Abstract

The goal of the work described is the development of an alternative neuroscience tool based on magnetorresistive sensors. These sensors, Spin Valves, are incorporated in silicon needles, to be inserted into the brain and measure the magnetic fields generated by the ionic currents generated by neuronal activity. The magnetic fields generated have extremely low intensities being hard to measure. To measure these signals, the sensors need to have low intrinsic noise, high sensitivities and low detectivities. To increase the signal-to-noise ratio of the acquired signal, processing techniques are required. Averaging of acquired measurements time locked to the stimuli provided to the cells was used. The steps involved in the microfabrication of the sensors and in their characterization are described. Micromachining and characterization were performed at INESC-MN. The biological experiments were performed in hippocampal slices from mice, since the pathways in this structure are well defined and studied. The in vitro experiments were done using a local field potential measurement setup at Instituto de Medicina Molecular. Micromachined sensors reached sensitivities of V/T and detectivities of tens of nT/Hz$^{1/2}$ at low frequencies and of nT/Hz$^{1/2}$ at high frequencies, without flux guides. When flux guides were incorporated the sensitivity increased and the detectivity decreased both by a factor of 10, reaching detectivity levels of hundreds of pT/Hz$^{1/2}$ at high frequencies. These probes were successful in measuring the magnetic field generated by the synchronized activity of the pyramidal cells in the hippocampus, that was in the order of tens of nT.

Keywords: microfabrication, magnetorresistive sensor, hippocampus, magnetic neuronal activity, magnetic field, detectivity

I. Introduction

There is a lot that we don’t know about the brain. Understanding how the brain works is a work in progress in life sciences specially in the neurosciences field.

Neurons in the brain form an intrinsic network of connexions between them and communicate
with each other by means of electric currents. The mechanisms involved in the generation of these electric fields is well known[1], [2], but little is known about the generation of magnetic fields, because these have very low amplitudes and therefore are hard to measure. Most of what we know about magnetic brain activity [3] arises from magnetoencephalography (MEG) that uses superconducting quantum interference devices (SQUIDs), which are very sensitive magnetometers that need to operate in magnetic shielded rooms at liquid helium temperatures. The alpha-rhythm observed over the posterior parts of the head is around 1-2 pT in amplitude. Pathological conditions like epilepsy may elicit spontaneous activity of even larger amplitudes. Evoked fields following sensory stimulation are weaker than pT by an order of magnitude or more. [4]

Electric activity in the brain arises from the synchronized activity of several neurons, firing action potentials. An Action potential is a short-lasting event in which the electrical membrane potential of a cell rapidly rises and falls, following a consistent trajectory. [5] In neurons there is a natural separation of charged particles across the membrane, which result in a resting potential of the membrane that is around -70mV. Sodium ions in the resting state are more concentrated in the extracellular space and potassium ions are more concentrated in the intracellular space. When the neurons receive sufficient excitatory input to rise its membrane potential up to a certain value, the permeability of the membrane to sodium ions increases and there is an inward current of sodium ions, rising the membrane potential and depolarising it. This depolarization causes an increase of the permeability to potassium ions, causing an outward current, decreasing the membrane potential, returning to its resting state. Action potentials are all-or-none responses. When recording electric brain activity what is being measured is the result of the added up activity of several neurons on a given area.

The flow of currents within an axon can be described quantitatively by cable theory that states that the neuron can be treated like an electrically passive, perfectly cylindrical transmission cable [6], [7].

In order to measure this magnetic activity, spin valve sensors are integrated in silicon needles that penetrate the extracellular space and there probe the magnetic activity at small distances from the source, where the intensity of the magnetic field is higher. These sensors need to combine high sensitivities with low intrinsic noises and low detectivities. Standard SV devices can achieve MR values of 9% at room temperature and when nano-oxide-layers are introduced next to the ferromagnets MR values of 20% can be achieved. [8]

MR sensors are currently being used to detect very weak magnetic fields in the order of nT and pT at room temperatures, not only in the measurement of magnetic brain activity but in other areas as well. At INESC-MN, research on systems capable to measure action potentials at room temperature are currently being developed. Silicon probes that incorporate giant magnetoresistance (GMR) and tunnel magnetoresistance (TMR) technologies have achieved detectivity levels of 30 nT/√Hz [9], [10]. These probes are currently being explored in the European project MAGNETRODES (Electromagnetic detection of neural activity at cellular resolution) that aims to develop a tool for magnetic imaging at a neuron scale in order to model the electromagnetic response of a neuron. Detectivities of 90pT/√Hz [10] have already been reported and for large areas and low aspect ratio devices detectivities of 46pT/√Hz were reached [11].
II. Methods

Sensor microfabrication:

The spin valve sensors were fabricated at the INESC-MN facilities in Lisbon. At INESC-MN there are a class 10, a class 100 and a class 10 000 clean rooms, where the microfabrication steps took place.

Spin valve sensors were incorporated in silicon needles. The microfabrication of these probes had several steps including five lithography steps, four deposition steps, one etching step and three liftoff processes.

The spin valve sensors had a stack composed of 20 Ta / 60 NiFe/ 70 IrMn / 33 CoFe / 25 Cu/ 23 CoFe / 28 NiFe/ 60 Ta. The thicknesses are measured in A. This stack was deposited onto a silicon substrate. This process was followed by a DWL lithography to define the shape of the sensors. Sensors were define with an 80X2\(\mu\)m\(^2\) area. An etching step in the Ion Beam Deposition/ ion milling system Nordiko 3600 was performed. The total thickness of the deposited stack was etched (figure 1-a).

Following the definition of the SV sensors, another optical lithography was performed. A 5000A thick layer of CZN was deposited in Ultra High Vacuum (UHV) I and flux guides (FG) were defined by liftoff (figure 1-b).

To avoid a short circuit between CZN and the aluminium contact leads a 3000A thick Al\(_2\)O\(_3\) insulating layer was deposited in UHVII. Previous to this step a lithography was needed to open vias so that the contact lead can connect to the sensor (figure 1-c). The vias were defined by liftoff.

In probes in which FG were not incorporated the steps of definition of flux guides and opening vias were skipped.

In order to perform measurements we need to be able to access to the SV by means of metal contacts. An optical lithography was performed in order to define the contact leads and a 3000A thick layer of aluminium was deposited in the Nordiko 7000 DC sputtering system (figure 1-d).

The last lithography was performed to give the sensor the needle shape and protect it with a Al\(_2\)O\(_3\) 3000A thick passivation, deposited at UHVII with a AlN\(_x\) 3000A thick passivation layer deposited in N7000 (figure 1-e) deposited on top.

\[\text{Figure 1} - \text{3D Schematics of the microfabrication process: a) SV sensor defined by etch; b) magnetic FG defined by liftoff; c) vias to access the sensor, defined by liftoff; d) Al contact leads defined by liftoff; e) passivation layer deposition}\]
A Scanning electron microscope image of a finished probe is shown in figure 2.

![Image of a finished probe](242x644 to 388x737)

**Figure 2 - scanning electron microscope image of a needle with flux guides**

**Experimental Setup:**

The micromachined needles were mounted in a PCB, where the MR sensors were wire bonded and the wires were protected with silicone gel. A hippocampal slice from mice was placed onto a local field potential measurement setup (figure 3-a). These slice is continuously perfused with gassed kreb's solution in order to maintain the normal metabolism of the cells. The flow of perfusion with kreb's was set to 3mL/min. The MR sensor was inserted in the CA1 area of the hippocampus slice and the stimulation electrode was inserted into the CA3 area of the hippocampus (figure 3-b). The frequency of stimulation was of 0.5Hz with an amplitude of 0.7mV. The PCB in which the needle was mounted was connected to the electronics by means of SMA connectors. In the electronics there is designed a double-sided Band Suppressed-Carrier Modulation (DSBSCM) circuit, composed of a Wheatstone bridge, a low noise instrum amplifier with a gain of 1000, a demodulator with gain 2, a low pass analogue filter with cut frequency at 70Hz and an amplifier with a gain of 100. The total amplification of this circuit is of 200 000. The carrier wave used in the modulation of the acquired signal had an amplitude of 500 mV\text{RMS} and a frequency of 20kHz. The output of this circuit was connected to an analogue-to-digital converter (ADC). The stimulation signal was also connected to the ADC to perform averaging time locked to the stimulation of the acquired signal. The ADC was in turn connected to a computer in which the acquisition software was implemented in MatLab.

In order to verify if the recorded signals were in fact produced by biological processes tetradoxin (ttx) was added to the kreb's solution that is perfusing the cells. Ttx is a reversible neurotoxin that inhibits action potentials in cell cultures.

![Image of LFP measurement setup](146x105 to 448x232)

**Figure 3 - a) LFP measurement setup; b) MR sensor inserted into the CA1 area and the stimulation electrode inserted into the CA3 area of an hippocamp slice from mice**
III. Experimental results

SV transport measurement characterization:

SV transport characterization for sensors incorporated in probes without flux guides is shown in figure 4-a, and for probes with FG is shown in figure 4-b.

![Figure 4](image)

Figure 4 - a) Magnetotransport curve of a SV sensor without FG with a MR of 4.71% and a sensitivity of 3.01V/T; b) Magnetotransport curve of a SV sensor with FG with an MR of 4.93% and a sensitivity of 33.4V/T

These measurements were performed with a current bias of 1mA passing through the sensor. Sensitivities of 3.01V/T and 33.4V/T were achieved for the probes with sensors without and with magnetic flux guides respectively.

SV noise characterization:

The noise measurement in SV sensor without flux guides incorporated in a sharp probe is shown in figure 5-a and for a probe with SV sensors with FGs the noise measurements are shown in figure 5-b.

![Figure 5](image)

Figure 5 – Noise measurements in spin valve sensors incorporated in silicon needles (a) without and (b) with flux guides
The noise and detectivity levels at both low and high frequencies extracted from figure 5-a and 5-b are shown in table 1.

<table>
<thead>
<tr>
<th>Sensor type</th>
<th>Noise @ 30Hz (nV/√Hz)</th>
<th>Noise @ 10kHz (nV/√Hz)</th>
<th>Detectivity @ 30Hz (nT/√Hz)</th>
<th>Detectivity @ 10KHz (nT/√Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without FG</td>
<td>83.6</td>
<td>4.92</td>
<td>27.7</td>
<td>1.63</td>
</tr>
<tr>
<td>With FG</td>
<td>75.2</td>
<td>4.42</td>
<td>2.41</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 1 – Noise and detectivity values at 30Hz and 10kHz for SV sensors incorporated in sharp probes without and with flux guides.

**In vitro experiments:**

To attest the viability of the hippocampal slice used an electric measurement was performed. The acquired measure is shown in figure 6.

![Figure 6 - Electric signal measured in the CA1 area of the hippocampus slice, exhibiting the stimulation artifact, the presynaptic volley and the population spike](image)

After verifying that the hippocampal slice had biological activity the electrode for electric measurements was removed from the slice and the probe with the MR sensor was introduced in the same area where viability was tested. After verifying that there was in fact a magnetic signal being measured (figure 7-a), ttx was added to krebs solution. The results after adding ttx are shown in figure 7-b.

![Figure 7 - a) Recorded magnetic signal exhibiting the same properties as the electric signal b) Recorded signal after adding ttx](image)
The amplitudes of the pre-synaptic volley and the population spike as well as their duration were measured. The results are presented in figure 8.

![Graph showing sensor output and magnetic field](image)

*Figure 8 – Characteristics of the acquired signal: 0.115 µV in amplitude and 6 ms in duration for the pre-synaptic volley and 0.033 µV in amplitude and a duration of 12 ms for the population spike*

The magnetic field that give rise to this signal was calculated and is shown in figure 9.

![Graph showing magnetic field](image)

*Figure 9 – Magnetic characteristics of the signal produced by the synchronized activity of the pyramidal cells of the hippocampus*
IV. Discussion

When comparing the sensitivity of the probes without flux, around 3V/T (figure 4-a) with the sensitivity of the probes with flux guides (figure 4-b), that is around 30V/T it’s possible to notice a gain in sensitivity of around 10, which is the expected value for the type of FG used. It’s also possible to notice that even though the stack of the SV sensors was the same for both probes the MR varies. This happens because the deposition methods are not uniform all over the wafer and therefore an equal result was not expected. In spite of this the values aren’t very disperse, showing that the measurements performed with the microfabricated probes are able to be reproduced.

Analysing figures 5-a and 5-b, the noise for both types of sensors (with and without FG) it’s possible to notice that the noise values are close and the graph has the same tendency, being dominated by the 1/f noise at low frequencies, decreasing and reaching the thermal noise value at higher frequencies. It’s possible to notice a peak at 50Hz due to the interference from the power grid. We can conclude that the FG don’t introduce any additional noise to the sensor. Looking at the values for the detectivity that should be as low as possible, the SV sensors without flux guides have a detectivity value of 27,7nT/sqrt(Hz), a value one order of magnitude above the 1,63 nT/sqrt(Hz) measured for the sensor with FG. The same difference in magnitude can be also be verified for the detectivity values at 10kHz, being measured a value of 1,63 nT/sqrt(Hz) for the sensor without FG and a value of 0,14 nT(sqrt(Hz)) for the sensor with FG. This is due to the gain of 10 introduced by the flux guides.

In the electric signal (figure 6) and magnetic signal (figures 7-a and figure 9), measured in the hippocampus, its noticeable the stimulus artefact, the pre-synaptic volley and the population spike. The biological phenomenon that causes the pre-synaptic volley is the depolarization of pre-synaptic cells. The amplitude of this pre-synaptic volley is directly proportional to the number of activated fibres on the hippocampus that is in turn dependent on the stimulation current provided. The population spike is caused by the movement on ions involved in the propagation of action potentials. This spike reflects synaptically induced firing and therefore can be classified as a field of excitatory postsynaptic potentials. Since in neurons the pyramidal cells are all oriented in the same direction, the extracellular signals from the generation of action potentials don’t cancel each other, but add up, giving rise to a signal that can more easily be recorded. The result obtained is the summation of the synchronized activity of several cells, not only one and therefore the amplitude of the recorded signal, contrary to happens with the action potential, depends on the stimulation provided, the higher the stimulation the higher the amplitude of the measured signal.

After the addition of ttx (figure 7-b) to the kreb solution the signal previously recorded was suppressed, remaining only in the recorded signal the stimulation artefact, confirming that it was caused by biological activity in the hippocampus slice.

In figure 8, the pre-synaptic volley on the magnetic signal measured has an amplitude of 0,115µV and a duration of 8ms, while the population spike has an amplitude of 0,033 µV and a duration of 12ms. The temporal values, when compared to the ones observed in an electric signal measured in the same slice (4,6ms for the pre-synaptic volley and 10,5ms for the population spike) are relatively
close, in spite of being slightly bigger. This might happen due to a delay in the spin valve response and also due to distortions in the signal introduced by the electronic setup.

In order to calculate the magnetic field associated with the biological activity of pyramidal cells in the hippocampus we must use the sensitivity of the sensor used, which is 3.01 V/T, meaning that a variation of 1T produces a variation of 3.01 volts in the sensor terminals. Since we are operating in the linear range of the magnetotransport curve, the can estimate the magnetic field that produced the changes associated with the change in voltage measured.

For the pre-synaptic volley, a magnetic field of 36.2 nT produced a change in voltage of 115nV, and for the population spike, a magnetic field of 10.9 nT give rise to a change in voltage of 33nV at the sensor terminals.

V. Conclusions

In order to perform this measurements MR sensors, SV sensors were incorporated in silicon needles and in vitro experiments were performed. The microfabricated devices had a sensitivity around 3V/T and it was demonstrated that this sensibility would be improved by a factor of 10 by incorporating FG into the device. For the SV sensors without flux guides sensitivities of 3.01V/T and detectivity levels of 27.7 nT/√Hz at 30Hz and of 1.63 nT/√ Hz at 10 kHz were achieved. Regarding the probes in which flux guides were included sensitivity of 33.4V/T and detectivity levels of 2.41/nT/√Hz at 30Hz and 140pT/√ Hz at 10kHz were achieved.

The experiments in hippocampal brain slices from mice were done by inserting the silicon needles containing the magnetoresistive sensors into the slice and using an electrophysiological chamber to perform the recordings of magnetic fields to measure the magnetic field generated by the synaptic currents. The magnetic neural activity in the hippocampus can be divided in two components one of high frequency, usually higher than 500Hz, that contains the action potentials and are called spikes, and a low frequency component, usually bellow 500Hz, called local field potentials and reflect the activity of several neurons. Both the pre-synaptic volley and the population spikes fall into the low frequency component of the electric brain activity. In spite of being an invasive method, the approach used in this thesis has the advantage of not measuring the magnetic activity at a distance and allows measurements right at its source.

Using an SV sensor without any flux guides and with a relatively high sensitivity and low detectivity levels, and performing a high level of averages it is possible to measure magnetic signals generated by the brain. In spite of this, these results are hard to reproduce since there are lots of variables that need to be controlled such as the proximity of the sensor to the stimulation electrode, the angle of insertion in the probe, among others. The results obtained weren’t possible to reproduce but magnetic brain activity in the pyramidal cells of the hippocampus was possible to be measured and this was verified by adding to the culture media a neurotoxin that blocks the firing of action potentials. The in vitro experiments yielded values of 36.2nT for the pre-synaptic volley and values of 10.9nT for the
magnetic activity generated by population spikes. These results were achieved with a stimulation of 0.7mV.

VI. Bibliography


