CHAPTER 20

Chronic multiunit recordings in behaving animals: advantages and limitations

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Abstract: By simultaneous recording from neural responses at many different loci at the same time, we can understand the interaction between neurons, and thereby gain insight into the network properties of neural processing, instead of the functioning of individual neurons. Here we will discuss a method for recording in behaving animals that uses chronically implanted micro-electrodes that allow one to track neural responses over a long period of time. In a majority of cases, multiunit activity, which is the aggregate spiking activity of a number of neurons in the vicinity of an electrode tip, is recorded through these electrodes, and occasionally single neurons can be isolated. Here we compare the properties of multiunit responses to the responses of single neurons in the primary visual cortex. We also discuss the advantages and disadvantages of the multiunit signal as opposed to a signal of single neurons. We demonstrate that multiunit recording provides a reliable and useful technique in cases where the neurons at the electrodes have similar response properties. Multiunit recording is therefore especially valuable when task variables have an effect that is consistent across the population of neurons. In the primary visual cortex, this is the case for figure-ground segregation and visual attention. Multiunit recording also has clear advantages for cross-correlation analysis. We show that the cross-correlation function between multiunit signals gives a reliable estimate of the average single-unit cross-correlation function. By the use of multiunit recording, it becomes much easier to detect relatively weak interactions between neurons at different cortical locations.

The discovery of the usage of electrical signals in the nervous system by Luigi Galvani in the 18th century led to new techniques to study brain functions. In the beginning of the last century it became possible to measure nerve impulses from brain tissue with microelectrodes. There are several methods to record the patterns of neural responses and many ways to analyze the recorded signal. Neural activity can be measured directly by recording the changes in membrane potentials over time, or indirectly by recording signals that are related to neural activity, such as magnetic field (MEG), blood flow, or blood oxygenation (fMRI).

A change in a neuron’s membrane potential is largely determined by the synaptic input to a neuron. Synaptic inputs usually last many milliseconds, and they come from thousands of neurons. All these inputs do not arrive at the same time, and the membrane potential of the target neuron therefore changes gradually, on a time scale of multiple milliseconds. One can measure this low frequency input signal by measuring the local-field potential (LFP) with electrodes inside the cortex or with EEG-electrodes on the scalp. If the synaptic inputs are sufficiently large, action potentials will be generated. These pulses are the carriers of information between nerve cells. Action potentials (spikes) are fast changes (~1 ms) in membrane potential and thus this type of activity has a high frequency. It is possible to record the membrane potential directly by using intracellular electrodes, but in many cases it suffices to have an electrode in the extracellular space, as long as it is sufficiently close to the cell. The extracellular electrodes readily measure...
somatic action potentials, but record dendritic or axonal spikes with some difficulty.

The method used to study brain functions depends on the question one likes to answer, given that every method has its advantages and disadvantages. Some methods, like EEG and fMRI, have the advantage of being noninvasive. EEG reflects the gradual change in the membrane potential of large populations of neurons (Creutzfeldt et al., 1966; Mitzdorf, 1988; Lopes da Silva, 1991). This technique has a good temporal resolution (~10 ms) but a poor spatial resolution (~2 cm). In contrast, the fMRI technique gives a relatively good spatial resolution (~1 mm), but a poor temporal resolution (~5 s). Moreover, fMRI does not accurately reflect the spiking activity of neurons in a particular brain area but seems to be related more to the input coming from other brain structures (Logothetis et al., 2001). Till date, the golden standard to investigate the way information is processed within brain structures still is to measure spiking activity with intracranial electrodes, i.e., using an invasive method. With an invasive method one gets the best spatial and temporal resolution at the same time. The most common method for measuring this is to record single units, and to analyze the spike rate of a single cell. Recording of single unit activity (SUA) can provide accurate information about that particular neuron during sensory stimulation or motor behavior. With single cell recordings, however, one usually skips the neurons with smaller spikes, and this method is therefore biased to large neurons. Moreover, it is usually difficult to follow the same cell over days or weeks. Recently, procedures have been developed to follow SUA by using chronically implanted electrodes (Nicolelis et al., 2003). But even if these new techniques are combined with advanced methods for spike sorting, it is not always possible to isolate SUA for extended periods of time, even if robust multiunit activity (MUA) can still be recorded from the same electrodes. This MUA-signal simply is the aggregate activity of a number of neurons in the vicinity of the electrode. There are methods for recording of MUA that also include the contribution of smaller spikes, and this reduces the sampling bias for large neurons. Here we will discuss the advantages and disadvantages of two types of MUA recording and compare the MUA responses and MUA-cross-correlation functions to SUA responses and SUA-cross-correlation functions.

**Methods**

During implantation of the electrodes, the monkeys were kept anesthetized, the skin overlaying the back of the head was opened and the bone over the occipital lobe was exposed. In order to be able to access the primary visual cortex (V1) a rectangular piece (2 cm × 3 cm) of the skull was removed over one hemisphere, and the dura was opened. Thereafter, Trimel coated platinum–iridium wires (California Fine Wire Company, USA; 25 μm diameter) were implanted with the aid of a binocular microscope in the operculum of one hemisphere of V1. The number of electrodes inserted was around 80. The electrodes have different lengths. Typically we used electrode lengths of 0.5 mm and 1.8 mm to record neural responses from the superficial and deep layers of the cortex, avoiding the middle layers. The electrode tip is usually between 40 and 120 μm. To prevent drifting of the electrode, they are glued to the meningeal cell layer (pia mater) of the cortex using histoacrylic glue. After insertion of the electrodes the dura is closed and the rectangular piece of skull placed back, leaving only the electrode wires outside the cranium. The wires were then connected to a pin connector, which was attached to the skull using titanium bone screws and dental cement (Fig. 1). Once the animal recovered from the operation recording of neural responses was started.

The chronically implanted electrodes have low impedances that vary between 100–300 kΩ at 1000 Hz. The low impedance values are due to the relatively large size of the electrode tip. However, occasionally electrodes may have higher impedances. The usual low impedance implies that neural responses are sampled across a relatively wide space around the electrode tip. Thus the spatial selectivity is less than that of a high impedance electrode. In other words, with the chronically implanted electrodes the activity of a number of neurons is picked up. We estimate that the signal comes from neurons near to (100–200 μm) the electrode tip, with strongest contributions from large neurons.
The recorded signal is amplified (40,000×) and then filtered between 750–5000 Hz (Filt-1 in Fig. 2). The filtered signal thus includes the high frequency component of neural activity, i.e., spike activity, since slow wave or low frequency signals such as LFP are filtered out. At this point the signal can be processed further in different ways. One of the most used methods is putting a threshold, or Schmidt trigger, to select only the larger spikes from the data. When large spikes occur one can reliably obtain single units. In our studies it was possible in a number of cases to record SUA from the chronically implanted electrodes by using a Schmidt trigger. Generally, however, we recorded two types of multunit activity, MUA_\text{S} and MUA_\text{E}.

MUA_\text{S} is the first type of multunit signal that is recorded when the trigger level of the Schmidt trigger is lowered, to record the action potentials of a number of single units that reached trigger level. The “S” of MUA_\text{S} stands for Schmidt-trigger. Thus, depending on signal quality one can get single units by setting the threshold level high and multunit when the level is low. Changing the Schmidt-trigger level will change a single unit recording into MUA_\text{S}. It should be noted that if the threshold is lowered too much, electrode noise might be included in the signal. However, this noise is constant in time, not correlated to task parameters, and different electrodes have independent noise. This implies that this noise will almost always be averaged out in subsequent analyses.

MUA_\text{E} is the second type of MUA that was recorded. The signal is full-wave rectified, i.e., negative values become positive (Rect in Fig. 2). The resulting signal is then low pass filtered (<500 Hz) to decrease the required amount of digital data (Filt 2 in Fig. 2). With this procedure, the final signal includes all the high frequency elements and reflects the envelope of the Filt 1 signal. The “E” of MUA_\text{E} stands for envelope. MUA_\text{E} provides an instantaneous measure of the number and size of action potentials of neurons in the vicinity of the electrode tip (Legatt et al., 1980; Brosch et al., 1995). MUA_\text{E} does not depend on the arbitrary positioning of a threshold level and therefore does not select only large spikes. This signal includes all high frequency signals although large spikes have more influence than small ones on the final signal. In principle, there are other high frequency components that may

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Fig. 1. Schematic illustration of chronically implanted electrodes in primary visual cortex of a monkey. Typically 50–80 electrodes are placed individually in the operculum of V1. The electrodes are Trimel coated platinum–iridium (Pt–Ir) wires with a diameter of 25 μm. The length of the electrode is determined by the distance between the position where the wire is bent and the end of the electrode tip. Usually the lengths of the electrodes are made to reach the upper or lower layers of the cortical mantle, avoiding the middle layers. Each electrode is attached to the pia mater of the cortex with a drop of histoacrylic glue. The wires of all electrodes are bundled and run underneath the skull from where they go to the connector that is fixed on top of the skull through a small opening in the skull. While not recording, the connectors are protected by a cap that covers the connectors. The length of the electrode tip varies around 40–120 μm.
contribute to the recorded signal, such as signals from axonal fibers or synaptic spikes. However, these signals are localized and much smaller than cellular action potentials. Indeed, if an electrode enters into the white matter, it is virtually impossible to record neuronal activity.

 MUAS simply counts how often the signal passes through the Schmidt-trigger level, and it is therefore measured in units of impulses/seconds. However, MUA_E is a continuous measure of the amount of high-frequency activity. The signal represents the envelope of high-frequency activity that can be expressed as a voltage. However, in our work we usually apply a normalization procedure. We first measure spontaneous activity, Sp, without any stimulus in the receptive field, as well as the peak

Fig. 2. The scheme illustrates the method used to record single- and multiunit activity. Signals from the chronically implanted electrodes were first amplified and then filtered between 750 and 5000 Hz (Filt1). In a number of cases it is possible to record single unit activity (SUA) from the chronically implanted electrodes by using a Schmidt trigger. Generally, however, we record two types of multiunit activity, MUAS and MUA_E. MUAS is recorded by lowering the trigger level of the Schmidt trigger, to record the action potentials of a number of single units that reach trigger level. MUA_E is the second type of MUA that is recorded by a full-wave rectification of the Filt1-signal (taking the absolute value of the voltage) followed by a low-pass filtering step at 500 Hz. MUA_E reflects the envelope of the Filt1 signal, and it thereby provides an instantaneous measure of the number and size of action potentials of neurons in the vicinity of the electrode tip.
response to a suddenly appearing stimulus, or the average peak response across a number of suddenly appearing stimuli, Pe. We then subtract Sp from the signal and divide by (Pe–Sp) to end up with a signal that varies between 0 and 1. It is important to stress that the values of Sp and Pe are determined once per recording site, and normalization can therefore not induce differences in response between conditions that are not present before normalization, or remove differences between responses that are present in the nonnormalized data.

**Results**

After 2–3 weeks of recovery one can start with the experiments. The advantage of using chronically implanted electrodes is that no daily penetrations of an electrode into the brain tissue have to be made. Instead the implanted electrodes are connected to a pin connector, which is mounted onto the skull of the animal. In this way the recording can start right away by plugging the animal to the recording system. Moreover, at the same time responses from numerous electrode sites are recorded. The sites are normally spaced far apart (>0.5 mm), so that signals at each electrode are independent. The properties of the responses that can be obtained using the chronic electrodes will be discussed.

**Response properties of MUA**

The envelope of the band pass filtered signal, MUA_E, provides an instantaneous measure of the number and size of action potentials near the electrode. This also holds for MUA_S, since it counts all spikes that reach detection threshold. Thus, both MUA signals sample the activity of multiple neurons that are close to the electrode tip. This implies that the RF properties of MUA, such as the receptive field size and tuning to orientation represent an average of the RF properties of several single cells. It is therefore expected that the RF sizes of the MUA at a particular electrode are somewhat larger than the size of the RFs of the SUAs that contribute to the signal. Moreover, the sharpness of tuning to for example, orientation is expected to be less sharp than the tuning of single neurons. In the primary visual cortex, however, cells nearby have similar RF properties, and they will therefore contribute similar features to the MUA responses. The tuning shared by nearby SUAs will show up in MUA_E and MUA_S.

In our experiments, we always first analyze the location and extent of the classical receptive field at each recording site by using a moving light bar. Figure 3A illustrates the method that was originally described by Kato et al. (1978). To determine the RF boundaries, light bars are moved across the RF in eight directions. In principle, four directions, at every 90°, suffice to define the RF, and the other four directions that differ by 45° provide a replication of this measurement. We determine the position of the light bar on the screen during the onset and offset of the visual response for each movement direction. In Fig. 3A it can be seen that the location of the bar moving at 0° during response onset, On_0, is to the right of the location of the 180° bar during offset of its response, Off_180. This difference can be explained by the processing delays in the visual pathways up to area V1. The left side of the RF is defined as the average of these two measurements, (On_0 + Off_180)/2. By taking the average there is an automatic compensation for the processing delays before V1. The other three RF boundaries are determined using an equivalent procedure.

We implant the electrodes on the surface of area V1 so that RFs are in the lower quadrant of the visual field, at eccentricities ranging from 1° to 6°. The sizes of the receptive fields are in the order of 1° (minimum 0.5 and maximum 2°). These values are slightly larger than the sizes of receptive fields measured with single unit recordings. It can be seen in Fig. 3B that the RFs that are sampled are slightly overlapping, and together they cover a relatively large region of the visual field. We usually repeat the RF measurements several times in the course of a number of months during which the electrodes can be used. The RF locations are stable across time. This is illustrated in Fig. 3B, where the initial RF mappings are shown in light grey. The same RFs were mapped after various intervals, but RFs can always be found at the same location. The shape of the RF is somewhat less stable across months, as would be expected if the population of neurons that contribute to the recordings gradually changes, or if the neurons themselves change their RF on a time scale of weeks.
Fig. 3. Response characteristic of multiunit activity. (A) RF boundaries can be determined by presentation of moving light bars in four directions. Arrows indicate the location of the light bar on the screen at the onset and offset of the visual response. It can be seen that the location of a 0° bar at response onset, On0, is shifted to the right relative to the location of the 180° bar during response termination, Off180. This difference can be explained by the visual latency, i.e., the processing delays before the visual information reaches area V1. To compensate for this latency, we define the RF boundary marked with a * as the average of these two locations, (On0 + Off180)/2. The other three RF boundaries are determined in the same way (not shown). We routinely repeat this measurement with four directions that differ by 45° (not shown), so that there are two independent measurements of the RF. (B) RF locations of MUA are stable across time. Shown are RFs that were measured repeatedly, with an interval of several weeks. Light grey RFs show the first measurement and darker grey the repeated measurement. Numbers show the number of weeks that elapsed between measurements. HM, horizontal meridian. VM, vertical meridian. (C) Orientation tuning of the MUA is relatively stable across weeks. Continuous curve, directional tuning profile of the MUA in A. Dashed curve, orientation-tuning curve of MUA at the same electrode that was repeated 6 weeks later. (D) Distribution of latencies of the MUA visual responses from chronically implanted electrodes. The latency of the sensory response was measured as the time that the response reached half of its maximum value. (E) Direction selectivity ratio from 96 MUA recording sites. Ratio was calculated by dividing the maximum response by the minimum response to moving bars of different directions.
or months. At present, we do not have data that distinguishes between these two possibilities.

The responses to the moving bars can also be used to determine orientation and directional tuning, as is illustrated in Fig. 3C. In most cases, tuning is stable across months. This is as expected, since the electrodes sample neuronal activity at one constant position of the orientation map in area V1, and these maps have been shown to be very stable in adult animals (Hubel, 1988; see also Gödecke et al., 1997). Directional tuning across a population of recording sites is illustrated in Fig. 3E ($N=96$; MUA$_S$). The results show that MUA responses are direction selective with a mean direction selectivity ratio (= maximum response divided by the minimum response) of 2.0. Thus direction tuning is apparent in MUA responses although it is less pronounced compared to the tuning of single cell responses. Furthermore, we found that the latencies of the onset of the visual responses are similar to single unit data (Fig. 3D; Lamme and Roelfsema, 2000). Thus, the principal RF features of neurons are maintained in the MUA responses, even though orientation tuning is less pronounced than in SUA responses. Our data are consistent with the claim that MUA represents the average of many SUA, and thereby confirm that MUA provides a truthful representation of average neural response in the vicinity of the electrode. This suggests that conditions where nearby neurons have similar response properties can be measured well with MUA. To investigate whether MUA can also be used to investigate perceptual and cognitive processing under conditions where neurons have similar response properties, we compared the MUA responses to SUA during a figure-ground detection task and a curve-tracing task.

**Figure-ground segregation**

The responses of a neuron to a stimulus can be modulated by stimuli that fall outside the classical receptive field. Typically, modulation of activity occurs at late stages of the stimulus-evoked response. Such contextual modulation has been shown to occur with figure-ground elements and reflect figure-ground segregation (Lamme, 1995; Zipser et al., 1996). The figure becomes segregated from the background when the responses to the figure elements become stronger than the neural responses to the ground elements. Modulation for figure-ground displays starts at the boundary (at $\sim 80$ ms), and is then followed by a process analogous to perceptual filling-in, so that in the end the whole figure surface is represented by elevated activity (at $\sim 150$ ms; Lamme et al., 1999). Previously we observed that the modulation for figure elements is absent when animals report not to have seen the stimuli (Supér et al., 2001a), or when animals are anesthetized (Lamme et al., 1998). That is, the responses to the figure and ground elements are similar and thus the figure does not segregate from the background. In addition, we have demonstrated that contextual modulation is directly representing whether stimuli are stored in working memory or not: The activity continues even when the stimulus is removed from the screen, yet this only occurs if the monkey has to remember the location of the stimulus, and does so correctly (Supér et al., 2001b). Finally, we observed an increase in the strength on contextual modulation just before the animal makes an eye movement towards the saccade target (Supér et al., 2004). Contextual modulation in V1 thus reflects whether (and how) stimuli are perceived, stored, and modulation appears to be associated with motor planning. Most of these results are obtained with MUA$_E$ recordings. Here we investigate the resemblance of contextual modulation obtained with SUA and MUA.

In previous studies, SUA (Lamme, 1995; Zipser et al., 1996) as well as MUA (Supér et al., 2001a, b) has been recorded during the texture segregation task, and similar results were obtained in the two types of studies. Here, we recorded SUA and MUA$_E$ activity in the primary visual cortex of monkeys that fixated to a small red dot on the centre of the screen. At stimulus onset a figure-ground texture was presented and the animals were allowed to make an eye movement toward the location of the figure. The figure was positioned so that it covered the receptive fields ($=$ figure condition) of the recorded neurons or so that background elements covered the receptive fields ($=$ ground, see Fig 4A). To know whether the two recording techniques are similar we compared
the SUA and MUA_E signals obtained in different sets of experiments.

In Fig. 4B we show that for figure-ground textures the SUA of the average population responses are comparable to the MUA responses. The responses for both methods there is a clear visual response that is followed by a sustained response. The visual transient response is caused by the abrupt transition of prestimulus pattern into a figure-ground texture and signals the visual elements, like the line orientation. Neurons in the primary visual cortex are orientation selective. Therefore we used complementary stimulus pairs, which mean that the orientations of figure and ground elements are alternated during the recording session. Since both orientations are used for the figure and the background textures the average visual stimulation is identical for figure and ground conditions. For both SUA and MUA_E around 100 ms after stimulus onset the responses to the figure become much stronger than the ground responses. This enhanced response is contextual modulation. Previous experiments have shown that the neurons with their RF on the border of a textured figure have an earlier onset of contextual modulation than when the RF is in the centre of the figure (Lamme et al., 1999). In the single unit recording, the RF was specifically positioned on the centre of a 4° square figure. This means that the enhanced SUA responses are an effect of the context of the receptive field stimulus and not the result of a difference in visual stimulation, i.e., the classical receptive field stimulation. Thus contextual modulated SU activity reflects filling-in of the figure and not the detection of the figure-ground border. In the MUA experiments, some RFs are located in the centre of the figure whereas other RFs may fall close to or on the border of the figure texture (Supér et al., 2001a). Thus with chronically recording MUA_E from multiple sites, it is possible to obtain border and centre information at different sites, which can be analyzed separately offline. However, clear differences in the strength of modulation have not been found between the border and the centre of the figure (Supér et al., 2003).

Not all sites show contextual modulation in a figure-ground task (Fig. 5). Intra-cortical EEG experiments to investigate the laminar organization of figure-ground segregation have shown different

![Figure 4](image-url)
temporal characteristics of the supra and infragranular activity (while no figure-ground activity was found in layer 4C; Lamme et al., 1993). This suggests to us that there is laminar specificity in the components of figure-ground activity. When doing single-unit recordings (Lamme, 1995) it was observed that ~70% of neurons show figure-ground modulation. When doing multiunit recordings, we also
observe modulation in about 70% of the sites (Lamme et al., 1998, 1999; Supér et al., 2001a). This would not be expected if the multiunit sites contained a random sample of single units (in that case we would expect weaker modulation in \( \sim 100\% \) of sites). This suggests that there are locations in the cortex where all neurons show modulation, and locations where none of the neurons show modulation. These may be different layers, but these may also be different structures in the horizontal sense, such as the blob and inter-blob regions.

Figure-ground modulation depends on feedback from extra-striate areas surrounding the primary visual cortex. The sign of contextual modulation can be positive (when the figure responses are stronger than ground responses) or negative (when ground responses are stronger than figure responses; Corthout and Supér, 2004). However, when using complementary stimulus pairs and comparing figure versus ground responses instead of comparing figure versus homogeneous texture responses, contextual modulation is almost always positive. The strength of modulation appears to correlate with saliency of the stimulus; high salient stimuli show strong modulation and low salient stimuli give weak modulation, and unseen stimuli give no modulation (Supér et al., 2001a, 2003).

In conclusion, the characteristics of SUA and MUA responses to figure-ground textures are very similar. Therefore, MUAE is an appropriate method to study figure-ground activity.

**Curve tracing task**

Next, we investigated the usefulness of multiunit recordings in a curve-tracing task that requires the perceptual grouping of contour segments into elongated curves. The task is illustrated in Fig. 6A. The monkey first looks at a fixation point on a computer screen. Then two curves and two larger circles appear on the screen. One of the curves connects the fixation point to one of the larger circles. This curve will be called target curve (T in Fig. 6A). The other curve is connected to the other circle but not to the fixation point, and will be called distractor curve. The monkey has to maintain gaze on the fixation point for 600 ms. Then the fixation point disappears, and the monkey makes an eye movement to one of the larger circles. He only receives a reward if he chooses the circle at the other side of the target curve, i.e., the circle that is connected to the fixation point. Thus to respond correctly in this task, the monkey has to group together all the contour segments that belong to the target curve, and segregate them from contour segments that belong to the distractor curve. This task has also been studied in humans using psychophysical techniques (Jolicoeur et al., 1986). Human observers solve this task by gradually spreading their attention across all the contour segments that belong to the target curve (Scholte et al., 2001; Houtkamp et al., 2003). The task is solved once all contours of one of the target curves have been labeled by attention. These attention shifts can also be monitored in area V1, where responses evoked by all contour segments that belong to the target curve are stronger than responses evoked by the distractor curve. Thus, labeling by attention in psychophysics corresponds to labeling with an enhanced neuronal response in area V1 (Roelfsema et al., 1998).

Here, we used chronically implanted electrodes to record neuronal activity in area V1 during curve tracing. We usually recorded MUA from the implanted micro-wires, but we occasionally also isolated single units. The first step was always to map the receptive fields (RFs) of the neurons, as was described above. The position of an example V1 RF during the curve-tracing task is shown in Fig. 6A. The RF falls on the target curve for the left stimulus and on the distractor curve for the right stimulus, but the contour crossing the receptive field is identical, which implies that the bottom-up input to the neurons from the LGN is the same.

Fig. 6B shows six example responses of single units (SUA) in area V1. Responses evoked by the target curve are shown as continuous lines, and responses evoked by the distractor curve as dashed lines. At time 0, the stimulus appears on the screen, and the sudden onset of a contour segment in the RF causes a transient response for most of the neurons. This initial response has a latency of about 40 ms, and it is presumably driven purely by the bottom-up input from the LGN. This input is tuned to the information inside the RF, which explains why the initial response does not discriminate between target and distractor
Fig. 6. Single-units during curve tracing. (A) In the curve-tracing task, the monkey first directs his gaze to a fixation point. Then a stimulus appears that consists of two curves and two larger circles. One of the curves is connected to the fixation point, and is a target curve (T). The other curve is a distractor (D). After a delay of 600 ms the monkey is required to make an eye movement to a larger circle at the other end of the target curve. If the left and right stimulus are compared, it can be seen how a small change in the stimulus close to the fixation point interchanges target and distractor curve. (B) Single-unit activity in area V1 during curve tracing. In general, the activity of the V1-neurons is stronger if their RF falls on the target curve (continuous curves) than when it falls on the distractor curve (dashed curves). The magnitude of this response enhancement differs between neurons. Numbers in the upper right corner of each panel indicate the significance of the response enhancement evoked by the target curve.
curve, since the two stimuli only differ at locations outside the RF. After a delay of around 150 ms, however, a difference between responses evoked by target and distractor curve emerges, since response evoked by the target curve start to be stronger. We evaluated the significance of this response enhancement by comparing the distribution of the single trial responses in a window from 200–600 ms with a U-test. In Fig. 6B, four of the V1 neurons have a significantly stronger response to the target curve than to the distractor curve. The other two neurons do not discriminate between target and distractor curve.

We also recorded multiunit activity from these electrodes, and for the purpose of the present study it is of interest to compare the properties of MUA_S and MUA_E, the two types of MUA that were defined above. To this aim, we simultaneously recorded MUA_S and MUA_E at 96 V1 recording sites. Examples of MUA_S responses evoked by the target and distractor curve are shown in Fig. 7A. Just as was the case for the single units, the initial MUA_S responses do not discriminate between the target and distractor curve whereas the delayed responses tend to be stronger for the target curve. The strength of this attentional response enhancement varies across recording sites. It is pronounced in the leftmost panel of Fig. 7A, intermediate in the middle panel, and nonsignificant in the rightmost panel. To quantify the strength of the response enhancement we defined a modulation index as (Tar-Dist)/Avg, where Tar is the response evoked by target curve after subtraction of spontaneous firing rate, Dist is the response evoked by the distractor curve, and Avg is the average of Tar and Dist. Figure 7C shows the distribution of the MUA_S modulation index across all recording sites. The distribution is shifted to the right of zero, which indicates that the responses evoked by the target curve are stronger than those evoked by the distractor curve (sign-test, P < 10^{-7}). The median modulation index for MUA_S was 0.19.

Figure 7B shows MUA_E responses that were recorded simultaneously at the same recording sites. The overall shape of the responses is similar to the shape of the MUA_S responses. This also holds for the strength and timing of the response enhancement evoked by the target curve. The distribution of the modulation index for MUA_E is shown in Fig. 7C. This distribution has a median of 0.17, which is similar to that for MUA_S. The variance of the distribution of the modulation index for MUA_E seems to be somewhat smaller than for MUA_S. This seems to suggest that MUA_E is a signal that is somewhat more reliable than MUA_S.

To investigate the reliability of MUA_S and MUA_E at individual recording sites, we compared the significance of the attentional effects at each recording site. In most of the cases, P-values for MUA_E were smaller than P-values for MUA_S (see Fig. 7A, B, D). At the population level, this difference was significant (sign-test, P < 5.10^{-5}). This can be explained, since MUA_S takes only those spikes into account that reach the trigger level for spike detection, whereas all spikes in the vicinity of the electrode can contribute to MUA_E. Pooling across more neurons increases the significance of the attentional effects.

We conclude that the results obtained with single units, MUA_S, and MUA_E in the curve-tracing task are similar. After the appearance of a stimulus in their RF, V1 neurons first exhibit a transient response at a latency of about 40 ms. The early responses are mediated by the shortest route from the retina to area V1, which passes through the LGN. This route primarily conveys information from inside the classical receptive field, which explains why the first spikes in area V1 do not discriminate between target and distractor curve, since the RF-contours were held constant (Fig. 6A). After about 150 ms, V1 neurons that have their RF on the target curve start to enhance their response. By the time the monkey is ready to respond, all contours that belong to the target curve have been labeled with an enhanced response in area V1. Importantly, the timing and magnitude of this response enhancement is similar for single units, MUE_S and MUE_E. It is likely that the delayed response enhancement is mediated by lateral connections between neurons in area V1 as well as by feedback connections from higher visual areas (Lamme and Roelfsema, 2000; Roelfsema et al., 2000). These routes can explain the delayed timing of the response enhancement, since they involve additional connections that are associated with further synaptic and transmission delays.

We noted an interesting difference between the reliability of MUA_S and MUA_E responses. MUA_S reflects the activity of a number of neurons that are sufficiently close to the electrode so that their action
Fig. 7. Multiunit activity during curve tracing. (A) MUA_S activity at three recording sites in area V1 during curve tracing. MUA_S evoked by the target curve tends to be stronger than that evoked by the distractor curve. The significance of this effect varies across recording sites. The scale on the Y-axis is in spikes/s. (B) MUA_E recorded at the same three recording sites. For MUA_E the responses are normalized, so that the peak of the response transient equals 1, and spontaneous activity equals 0. Note the similarity of the shape of the MUA_S and MUA_E responses, as well as the similarity of the response enhancement. (C) Distribution of the modulation index for MUA_S and MUA_E during curve tracing (96 recording sites where both signals were recorded). The index is positive if the response evoked by the target curve is larger than the response evoked by the distractor curve. Both distributions are shifted toward positive values (sign-test, $P < 10^{-5}$). The median modulation index for MUA_S equals 0.19 and the median modulation index for MUA_E equals 0.17. Cases where the response modulation was significant are indicated in black. (D) Comparison of the significance of the response modulation between MUA_S and MUA_E recordings. Abscissa, $P$-value for MUA_S. Ordinate, $P$-value for MUA. Most points are above the diagonal, which indicates that the significance tends to be higher for MUA_E.
potentials can reach the trigger level for spike detection. More cells contribute to MUAE and pooling across neurons averages out the noise that is due to the Poisson-like statistics of spike trains. Pooling across neurons is only beneficial if the difference between the responses to stimuli is consistent across the population. This holds for the attentional effect during curve tracing, since the large majority of neurons enhance their response if the contour segment in their RF belongs to the target curve, and there are only few neurons that have the opposite effect (Fig. 7C).

**Cross-correlations between MUA-signals**

In the results described so far, the activity of single and multiunit signals were related to the appearance of the stimulus. The implanted electrodes give access to neuronal activity at many different locations in the cortex at the same time. This provides the opportunity to also relate neuronal activity at one location to the activity at another location in the cortex. The most straightforward way to study these relations is by cross-correlation analysis (Perkel et al., 1967). We will therefore investigate the relation between cross-correlation analysis carried out with SUA and MUA.

In the case of spike-trains, like SUA or MUAS, the cross-correlation function describes the probability of detecting an action potential at one electrode at different points in time before and after the detection of an action potential at another electrode. However, in the case of analog signals, like MUAE, the cross-correlation function describes the linear correlation between the signals at two electrodes as a function of time lag. The MUAE cross-correlation function can differ from the MUAS cross-correlation for three reasons. First, the MUAE signal is filtered below 500 Hz, which implies that the narrowest peak that can occur in the cross-correlation functions has a width of approximately 2 ms. In theory, peaks in the cross-correlation function between two MUAS signals can be much narrower, although such precise correlations are not found in the cortex (Engel et al., 1990; Nowak et al., 1995, 1999; Livingstone, 1996). Second, in MUAS all spikes that reach the Schmidt trigger level are treated equally, whereas in MUAE larger spikes contribute more to the cross-correlation function than smaller spikes. Third, in MUAE small spikes also contribute to the cross-correlation function, whereas small spikes are not detected in MUAS and they do not contribute to the cross-correlation function at all. Despite these differences, in practice MUAS and MUAE cross-correlations have a similar structure, as will be shown below.

The “raw” cross-correlation function is defined as follows:

\[
C_{1,2}^{\text{Raw}}(\tau) = \frac{1}{N_{\text{Tr}}} \sum_{t=1}^{T} \sum_{t=-T}^{T} S_{1}^{\text{Tr}}(t)S_{2}^{\text{Tr}}(t + \tau)
\]  

(1)

Here, \(S_{i}^{\text{Tr}}(t)\) is the response strength at site \(i\) in trial \(\text{Tr}\) at time \(t\). For MUAS, \(S_{i}^{\text{Tr}}(t)\) is the number of spikes detected in a particular time bin (of e.g., 1 ms). For MUAE it represents a single sample of the analog signal. In Eq. (1) \(\tau\) is time-lag, \(T\) is the length of the computational window, and \(N_{\text{Tr}}\) is the number of trials. The presentation of the stimulus causes correlated changes in activity at different electrodes that are not due to the interactions between the neurons. These stimulus-locked correlations can be estimated by computing the correlation between the PSTHs, known as “shift-predictor”:

\[
\text{SP}_{1,2}(\tau) = \sum_{t=-T}^{T} P_{1}(t)P_{2}(t + \tau)
\]  

(2)

where \(P_{i}(t)\) is the PSTH,

\[
P_{i}(t) = \frac{1}{N_{\text{Tr}}} \sum_{t=1}^{T} S_{i}^{\text{Tr}}(t)
\]  

(3)

To estimate the neuronal interaction unconfounded by the stimulus-locked correlation, it is common to take the difference between the raw correlation function and the shift predictor:

\[
C_{1,2}(\tau) = C_{1,2}^{\text{Raw}}(\tau) - \text{SP}_{1,2}(\tau)
\]  

(4)

It is of interest to consider an alternative method for the subtraction of the shift predictor. The method first subtracts the average response (i.e., the PSTH) from the responses on individual trials, before subjecting these single trial responses to cross-correlation analysis. This is mathematically equivalent to subtraction of the shift-predictor, since if
Eqs. (1)–(4) are combined it can be seen that
\[ C_{1,2}(\tau) = \frac{1}{N_{Tr}} \sum_{T=1}^{N_{Tr}} \sum_{t=1}^{T} (S_{1}^{Tr}(t) - P_{1}(t)) \times (S_{2}^{Tr}(t + \tau) - P_{2}(t + \tau)) \]

(5)

An important question is whether the MUA–MUA cross-correlation function gives an appropriate description of the underlying SUA–SUA interactions. Fortunately, there exists a simple relationship between MUA – MUA cross-correlations and the cross-correlation functions between SUA at two recording sites. Let us assume that \( N_{1} \) single units contribute to MUA at site 1, and \( N_{2} \) contribute to MUA at site 2. Thus,

\[ S_{MUA1}^{Tr}(t) = \sum_{i=1}^{N_{1}} S_{i}^{Tr}(t), \quad P_{MUA1}(t) = \sum_{i=1}^{N_{1}} P_{i}(t) \]

(6)

\[ S_{MUA2}^{Tr}(t) = \sum_{i=1}^{N_{2}} S_{i}^{Tr}(t), \quad P_{MUA2}(t) = \sum_{j=1}^{N_{2}} P_{j}(t) \]

(7)

where \( S_{MUA1}^{Tr}(t) \) is MUA at time \( t \) in trial \( Tr \) at site 1, which equals the sum of all SUAs \( S_{i}^{Tr}(t) \) at this site. \( P_{MUA1}^{i} \) is the MUA PSTH, which equals the sum across \( P_{i}(t) \), the single-unit PSTHs. The shift-predictor corrected MUA – MUA cross-correlation is defined as in Eq. (4):

\[ C_{MUA1,MUA2}(\tau) = \frac{1}{N_{Tr}} \sum_{T=1}^{N_{Tr}} \sum_{t=1}^{T} S_{MUA1}^{Tr}(t)S_{MUA2}^{Tr}(t + \tau) - \sum_{t=1}^{T} P_{MUA1}(t)P_{MUA2}(t + \tau) \]

(8)

We combine Eqs. (6)–(8) to obtain:

\[ C_{MUA1,MUA2}(\tau) = \frac{1}{N_{Tr}} \sum_{T=1}^{N_{Tr}} \sum_{t=1}^{T} S_{i}^{Tr}(t)S_{j}^{Tr}(t + \tau) - \sum_{i=1}^{N_{1}} \sum_{j=1}^{N_{2}} P_{i}(t)P_{j}(t + \tau) \]

\[ = \sum_{j=1}^{N_{2}} \left( \frac{1}{N_{Tr}} \sum_{T=1}^{N_{Tr}} \sum_{t=1}^{T} S_{i}^{Tr}(t)S_{j}^{Tr}(t + \tau) \right) - \sum_{i=1}^{N_{1}} \sum_{j=1}^{N_{2}} C_{i,j}(\tau) \]

(9)

Thus, the MAU – MAU cross-correlation function \( C_{MUA1,MUA2}(\tau) \) equals the sum of all \( N_{1} \times N_{2} \) between-electrode SUA–SUA cross-correlation functions \( C_{i,j}(\tau) \). It is easy to derive a similar result for the MUA – MUA cross-correlation functions, which equal the weighted sum of the SUA–SUA cross correlation functions, with weighting factors that are determined by the size of the action potentials of the neurons that contribute to the two MUA signals. An advantage of MUA–MUA cross-correlation functions over MUA and SUA cross-correlation functions is that they are computed from two analog signals. Therefore, the magnitude of the correlation can be expressed as a correlation coefficient.

These results, taken together, have important implications for the utility of MUA recording. MUA–MUA cross-correlation functions give an unbiased estimate of the average cross-correlation function between the single neurons at two different cortical locations. Thus if the aim is to characterize the average interaction, then it is beneficial to use MUA recordings. By averaging across many cross-correlation functions, the MUA results are less prone to variability caused by the random sampling of single cells. Moreover, MUA–MUA cross-correlation functions have more spikes in every bin, which makes them less noisy. Some studies have compared the results obtained with SUA and MUA cross-correlation analysis, and found that it is easier to detect the underlying neural interaction by using MUA, but that MUA and SUA cross-correlations are indeed similar in all other respects (Nowak et al., 1995, 1999).

To make these considerations more explicit, we will now compare cross-correlations between SUA, MAU, and MAU obtained during curve tracing. We will first consider results from SUA–MAU pairs, where SUA was recorded at one electrode and MUA at the other. Three representative cases from electrodes in area V1 that were separated by more than 1 mm are shown in Fig. 8A. The cross-correlation functions were computed in a window from 200 to 600 ms after the appearance of the stimulus. They have a small number of coincidences per bin, and it is therefore difficult to detect the relatively weak interactions, even though these cross-correlation functions were accumulated over more than 50 stimulus repetitions. The number of
coincidences per bin increases for MUA$_S$–MUAS cross-correlation functions, and this makes it easier to detect the neural interactions (Nowak et al., 1995, 1999). Three MUA$_S$–MUAS cross-correlation functions that were obtained in a single session are shown in Fig. 8B. The two leftmost cross-correlograms suggest weak synchrony. However, more robust results are obtained when the correlations between MUAE are evaluated, since here even more neurons contribute to the recordings. This can be appreciated in Fig. 8C, which shows the interactions between MUAE at the same electrode combinations as Fig. 8B. Note that the shapes of the cross-correlation functions are similar, but that the signal to noise ratio has been improved. Figure 8C also illustrates the use of a curve-fitting procedure to extract the significant structure in the cross-correlation function, which has been described by König (1994).

These results, taken together, imply that MUA recording has a number of advantages over SUA
recording in correlation analysis. This is why most of the studies on the interactions between neuronal activity at cortically separate locations used MUA (e.g., Gray et al., 1989; Engel et al., 1990; Brosch et al., 1995, 1997, Nowak et al., 1995, 1999; Kreiter and Singer, 1996; Castelo-Branco et al., 2000; Gail et al., 2000; Maldonado et al., 2000). One issue that is sometimes raised is whether action potentials of a single neuron might contribute to the signal at two different electrodes. Such a contribution would show up as an artifactual correlation between the two electrodes. However, the probability that that happens is minimal with an electrode spacing of more than 500 µm. Gray et al. (1995) showed that action potentials in the cortex decrease to 10% of their size when the electrode is removed from the soma by 65 µm. This ensures that the same cell cannot contribute to two MUA signals if the electrodes are separated by more than 200 µm.

Discussion

In coming years, single unit recording is likely to remain the gold standard for the investigation of the function of individual neurons in various brain regions. We note, however, that even this method has some limitations. In the cerebral cortex there are many different types of neurons. Thus, neurons are heterogeneous and have diverse electrical properties. SUA recordings provide a biased sample of the neuronal population, since they overemphasize activity from large neurons (pyramidal neurons). Therefore a set of SUA recordings may not provide an accurate representation of the entire neuronal population. A further limitation is that with the present technology, it is very difficult to study a single neuron for extended periods, across days or weeks.

In the present study we have compared MUA recordings to SUA, to shed light on the source of the signal underlying MUA. The results obtained with MUAS are quite intuitive. To record MUAS, the Schmidt trigger level is lowered, so that multiple action potentials can reach this threshold. This implies that MUAS is simply the sum of the spikes of all neurons that reach the trigger level. The measurement of MUAE is slightly more complex. A band pass filter (500–5000 Hz) appropriate for action potential detection is followed by a rectification step (negative potentials become positive) and a low pass filter. This allows measurement of the envelope of activity in the action potential frequency band. Are the action potentials fired by neurons in the vicinity of the recording electrode the only source of the MUAE signal? Although it is difficult to conceive of an experiment that would give definitive proof, our results suggest that the answer to this question is “yes”. Of course, there are other types of electrical events in the cortex. One type of event is the postsynaptic potential, but its time-course is too slow to contribute to MUAE. Furthermore, there are axonal spikes and synaptic spikes that do have power in the frequency band between 500 and 5000 Hz. However, axonal spikes are small, which explains why it is virtually impossible to record neuronal activity in the white matter, below the cortex. Dendritic spikes are presumably even smaller, and it is virtually impossible to detect them without specialized recording methods (e.g., Chen et al., 1997). These considerations imply that MUAE measures the action potentials fired by neurons with somas close to the recording electrode. This conclusion is supported by our comparison of SUA to MUA during figure-ground segregation and curve tracing. The MUA responses in area V1 are similar to SUA in their timing of the visual response as well as in the timing of the modulation of this response by the context. Moreover, the strength of the modulation by figure-ground segregation and attention for MUA and SUA are also similar. This supports our conclusion that MUA is just the sum of a number of SUA. We note that MUA differs in this respect from other widely used signals, such as optical imaging, and the BOLD signal that is picked up by fMRI. It is still unclear how the vascular events that underlie these signals depend on action potentials, postsynaptic potentials, and other neuronal events (Vanzetta and Grinvald, 1999; Logothetis et al., 2001).

There are some limitations in the use of MUA compared to SUA. MUA pools action potentials fired by different neurons together. It can therefore not be used to assess the distribution of the response properties of individual neurons in a particular brain region, or to measure the sharpness of tuning. If, for example, the aim is to investigate how a particular stimulus variable influences firing rates, but different
neurons change their response in opposite ways, the tuning of the individual cells may get lost in the MUA signal. If nearby neurons respond similarly, on the other hand, it can even be advantageous to pool across neurons, since in this case pooling increases the signal to noise ratio. In our own work we have used MUA to measure the contextual modulation of neuronal responses in the visual cortex as a function of figure-ground segregation, working memory, and attention. These factors have a consistent influence on firing rates. Responses to figures are enhanced compared to responses evoked by a background. Similarly, responses evoked by attended stimuli are enhanced relative to responses evoked by unattended stimuli. In these cases MUA recording is useful, since it is relatively easy to get a good impression of how these contextual effects influence the firing rate of the entire population of neurons in a particular brain region. In many studies single cells are isolated, but the final result is presented as a population average, the average response when all neurons are pooled together. By using MUA recording, it is possible to measure this population response reliably after fewer recordings, since multiple cells contribute to the response at every site.

MUA recordings have some clear advantages for cross-correlation analysis. If the aim is to characterize the average cross-correlation function, or how the average cross-correlation function depends on certain behavioral or stimulus-related parameters, MUA cross-correlograms can quickly give a reliable impression. Here we demonstrated that the MUA–MUA cross-correlation is the sum of all SUA–SUA cross-correlations between the neurons that contribute to two MUA recordings. If \( N \) neurons contribute to a MUA recording, the number of SUA–SUA cross-correlograms that are averaged together in the MUA–MUA cross-correlogram equals \( N^2 \). This explains why MUA recordings are widely applied in cross-correlation analysis, especially if the focus is on the weak neuronal interactions that occur across large cortical distances.

To conclude, MUA obtained with chronically implanted electrodes provides a reliable and useful technique to record neural spiking activity. With the chronically implanted electrodes one is able to record neural responses over a long period of time. In our experience, high quality recordings of neural activity can be made up to several months with the Pt–Ir electrodes and to over a year with commercially available Utah array electrodes. With such long and stable recordings it is possible to study neural effects that change relatively slow, e.g., learning and memory processes. Moreover, chronically implanted electrodes can be used in freely moving animals and permit study of neural responses in animals during more natural behavior. Finally, the long-term existence of chronically implanted electrodes may be useful in developing prostheses. For example, in the future, disabled people may have electrodes implanted in a particular brain area so that neural activity can be used to control the movement of prosthesis. In that case, one may wish to rely on a neuronal population rather than on a single cell.

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**References**


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