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Octopus visual system: a functional MRI model for detecting neuronal electric currents without a BOLD confound

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Abstract

Purpose—Despite the efforts that have been devoted to detecting the transient magnetic fields generated by neuronal firing, the conclusion that a functionally relevant signal can be measured with magnetic resonance imaging (MRI) is still controversial. For human studies of neuronal current MRI (nc-MRI), the blood-oxygen-level-dependent (BOLD) effect remains an irresolvable confound. For tissue studies where hemoglobin is removed, natural sensory stimulation is not possible. This study investigates the feasibility of detecting a physiologically induced nc-MRI signal *in vivo* in a BOLD-free environment.

Methods—The cephalopod mollusc *Octopus bimaculoides* has vertebrate-like eyes, large optic lobes (OLs) and blood that does not contain hemoglobin. Visually evoked potentials were measured in the octopus retina and OL by electroretinogram and local field potential. nc-MRI scans were conducted at 9.4 Tesla to capture these activities.

Results—Electrophysiological recording detected strong responses in the retina and OL *in vivo*; however, nc-MRI failed to demonstrate any statistically significant signal change with a detection threshold of 0.2° for phase and 0.2% for magnitude. Experiments in a dissected eye-OL preparation yielded similar results.

Conclusion—These findings in a large hemoglobin-free nervous system suggest that sensory evoked neuronal magnetic fields are too weak for direct detection with current MRI technology.

Keywords

functional MRI; retina; optic lobe; electroretinogram; local field potential; cephalopod

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INTRODUCTION

Functional magnetic resonance imaging (fMRI) is a leading research tool for noninvasive, *in vivo* investigation of neural activation. Conventional fMRI is based on the blood oxygen level-dependent (BOLD) mechanism, which provides only an indirect measurement of the underlying neural activity and, because of neurovascular coupling, is limited in its spatial and temporal resolution. Recently, there has been great interest in developing alternative MRI techniques that are independent of hemodynamics (1), such as diffusion imaging based on cell swelling (2), Lorentz effect imaging (3) and molecular imaging (4–6). These methods aim to detect changes more closely tied to neural activity than the BOLD effect is, and should in principle offer improved temporal or spatial resolution, or both.

One approach that has generated much interest and debate is neuronal current MRI (nc-MRI), which aims to detect the small magnetic field changes in the brain caused by electrical currents generated during neuronal activation. Such an effect undoubtedly exists and its successful detection would constitute a direct measurement of neural activity. Phantom experiments on nc-MRI have yielded promising results (7–9), but the conclusions from human experiments have been controversial. Detection of a nc-MRI signal has been reported by several groups (10–18), but others have failed to find such effects with current MRI sensitivities (19–23). One important confounding factor in the *in vivo* nc-MRI studies that yielded positive results is the inescapable presence of the BOLD effect. Efforts have been taken to separate the true neuronal response from the BOLD response by utilizing their very different timing characteristics (11,19,23). However this method cannot guarantee complete elimination of the BOLD signal, especially if the hemodynamic response function varies from trial to trial. Thus, a crucial step in the development of nc-MRI is to demonstrate the detectability of the nc-MRI signal under conditions where the BOLD effect is clearly separated or eliminated. Towards this end, tissue preparations without blood have been used to study neuronal current effects using MR spectroscopy. Successful detection of signal magnitude, phase changes or both have been reported in snail ganglia (24), rat brain organotypic cultures (25) and earthworm median giant fiber (26). These studies, although promising, required the use of drug treatments or electric shock to stimulate the tissue, which is likely to have elicited a much greater neuronal response than would be expected in *in vivo* studies employing natural stimulation. To study nc-MRI with sensory stimulation while removing hemodynamic confounds, Luo *et al.* (27) developed an *in vitro* bloodless turtle brain model. Electrical recordings demonstrated clear responses to visual stimulation, but no MRI signal changes were detected.

In this study, we aimed to detect an nc-MRI signal in live octopuses with visual stimulation. Cephalopod blood uses copper-based hemocyanin, instead of iron-based hemoglobin, for oxygen transport. Hemocyanin has been previously found to not differ in its magnetic susceptibility between its oxygenated and deoxygenated states (28–30), and consequently can cause no BOLD MRI effect. Thus, this animal model affords the same advantage as the bloodless turtle brain model (27) in allowing the study of nc-MRI from sensory stimulation in a BOLD-free environment. It has the added advantage that such investigation is carried out *in vivo* in an intact animal. Octopuses have large eyes and optic lobes (OLs) (Fig. 1) that

generate strong electrical responses to visual stimulation. nc-MRI was performed for both the retina and OL to examine the feasibility of direct detection of neural activity-generated magnetic fields. In addition, electroretinograms (ERGs) and OL local field potentials (LFPs) were recorded to characterize the electrical responses to visual stimulation and to confirm tissue health and responsiveness after scanning. The amplitude of ERGs and OL LFPs were found to be up to 6 mV and 0.7 mV, respectively. For comparison, the typical ERG amplitude recorded from human (31) and rat (32) eyes is ~0.5 mV and the LFP studied in the bloodless turtle optic tectum (27) was ~0.3 mV. Although it is difficult to link quantitatively the amplitude of such electrophysiological measurements to the magnitude of neural activity-elicited magnetic field, these observations indicate that the octopus visual system generates a stronger electrical response than that observed in many animals, making it an ideal candidate for nc-MRI studies. In addition to *in vivo* imaging, we also performed nc-MRI scans on isolated octopus eye/OL preparations with the optic nerves intact. This preparation was developed in an effort to increase the signal-to-noise ratio (SNR) and reduce the influence of seawater on the MRI signal.

METHODS

Animal preparation

Octopus bimaculoides adult females weighing between 50 g to 110 g were wild-caught off the southern California's coast and supplied by Aquatic Research Consultants (San Pedro, CA). The octopuses were dark-adapted overnight and anesthetized by immersion for 15 minutes in 1.2% – 1.8% ethyl alcohol diluted in artificial seawater (ASW; Tropic Marin, Montague, MA), the concentration of ethanol varying according to animal size and activity. To ensure illumination of the retina, a piece of the eyelid covering one eye was removed and the part of iris covering the lens was resected. The anesthetized animals were then ready for *in vivo* electrophysiological recording or nc-MRI scans.

For the isolated eye/OL preparation, the skin surrounding one eye was removed to expose the orbit and the white body. The transparent membrane that covers the back of the eye and the white body was opened and the white body was carefully removed, thereby exposing the OL and the optic nerves but leaving the optic nerves intact. The optic tract connecting the OL to the central brain was severed and any tissues remaining attached to the eye were removed. The eye/OL explant with optic nerves attached was lifted from the head and quickly transferred to oxygenated ASW.

Visual stimulation

Visual stimuli were produced by a white light LED controlled by transistor–transistor logic pulses from a PC running E-Prime (Psychology Software Tools, Pittsburgh, PA). During the nc-MRI scans, the E-Prime paradigm was triggered by transistor–transistor logic pulses from the scanner. The time delay between the stimulus onset and image acquisition was controlled by E-Prime. The light was carried to the eye with a fiber optic cable. In all experiments the exiting end of the cable was placed ~5 mm away from the lens of the eye to be stimulated. The nc-MRI experiments were performed with inter-stimulus interval (ISI) =

2 s, and flash duration = 50 ms and 100 ms for the *in vivo* and *in vitro* studies respectively (see Results).

Electrophysiology measurements in live octopuses

To determine the optimal parameters for visual stimulation and nc-MRI pulse sequence, neural responses in the retina were measured with ERGs in both live octopuses and isolated eye/OL preparations. For *in vivo* ERG measurements, we used procedures similar to those described by Hamasaki (1968). Thin copper wires were placed in each eyelid. The wires stabilized the eye and allowed it to be lifted above ASW to prevent short-circuiting. The ERG signal was picked up with a homemade cotton wick electrode (32,33). The electrode was impregnated with ASW and its tip was placed on the anterior surface of the eye adjacent to the lens. The reference electrode was placed in the ASW bath, which was grounded.

For LFP recordings in the OL, an incision was made in the skin behind the orbit. The transparent membrane covering the white body as well as part of the white body were removed to expose the optic lobe. A stainless steel electrode was inserted ~1 mm into the optic lobe and the reference electrode was placed in the ASW.

To establish the optimal stimulation parameters, ERGs and OL LFPs were recorded in 3 and 2 animals respectively, over a wide range of visual stimulus conditions. The stimulus duration was varied from 1 ms to 100 ms, and the ISI ranged from 200 ms to 5 s, and each recording condition was repeated 10–20 times.

In addition, for all animals that underwent nc-MRI scan, ERGs were recorded both before and immediately after the scan. OL LFPs were measured in these animals only after the scan, as the recording process progressively shut down activity in the OL (see Results).

Electrophysiology measurements in dissected eye/OL preparations

ERGs and LFPs were recorded from the eye/OL explants in procedures similar to those employed for *in vivo* measurements. For ERG recording, the anterior part of the eye was lifted above ASW. The tip of a cotton wick electrode was placed on the surface of the eye next to the lens. For LFP recordings, the OL was slightly raised so that its dorsal surface was above ASW. A stainless steel electrode was inserted ~1 mm into the OL. For both ERG and LFP recordings, the reference electrode was placed in the ASW, which was grounded. To determine the effect of stimulus duration and ISI, ERGs and OL LFPs were recorded in 4 and 2 explants respectively, over a range of stimulus durations and ISIs. For the 6 eye/OL preparations that underwent nc-MRI scans, ERG was recorded both before and after the scans, and LFP in the OL was only recorded after the scans.

nc-MRI acquisition

nc-MRI scans were performed on a Bruker 9.4 T animal scanner (Bruker, Karlsruhe, Germany) with a purpose-built 2-cm-diameter receiver coil. For *in vivo* study, the head of the animal was restrained in a cylindrical tube with an inner diameter of 2 cm, while the mantle lay in a larger container to allow the octopus to breathe freely. The container was filled with ASW, which was slowly bubbled with oxygen to facilitate respiration. During nc-

MRI scans, the ethyl alcohol concentration was lowered to 0.6% – 1%. For *in vitro* study, the eye/OL preparation was placed in a cylindrical tube with a 1.5 cm inner diameter and oxygenated ASW was slowly circulated through the tube. For both the *in vivo* and *in vitro* preparations, a few small cotton balls were placed in the tubes to help fix position and reduce sea water volume.

A single shot gradient echo (GE) EPI sequence was used to capture the phase shift caused by neuronal magnetic field. A GE sequence was chosen over a spin echo (SE) sequence because the durations of ERG and OL LFP responses are long compared to the optimal echo time (TE), and phase cancellation is likely to occur in a SE sequence. The following scan parameters were used: FOV = $2 \times 2 \text{ cm}^2$, matrix size = 64×64 (in-plane resolution = $0.31 \times 0.31 \text{ mm}^2$), slice thickness = 1 mm, TR = 1 s and flip angle = 70° (Ernst angle). T_1 of both the retina and the OL were found to be in the range of 900 – 1100 ms. For *in vivo* studies, two slices were employed to capture potential signal changes in the retina and the OL respectively. The time delays between visual stimulation and MR acquisitions were chosen so that the two slices corresponded to the peak electrical activities in the retina and the OL (Fig. 2C and D). The acquisition windows were placed on the plateau of the electrical responses instead of on their slopes because the neuronal magnetic field is mainly caused by a quasi-static distribution of neuronal currents, not by the change of electrical potentials (34). The optimal TE for detecting signal phase and magnitude changes was found to be 18 ms and 27 ms, respectively, using methods described by Luo and Gao (35). Eight animals were scanned. In six animals, TE was set at 18 ms to optimize the detection sensitivity for signal phase change. In the other two animals, TE was set as 27 ms to optimize the sensitivity to signal magnitude change. Emphasis was placed on detection of phase change because previous simulation studies indicated that phase change was likely to dominate for physiologically-evoked activity (36). Each animal was scanned for two hours with TR = 1 s and 8 dummy scans, and an ISI of 2 s was used. This yielded a total of 7200 time points, which were evenly divided between the rest and the activated states to maximize statistical power. Anatomical images of the same slices were acquired using the rapid acquisition with relaxation enhancement (RARE) sequence, with TR = 2 s, TE = 57.8 ms, flip angle = 80° , RARE factor = 8 and matrix size = 128×128 .

For nc-MRI scans of the dissected eye/OL preparations, three slices were prescribed to capture the neuronal activity. The second slice was acquired during the peak electrical activities in both the retina and the OL. The other two slices covered the rising and falling periods of the activity (Fig. 3C and D). In these tissue preparations, physiological noise was assumed to be negligible and TE was set to be equal to T_2^* of the tissue ($\sim 14 \text{ ms}$) to maximize detection sensitivity to phase changes (34). Six preparations were scanned with parameters similar to the *in vivo* studies, except that the scan duration was one hour and TE was 14 ms.

nc-MRI data analysis

The nc-MRI data were analyzed with the AFNI software package (37). Both the magnitude and phase images were first analyzed at the voxel level. No slice timing correction was performed since the acquisition time of the two slices were each aligned according to their

own peak activity. 2D motion correction was performed within each slice. The estimated motion was less than 0.1 mm for *in vivo* scans and negligible for *in vitro* scans. The magnitude and phase time series at each voxel were high-pass filtered with a cutoff frequency of 0.02 Hz to remove slow temporal drift. The residual time series was analyzed using the general linear model (GLM). A regressor was constructed with an impulse response at every other TR, without convolution with the hemodynamic response function. To take into account the decay of electrical response in the visual system over course of the scan, the regressor was modeled with a linear decay over time. The rate of decay was determined for each animal based on the ratio of ERG amplitudes recorded before and after the scan. This ratio was used for both retina and OL since only ERG was recorded both before and after the scan. The regressor was then independently fitted to the time course at each voxel and a t score was calculated. The resulting t statistic map was thresholded at the $p < 0.01$ level to search for evidence for activation in the visual system. The statistical map was then corrected for multiple comparison to the $p < 0.05$ level by clustering. The cluster size was determined by Monte Carlo simulation.

Since a cluster analysis might miss highly localized activations, the nc-MRI data (both magnitude and phase) were also analyzed with a permutation test (38). Five thousand random relabelings of the original time series were created for each voxel, which was considered sufficient as it theoretically allows for a smallest obtainable p-value of 0.0002 and asymptotic behavior is typically reached with 1000 relabelings (38). A t-score was obtained from each relabeling by analysis with the same GLM method described above. The distribution of the t score was used to calculate the p value at each voxel. The p-statistic map had a threshold of $p < 0.01$. Correction for multiple comparison was performed using the maximal statistic method (38), where the maximal statistic was chosen to be the maximum of the absolute value of the t score obtained from each relabeled dataset. The distribution of the maximal statistic was used to calculate the corrected p value, which was then thresholded at the $p < 0.05$ level to determine whether any voxel was activated in the visual pathway.

nc-MRI data was also examined in a region of interest (ROI) manner. ROIs for the retina and the OL were manually drawn on the magnitude images for each of the animals and for the tissue preparations. A ROI-averaged time series was generated for each of the ROIs. The resulting time series were examined using the same GLM and the same permutation test procedures as described for the voxel-wise analysis.

RESULTS

ERG

The ERG recordings were very robust and consistent across animals. Fig. 2A shows a typical ERG recording in a live octopus responding to white light flashes of varying durations with ISI = 2 s. The latency to response was 10–14 ms with a negative deflection of up to –6 mV peaking at ~35 ms. The width of the negative peak increased with the duration of light stimulation. The amplitude of the response increased as the duration of light stimulation was increased from 1ms to 10 ms but then plateaued, suggesting that flashes longer than 10 ms are sufficient to elicit the maximal electrical response. Fig. 4A shows

ERG responses to repeated stimulations with different ISIs. There was little adaptation effect observed for ISIs greater than 1 s. For ISIs less than 1 s, the latter trials have significantly smaller amplitude than the first trial, and the ERG does not have sufficient time to return to base line. Based on this information, an ISI of 2 s was chosen for nc-MRI scans. Our findings agree well with previous studies of ERGs in octopus eyes (33,39). Fig. 3A shows a typical ERG recording from the dissected eye/OL preparation. The response latencies were similar to those seen in the *in vivo* recordings, but the ERG took longer to reach peak amplitude. In addition, a longer stimulus duration (~ 200 ms) was required to elicit the maximum response. The peak amplitude measured *in vitro* (up to -1.2 mV) was considerably smaller than that found *in vivo*.

For all the animals and eye/OL dissections that underwent nc-MRI scans, an ERG was recorded both before and immediately after the scan, to confirm the vitality of the tissue during the scan (Figs. 2C and 3C). The average ERG amplitude from eight live animals was -3.75 ± 0.96 mV before scanning and -1.32 ± 0.43 mV after scanning. The average ERG amplitude from the six eye/OL preparations was -2.67 ± 1.62 mV before scanning and -0.44 ± 0.23 mV after scanning.

LFP in the OL

LFP recordings in the OL were more complex in shape than the ERGs and varied with the location of electrode. Fig. 2B shows a typical OL response to light flashes of differing durations recorded in one animal with an ISI = 2 s. The latency was between 20 – 30 ms, with a positive deflection which reached its peak between 70 – 100 ms. In some cases, a weaker peak was observed at ~ 60 ms. The amplitude of the main deflection was between 0.3–0.6 mV, and remained stable for stimulation durations from 10 ms to 200 ms. The width of the deflection increased with the stimulus duration. Fig. 4B shows OL LFP responses to repeated stimulations with different ISIs. Similar to the ERG results, the adaptation effect is only important for ISIs less than 1 s so an ISI of 2 s was employed. Fig. 3B illustrates a typical OL response from an isolated eye/OL preparation. The *in vitro* recordings had a similar timing to the *in vivo* recordings but with reduced amplitudes (0.07–0.15 mV). In a few recordings with stimulus durations longer than 50 ms, a second positive deflection was observed around 135 ms (Fig. 3B). The reproducibility of this additional peak was, however, not reliable and it was not studied in nc-MRI scans.

The recording of LFPs in the OL was less robust than the ERG recordings. After insertion of the electrode, the LFP weakens over time and typically vanishes after 10–20 mins. This phenomenon was also reported by Boycott *et al.* (1965), who suggested that the octopus responds to injury with local vascular spasms that compromise activity in the injured region. For this reason, in the live animal and *in vitro* preparations that underwent nc-MRI scans, LFPs were only recorded after the scan to confirm tissue vitality. The average LFP amplitude after scanning was 0.07 ± 0.02 mV for the eight live animals and 0.04 ± 0.02 mV for the six eye/OL preparations.

nc-MRI

Fig. 5A shows p-statistic maps for phase change from one animal overlaid on the corresponding anatomical images. If there were any statistically significant MR signal change due to visual stimulation, a concentration of activated voxels would be expected in the retina in the 1st slice and in the OL in the 2nd slice. The activated voxels were, however, scattered across the image. No activated voxels were found in the retina in any of the eight animals. A few activated voxels were seen in the OL in two animals, but appeared to be distributed at random. The statistic map was then corrected for multiple comparisons using a cluster analysis approach. After this procedure, no cluster survived at the $p < 0.05$ level. The results from magnitude images were similar.

Representative p-statistic maps generated with the permutation test are shown in Fig. 5B, thresholded at the $p < 0.01$ level (uncorrected) and overlaid on EPI images. The activation patterns were similar to those obtained with the GLM model, but with a few additional activated voxels. No statistically significant activation was found in the retina or OL. The p-statistic map was then corrected for multiple comparisons using the maximal statistic method (38). After this procedure, no voxel survived at the $p < 0.05$ level.

The temporal signal-to-noise ratio (tSNR) for signal magnitude in the retina and OL was between 25 and 40. The temporal phase stability was between 2° and 4° . Based on power analysis (40), we estimate the detection thresholds in our experiments to be less than 0.2% for magnitude and 0.2° for phase at the $p < 0.05$ level.

The statistical results for *in vitro* study were similar to those from *in vivo* studies. For both phase and magnitude images, the activated voxels appeared to be scattered at random, and no statistically significant activation was observed in any of the preparations. Fig. 6 shows representative p-statistic maps for phase change in one tissue preparation, with the threshold set at the $p < 0.01$ level (uncorrected). After correction for multiple comparisons, no cluster or voxel survived at the $p < 0.05$ level.

The tSNR of signal magnitude for *in vitro* study was between 60 and 90 and the phase stability was between 0.5° and 1.5° . Based on power analysis (40), we estimate the detection threshold in this experimental setting to be better than 0.1% in magnitude and 0.1° in phase at the $p < 0.05$ level.

In ROI analysis, none of the ROI-averaged time series from the 14 animals under study showed evidence for activation at $p < 0.05$ level, either with the GLM or permutation test.

DISCUSSION AND CONCLUSION

In this work we adopted a unique animal model for studying the feasibility of detecting neuronal current-induced magnetic fields by MRI. Under our experimental conditions, no statistically significant phase or magnitude change was observed in the octopus visual system at the $0.2^\circ/0.2\%$ level for the *in vivo* study and at the $0.1^\circ/0.1\%$ level for the *in vitro* study. The results from the present study and those from our previous work on bloodless turtle brain model (27) suggest that sensory evoked potentials are too weak for direct nc-MRI detection. These results are consistent with the conclusions from previous human

studies in which the BOLD effect was carefully controlled (19,21–23). They suggest that previous positive findings (10,11,13,17) were not due to true neuronal magnetic field changes, and were more likely the result of residual hemodynamic effects.

Previous reports (24–26) using isolated tissue preparations yielded results in favor of detection of nc-MRI signal changes. The difference between the conclusions of the present work and those of previous studies might in part be explained by two factors. First, vastly different sources of neuronal magnetic field were employed to search for MR signal changes. In the study by Park *et al.* (2004) using snail ganglia and that of Poplawsky *et al.* (2012) with earthworm median giant fibers, the experiments were designed to capture the magnetic field induced by action potentials in axons. The action potential produces a dipolar magnetic field close to the axon, which could be as large as 1.8 nT at its surface (24). Away from the axon's surface, however, the magnetic field becomes quadrupolar and decays rapidly with distance (34). By isolating very large axons or axon bundles, it was possible to place the radiofrequency (RF) coil in the close proximity to the axon and thereby detect a large dipolar magnetic field. Such a condition is challenging to replicate in *in vivo* studies, where the RF coil is likely at best to be a few centimeters away from the neural source and the quadrupolar axon magnetic field would be too small to be detected. On the other hand, processes such as postsynaptic potentials and voltage-gated membrane potentials generate dipolar magnetic fields that are likely to dominate at large distances (34). The aggregated effect of these activities can be captured by the LFP, which lasts tens to hundreds of milliseconds. Consequently, most nc-MRI studies to date (10,11,13,19,20,22), including the present work, have targeted these slower, more extended processes. The unique conditions in Park *et al.*'s and Poplawsky *et al.*'s experiments afforded the advantage of being able to detect the field changes related to action potentials, which is different from the objective in most *in vivo* nc-MRI studies. The study on rat brain tissue culture (25) reported on induced epileptic activity, which is known to produce an at least 40 times larger equivalent current dipole than does physiologically-evoked activity (34).

A second factor that may have contributed to the negative findings of the present study is the relatively low temporal SNR. This was in part the result of using a small voxel size ($0.31 \times 0.31 \times 1 \text{ mm}^3$), which was chosen in an effort to reduce partial volume effects in the octopus retina ($\sim 500 \mu\text{m}$ thick (41)). A second, more important contributor to the low SNR is the complication of imaging with the presence of ASW. The conductivity and the dielectric effect of the ASW lowers the Q factor of the RF coil, thereby reducing the SNR (42). A 60% decrease in the coil Q factor due to the presence of ASW was found in our experiment. Similar finding has been previously reported at 4.7 T (43). We attempted to reduce the salinity of the ASW, but found that the reduction necessary to shift the SNR was incompatible with animal survival and explant tissue health. Blackband *et al.* (43) made similar observations. Our experiments were consequently conducted with full-strength ASW, but we reduced the amount of ASW “seen” by the RF coil by fitting the animal head snugly within a small tube and squeezing cotton balls into the spaces between the head and the tubes. The experiments on the dissected eye/OL preparation were also in part motivated to reduce the amount of ASW within the coil.

Theoretical simulations of the nc-MRI effect have given a wide range of predictions regarding the expected signal change. Optimistic simulation schemes using current dipole models yielded results up to a few percent of magnitude change and 1° of phase change (44,45). Other, more conservative simulations drawing on physiological data on human neurons have led to predictions of signal changes below 10^{-5} in magnitude and 10^{-3} degree in phase (36,46). The results of the present study indicate that nc-MRI signal change could not be detected in the octopus visual system at the 0.2%/0.2° level *in vivo* and at the 0.1%/0.1° level *in vitro*. Though it is difficult to relate these conclusions directly to human studies, they strongly suggest that the conservative simulation models (36,46) are more realistic.

In conclusion, a novel animal model was developed that allowed both *in vivo* and *in vitro* study of nc-MRI effect without BOLD contamination. However, no statistically significant signal change was observed with sensory stimulation. These results suggest that sensory evoked activities are too weak for direct detection with current nc-MRI schemes. It is possible that stronger and more coherent neuronal activations, such as is seen with spontaneous neural oscillations (12) and epileptic seizures (14,18), could be detected with nc-MRI, but it is likely that very different strategies, such as measurement of the neuronal magnetic fields using more sensitive superconducting quantum interference devices (SQUID) (47), will be necessary for functional nc-MRI.

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Fig. 1.

MRI slice through the *Octopus bimaculoides* visual system acquired with a T₂-weighted RARE sequence. Retinal fibers project topographically across the surface of the optic lobe, with most ending in a superficial plexiform layer (arrows), but some traveling deeper to a radial layer (r) of irregular columns of cells and fibers. The optic lobe's center (c) is composed of islands of cells enmeshed in a network of axons and dendrites. Output fibers of the lobe travel by the optic tract (asterisk) to the central brain. VL, vertical lobe of the central brain.

Fig. 2.

In vivo measurement of electrical response in the octopus visual system. (A) ERG responses to light flashes of varying durations. (B) LFPs in the OL elicited by light flashes of differing durations. Measurements in A and B were made immediately after electrode implantation. (C) ERG responses in one octopus before and after two hours of nc-MRI scanning. (D) LFP recorded in the OL after two hours nc-MRI scanning in the same octopus presented in C. For all plots, the light stimulus began at 0 ms. In C and D the stimulus duration was 50 ms, which is indicated by the black boxes on the bottom axis. The grey boxes on the top axis show the acquisition windows of the two MR slices. All curves were obtained by low passing the raw data at 100 Hz and averaging it over 10 – 15 repetitions.

Fig. 3.

In vitro measurement of electrical response in dissected octopus visual system. (A) ERG responses to light flashes of varying durations. (B) LFP recordings in the OL induced by light flashes of differing durations. Measurements in A and B were made immediately after electrode implantation. (C) ERG responses in one octopus before and after one hour of nc-MRI scanning. (D) LFP recorded in the OL after one hour nc-MRI scanning in the same explant presented in C. In all the plots, the light stimulus began at 0 ms. In C and D the stimulus duration was 100 ms, which is indicated by the black boxes on the bottom axis. The grey boxes on the top axis show the acquisition windows of the three MR slices. All curves were obtained by low passing the raw data at 100 Hz and averaging over 10 – 15 repetitions.

Fig. 4.

In vivo ERG (A) and OL LFP (B) responses to repeated stimulations. Four ISIs from 0.2 s to 2 s were tested using a stimulation duration of 50 ms. An ISI of 2 s was chosen for the nc-MRI scans of both structures.

Fig. 5.

(A) RARE images from an *in vivo* nc-MRI scan. The color map shows the statistical test results using the GLM thresholded at the $p < 0.01$ (uncorrected) level. (B) EPI images for the nc-MRI scans of the two corresponding slices. The color maps show the statistical test results from the permutation test, thresholded at the $p < 0.01$ (uncorrected) level. The acquisition windows of the first and second slices corresponded to the peak activity in the retina and the OL respectively (see Fig. 2C and D). The B_0 field was perpendicular to the slices and hence also perpendicular to the photoreceptor cells in the retina.

Fig. 6.

(A) RARE images from an *in vitro* nc-MRI scan. The color map shows the statistical test results using the GLM, thresholded at the $p < 0.01$ (uncorrected) level. (B) EPI images for the nc-MRI scans of the three corresponding slices. The color maps show the statistical test results from the permutation test, thresholded at the $p < 0.01$ (uncorrected) level. The acquisition window of the second slice corresponded to the peak activity in the retina and the OL. The first and third slices covered the rising and falling period of the electrical activity (see Fig. 3C and D). The B_0 field was perpendicular to the slices, and hence also perpendicular to the photoreceptor cells in the retina.