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Dates of Rotation: 2015.3.23 – 2015.5.27

There were two goals in this rotation. First, developing an automated behavior tracking and feedback control system that facilitates the simultaneous recording of cortical AMPAR dynamics in behaving animals under two-photon microscope. Second, a more naive one, that obtaining some practical understandings of biochemistry data through learning a relevant technique.

AMPARs play crucial roles in LTP and LTD expressions (Huganir and Nicoll, 2013). Using two-photon imaging and pH-sensitive fluorescent labeling, Zhang et al. were able to observe and quantify AMPARs trafficking *in vivo* with an unprecedented spacial-temporal precision (Zhang et al., 2015). In order to study the relationship between AMPARs dynamics and learning, a task of reaching and retrieval of food pellets was adopted to train the mice (Chen et al., 2014). As one of the long term goals, we are curious about the “online” AMPARs dynamics in motor cortex while the animal is going through trial-and-error. To that end, an automated behavior tracking and feedback control system (nicknamed R5, stands for **R**eaching and **R**etrieval Behavior System for **R**ichard **R**oth in **R**ichard Huganir’s Lab) is developed for manipulations in the dark under two-photon microscope and for recording of behavior data. In addition to automatic discrimination of trial results (e.g. hit, miss, and fail), R5 provides more quantitative measurements of behavior including real-time paw kinematics in 3-dimensional space up to 120Hz sampling rate (requires high-end computer). The detailed characterization may allow us to better correlate the AMPARs dynamics with behavioral significance and provide further insight to the systemic mechanisms of the underlying learning process (Wu et al., 2014). The rules for real-time feedback interaction with the animal is programmable with graphical user interface to certain extent, which enables the design of more complex tasks.

Subunits of AMPARs and NMDARs are actively modulated by various molecular players in response to specific neuronal activities (Huganir and Nicoll, 2013). To corroborate the AMPARs dynamics observed via imaging and to further investigate the underlying mechanisms, the quantity of GluA1, S831 phosphorylation of GluA1, S845 phosphorylation of GluA1, GluA2, GluN2B, and PSD95 (for normalization) in synaptosome extracts from the motor cortex of three groups of mice were measured using Western blots. The first group was WT (n = 3), as one control. Mice in the second (n = 3) and third group (n = 3) were trained on the reaching and retrieval task for two and four days, respectively. Measurements from the hemisphere corresponding to the unpreferred paw were used as another control. Behaviorally, the success rate of 4 out of 6 mice in the two training groups generally increased overtime. Biochemically, however, the signal to noise ratio (based on the absolute blot intensity) of most quantifications, especially those in earlier experiments, were too low to be accountable. Possible reasons for the failure include inconsistent dissection of the motor cortex (some tissue blocks were too small) and the prolonged operation time of synaptosome purification.

References

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