Resting State Connectivity in the Teleost Fish: An Exploratory Study

Alex TL Leong[1,2], Russell W. Chan[1,2], Iris Y. Zhou[1,2], Adrian Tsang[1,2], and Ed X. Wu[1,2]

[1]*Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China,* [2]*Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China*

INTRODUCTION Resting state fMRI (rs-fMRI) has become one of the most widely used method to study the basis of functional connectivity in different brain regions in the absence of external stimuli. It has been applied to study the brain dynamics in mammals including humans [1], macaques [2] and rodents [3]. However, there is a clear unexplored possibility of resting state modality being utilized to study the spontaneous fluctuations of low frequency hemodynamic changes in simpler forms of vertebrates to understand primitive brain functions that has survived the course of evolution. In this study, we investigated for the first time the possibility to identify resting state network using the BOLD signal in the teleost fish which is one of the most diverse species in the world and is considered a much simpler life form with a rich evolutionary significance.

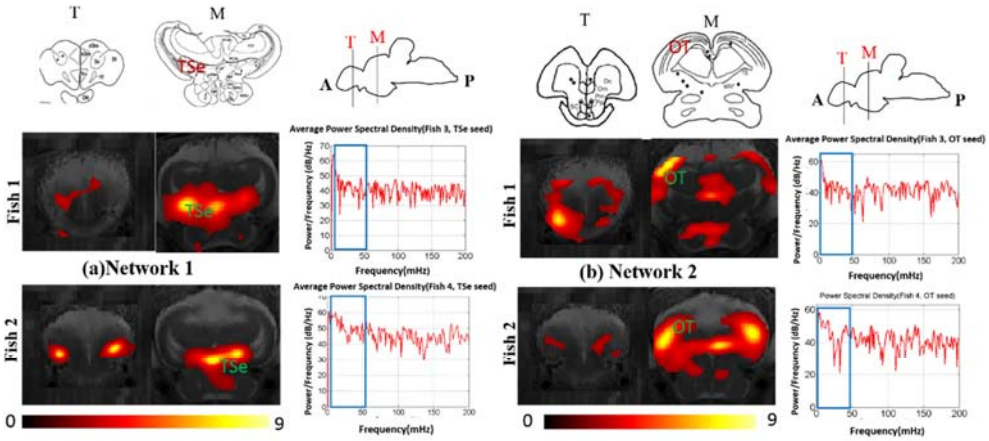

Fig. 1 Top row: Structural connectivity map taken from [4, 6] at the level of telencephalon, T and midbrain, M. Middle and bottom row: Spatial ICA map thresholded at z>1 and the average power spectral density extracted from locations with the highest positive activation namely the Torus Semicircularis, TSe and Optic Tectum, OT for fish 1 and 2 respectively. Lateral line auditory network (network 1) and somatosensory integration network (network 2) delineated from ICA have comparable spatial localization with fish neuroanatomy (top row). Both networks have consistent low frequency band dominance (indicated by the blue box at each power spectral density map).

METHODS **Animal Preparation:** Adult carp fish, (150~200g and 9~11cm in length) is used in acquiring the rs-fMRI scans (N=6). The carp fish is first anesthetized with 150mg/L of clove oil (50% eugenol, Baidyanath Pharmaceuticals) and subsequently lowered to 40mg/L during scans. **Data acquisition:** Rs-fMRI scans were acquired for the alive fish using a 7T Bruker scanner with single shot GE-EPI sequence (TR/TE=750ms/18ms, Flip angle=50˚, FOV=32x32mm^2, matrix =64x64 and 10 contiguous 1mm slices). In addition, rs-fMRI scans were also acquired from freshly euthanized adult carp fish (N=3). **Data analysis:** All rs-fMRI scans were slice-timing corrected, Gaussian smoothed and detrended in MATLAB. However, the usual practice of temporal band pass filtering was not employed due to the exploratory nature and novelty of the study. Individual Independent Component Analysis (ICA) is first performed using GIFT followed by seed based analysis on networks that are of importance. Spectral analysis is also performed for further quantification of the difference in resting state networks between the alive and dead fish.

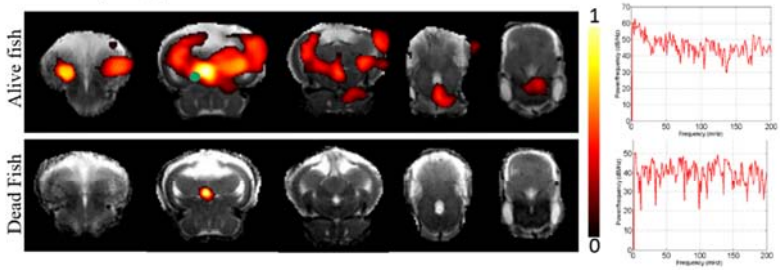

Fig. 2 Similar seed location (green circle) is used for seed based analysis to generate the CC maps thresholded at r>0.5. Distinct differences in the power spectral density for the dead fish indicates no low frequency band domination.

RESULTS Long-range networks were found in 2 out of the 6 alive fish that are functionally localized at the torus semicircularis [4] and at the optic tectum [5] with some somatosensory integration areas in the telencephalon [4] (**Fig. 1**). Spectral analysis of the first and second long range networks show similar low frequency band (<0.05Hz) domination (**Fig. 1).** ICA and spectral analysis performed on the 3 dead fish shows no spatial specificity in any functionally relevant networks and without any low frequency band domination. The mean cross correlation map of one representative dead fish and alive fish is shown in **Fig. 2.** The 4 other fish show sparse unilateral and local networks which are generally of lower interest in rs-fMRI and hence the data are not reported but will be kept for analysis in our further study.

DISCUSSION AND CONCLUSION

Despite some notable SNR limitations due to the small brain size of the fish, we were still able to show distinct functional networks in the alive fish with low frequency dominance. The lower SNR of the functional images compared to similar protocols performed on rodents is sufficient as it was able to demonstrate differences between the alive and dead fish. This allows the quantification of functional connectivity to show dominance in the low frequency regime and reject the notion of spurious activations due to noise. A close inspection of the power spectral density maps show dominance in the frequency bands of <0.05Hz and this correlates well with the power spectra of neuronal activities in a recent recording study on zebrafish[7]. **Fig.1a** shows a prominent auditory lateral line network in the fish centered in the torus semicircularis and parts of the ventral telencephalic regions [4]. A visual and sensory integration network (**Fig 1.b**) is also seen centered at the optic tectum and parts of the dorsal and ventral telencephalic regions which have numerous known neuroanatomical projections to other mechanosensory areas. Although such important functional networks can only be seen with close anatomical specificity to fish neuroanatomical studies in two fish, it still provides us motivation to further investigate the nature of resting state networks in fish as the effects of anesthesia on fish has been suggested to vary significantly for fish of the same species [8]. Therefore, future study would involve studying the effects of anesthesia levels on fish physiology as it is crucial for establishing a rs-fMRI study. In conclusion, this exploratory study has shown the feasibility to reveal RSN in fish and paves the way for resting state to be a prominent tool in unlocking the mystery of the brain of a simpler life form.

REFERENCES [1]Biswal B, et al. Magn Reson Med 1995;8:700-11. [2] Vincent JL, et al. Nature 2007;447:83-86. [3]Pawela C, et al. Magn Reson Med 2008;59(5):1021-1029. [4]Yamamoto N, et al. J Comp Neurol 2010;518:2475-2502. [5] Luiten PGM, Brain Res 1981;220:51-65. [6] Pérez-Pérez MP, et al. Vis.Neuroscience 2003;20:397-410 [7] Ahrens MB, et al. Nature Methods 2013;10:413-420.[8] Neiffer DL, et al. ILAR J 2009;50(4):343-360.