Locomotion in Tethered *Drosophila melanogaster* through Virtual Worlds

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A fundamental goal of neuroscience is to understand how neural circuits transform sensory signals into sensory-guided behavior. Virtual Reality (VR) systems provide an experimental platform where animals perform naturalistic-like behaviors under precise control of sensory stimuli. In these systems, animals are usually restrained, and walk on the spot on moving treadmills, which, in turn, track the animal's locomotion and close the loop between behavior and the virtual environment to recreate an immersive experience [reviewed in 3]. Furthermore, restrained animal preparations are ideal for optically recording the activity of large populations of neurons in simultaneous with behavior [GCaMP6]. We recently developed *FlyVRena*, a VR system for tethered walking *Drosophila* that is based on a tracking system [4], a novel software platform, and a high-speed projection system [5]. Using FlyVRena, we studied visually guided locomotion in flies, an ethologically relevant task for this highly visual animal. Tethered flies walking on a spherical treadmill (an air-suspended ball) can perceived and interact with 3D-VR worlds as showed in spatial orientation tasks (Cruz *et al*, unpublished). However, because both the animal's restrained position and the moving treadmill itself constrain the locomotive behavior, it is difficult to make comparisons to natural walking conditions. Because our goal is to understand the link between neural activity and oriented walking, it is necessary to characterize those constrains, and analyze the consequent imposition they exert on locomotion. Here we present our attempts to characterize locomotion in tethered *Drosophila* walking on a ball through visual VR worlds. Using the dynamical signals from the treadmill tracking system, which measure the ball rotations, we first noticed different modes of ball motion induced by the fly. Notably, a subset of these modes correlates with high behavioral performance in orientation tasks, suggesting that they may represent naturalistic walking gates. To analyze the correspondence between ball rotations and walking under visual stimulation, we recorded the animal's locomotion using both the tracking system and videography [6]. Previous work has attempted to characterize leg movements with ball rotations [7]; however, this technique only detects the x-y position of the legs, which prevents recording other behaviors such as pushing and pulling of the ball, or abdomen movements. We therefore set two orthogonal cameras to measure leg and abdomen movement in 3D. Using this information, we developed a decoder to extract ball rotations associated with coordinated walking. Furthermore, we compared different kinetic parameters of free walking fly trajectories with those from reconstructed virtual trajectories in order to explore the effects of the constrains imposed by the VR system. The results we present in this work provide an unbiased description of the fly's maneuvering of the ball.

References

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