (Feingold et al., 2011). In addition these accomplishments validate 1) reliable adaptation of the microdrive to the standard NHP skull chamber to allow similar probe placements in the same brain regions on different occasions, and 2) little if any significant damage resulting from penetration with several small recording probes (~ 0.3 mm) containing multiwire (~ 0.02 mm) recording capability (Figure 1). This device therefore, has the potential to characterize neuronal circuitry relevant to normal and disease states in human subjects by recording simultaneously large assemblies of neurons from several different brain regions on different occasions.

Additionally, a practical necessity for NHP recording of this type during behavior is to assess activity from single neurons simultaneously in cortical and subcortical areas in order to strengthen further application of current neuroprostheses for clinical purposes where the number of neurons is a key feature (Wessberg et al., 2000) (Nicolelis, 2001) (Berger et al 2011). From this perspective the rare demonstration of simultaneous recording in cortical and subcortical structures (e.g., M1 and Putamen) in primate brain correlated with behavioral performance (Opris et al., 2011) has direct relevance to the use of the device shown here for achieving accurate functional models for human brain disorders.

Finally, a major feature of this new technology is its applicability for both cortical and subcortical recording in primate brain with very high resolution 17–25 μm diameter probes, which allows placement within the anatomic constraints of functional circuits across relatively large expanses of tissue. Future applications of recording technologies in NHPs as well as in humans, suggest that multi electrode arrays (MEA) that have anatomic conformation will be necessary required for clinical applications. Given the versatility of the tetrode microdrive described here, it will also provide the means to map and construct relevant conformal MEAs that can be utilized for the treatment of system disorders when combined with other stimulation technologies (Berger et al., 2011).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Recording from cortical and subcortical regions simultaneously in behavior primates
- Primary motor cortex, putamen and hippocampus were simultaneously recorded, as well as PFC layers
- New microdrive device for recording from large numbers of neurons in several areas of primate brain
- Device fits within the standard primate craniotomy-access cylinder
- This technology has a wide potential application for study neurological

A novel tetrode-based microdevice for monkeys

Figure 1.

Multi-tetrode NHP microdrive designed for simultaneous recording in multiple primate brain structures. A: Overall construction of the microdevice is illustrated showing all major components configured for insertion. A: 1–3 in red shows the acrylic solid base that provides physical support during positioning of tetrodes with drive screws. Upper components include the connector (A: a.1), the electronic interface board/EIB (a.2), gold connector pins (a.3) that clamp to the recording microwires (0.02 mm). Also shown are the tops of the drive screws that move the wires vertically (A: b.1–b.7) with each complete turn corresponding to 0.28 mm in linear displacement. Each tetrode has one half moon screw (Gray et al., 1995; Nguyen et al. 2009). In the lower part of A is shown the protruding recording tetrode probes (c.1–3) with associated stainless steel guide tubes (0.3 mm outer diameter) and lengths of recording wires extended different distances (~10.0 and ~1.5 mm respectively i and ii). The short 23 gauge cannula (c.1) connects to the system ground. The sections that extend below the dotted line (c2) are the only areas that contact the brain. The lower left inset (Ax) shows the actual size of the microdevice fully loaded and ready to insert with screws that secure the microdrive to the outer rim of the skull well shown in B. Calibration: 5.0 mm, the same for B and C panels. B: View of the bottom housing of the microdevice (Ac). The inset (z) shows one cannula (z.1, ~0.9 mm) with the tiny tetrode wires (z.2) extended for visualization, and this cannula (z3) as part of a microdrive configured for PFC layers recordings. The dotted discontinuous line represents the dura mater limit. C: Zoomed lower view of guide cannulae with their respective tetrodes protruding as shown in B in relation to diameter of standard NHP skull well. D: Reconstruction of electrode locations based on magnetic resonance imaging (MRI) and stereotaxic coordinates (Paxinos et al. 2003) in which medial line of the wells were +3.2mm and -11mm from bregma for PFC and motor cortex/striatum/Hippocampus, the respective right and left wells. Red dots indicate the average tetrode center, and the blue circles indicate the approximate recording areas of each tetrode within a radius of 150 μ m (Buzsaki, 2004). E: Cross section views of: (i) a guide cannula (outer-inner diameter of 0.31–0.15 mm), (ii) tetrode and (iii) hexatrode microwires are displayed. The impedance values are shown in supplementary figure. Calibration: 0.05 mm.

Cluster separation of tetrode and hexatetrode recorded together

Figure 2.

Cluster separation of multiple cell recordings and real-time firing of single neurons with tetrode microdrive device during performance in the DMS task. Left: Cluster separation of single neurons simultaneously recorded from motor cortex (A), putamen (B) and hippocampus (C) in 2 NHPs, or from the PFC (D) in different cell layers in 2 other NHPs while performing the DMS task. Superimposed colored waveforms (A–D) show peak-to-peak amplitude separations of only one wire where at least two well separated clusters of cells were recorded. To the right of each waveform plot the cluster space of spikes of respective waveforms (A.1, B.1, C.1, D.1) shows the cell separation in 3-dimensional space. Below each quadrant (A.2, B.2, C.2, D.2) is shown the action potential occurrence in time of the respective waveforms, dissociated by color and amplitude. Multivariate pseudo F statistic (MANOVA) used for unit separation rectification for which F (D2), J3 (2D) and Davies–Bouldin index (DBi) (D2) values are displayed (A2, B2, C2, D2) for each area/quadrant as follows: A: F = 34.84; J3 = 12.12; DB = 0.12; B: F = 16.0; J3 = 0.26; DB = 0.24; C: F = 20.1; J3 = 17.1; DB = 0.5; D: F = 21.7; J3 = 16.4; DB = 0.2. Right: Comparison of spike trains of prefrontal cortical layer 5 neurons recorded from a single tetrode (top) or hexatetrode (bottom).. Both tetrodes and hexatetrodes discriminated the temporal pattern of neuron spiking (tick marks) from clusters of simultaneously recorded cells (different lines), but the extended capacity of the hexatetrode is shown by additional spike trains indicated by the blue traces at the bottom.

Simultaneous Multiple Tetrode Recording in NHPs Performing DMS Task

Figure 3.

Task-related of neurons recorded with the NHP tetrode microdrive from brain regions indicated in illustration (center) and in Figure 1D, during performance of the DMS task shown in the diagram below. The task consists of 4 main phases: Focus (start trial) Ring (F), Sample (S), Delay (D) and Match (M) with a juice reward delivered for a correct match response (MR) in the M phase (red arrow). A: Perievent histograms (PEHs) bracketing (± 2.0 s) the occurrence of the match response (MR) show simultaneous recordings from neurons in motor cortex (M1, $n=6$), striatum (CPu, $n=6$), and hippocampus (Hipp, $n=6$) all in the same DMS session. Vertical line indicates onset of Match phase of the task. B: PEHs show the associated firing rates of neurons ($n=10$) simultaneously recorded in different layers of PFC in another NHP performing the same task. C: DMS Task parameters: number of distracter images in Match Phase varies randomly from 2–7 across trials with delay interval duration between 10–90; D) trajectory of hand movement during the task. The brain diagram with the corresponding recording locations (purple and green shadows show the tetrode paths in both cylinders implanted over animal's head).

Simultaneous recordings across brain regions during a visuomotor task

Figure 4.

Simultaneous recordings of individual trial rasters and PEHs recorded with the NHP tetrode microdrive in two other DMS recording sessions. Recordings are shown for all four phases of the DMS task for clusters of simultaneously recorded cells from the same areas of interest shown in Figure 3. For comparison purposes only 4 cells are shown for each brain area. **A:** Rasters and PEHs obtained from cells recorded with two tetrodes in primary motor cortex (upper, red) and two tetrodes positioned in the CPu (lower, purple) in the same session. **B:** Simultaneous recording with two tetrodes in PFC layer 2/3 (upper green) and two other tetrodes in layer 5 (lower green) in the same session. In all cases firing activity on correct trials is plotted in rasters and PEHs (± 1.0 s) for events in the DMS task (Figure 3C). The phase of task is shown above each respective column: Focus, Sample, Match M (onset) and MR Reward, and vertical lines indicate occurrence of behavior or stimulus presentation in each task phase. Average cross-correlations within ± 50.0 ms (1.0ms resolution) are shown for all cells recorded with the same tetrodes in each illustrated brain area during the DMS task. Some cell pairs in A show intra-region interactions between neurons in the motor cortex, via common modulations during Match presentation however this firing is associated with a wide range of cross correlation between all cell pairs shown in the same rastergrams and PEHs (A Motor Cortex). The remaining cross-correlograms confirm the same observation that neurons recorded on the same tetrodes wires do not show cross-correlated activity during performance of the task.