Sequential synaptic activation in the brain for voluntary initiation of walking in the crayfish, *Procambarus clarkii*.

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Prior to the initiation of voluntary behavior, the readiness or preparatory neural activity can be recorded in specific regions of the animal brain. However, the neurons and synaptic mechanisms mediating the activity in the brain are unknown. Intracellular recording and staining of the descending neurons in the brain of freely behaving crayfish revealed that the readiness discharge was shaped not only by synaptic excitation but also by subsequent inhibition. We also found other two types of descending neurons: One is the continuation type neurons, which increased their spike discharge rate a few hundreds milliseconds prior to the behavioral onset and maintained it during walking. The other is the termination type neuron, which increased their spike discharge rate before the behavioral offset of walking. These neurons tended to project their dendrites to the medial protocerebrum and extended their axon collaterals in the tritocerebrum. We additionally identified local neurons whose synaptic activities showed significant changes before the behavioral onset. It is therefore, suggested that the collaterals of descending neurons activate those recurring local interneurons. We propose that the readiness discharge is mediated by sequential synaptic events in the protocerebrum and tritocerebrum without feedback signals from downstream ganglia.