Journal of Insect Physiology 108 (2018) 48–53

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Short Communication

The use of MEMRI for monitoring central nervous system activity during intact insect walking

Daniel Knebel[a,b], Yaniv Assaf[b,c], Amir Ayali[a,b,*]

[a] School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
[b] Sagol School of Neuroscience, Tel Aviv University, Tel Aviv, Israel
[c] Department of Neurobiology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

ARTICLE INFO

Keywords:
Locust
Locomotion
Walking
Manganese enhanced magnetic resonance imaging (MEMRI)
Subesophageal ganglion (SEG)
Higher motor centers

ABSTRACT

Background: Monitoring neuronal activity in the intact behaving animal is most desired in neuroethological research, yet it is rarely straightforward or even feasible. Here we present the use of manganese enhanced magnetic resonance imaging (MEMRI), a technique allowing monitoring the activity of an animal's nervous system during specific behavioral patterns. Using MEMRI we were able to show activity in different ganglia of the central nervous system of intact locusts during walking.

Results: We injected two groups of locusts with manganese, which serves as a magnetic contrast agent. One group was forced to walk on a treadmill for two hours, while the other was immobilized and served as a control. Subsequently, all animals were scanned in a T1 MRI protocol, and the accumulation of manganese in the neuronal tissues that were active during walking was demonstrated by comparing the scans of the two groups. Two neuronal sites showed significantly higher T1 signal in the walking locusts compared to the immobilized ones: the prothoracic ganglion, which locally controls the front legs, and the subesophageal ganglion, a head ganglion which takes part in initiation and maintenance of walking.

Conclusion: MEMRI is a potent, non-invasive technique for monitoring neuronal activity in intact locusts, and arthropods in general. Specifically, it provides a promising way for revealing the role of central and high-order neuronal structures in motor behaviors such as walking.

1. Introduction

Revealing the functional role of different neuronal centers in animal behavior is in the essence of neurobiological studies, and specifically in neuroethology: the study of neural mechanisms that underlie natural behavior. This is particularly challenging in the case of the central nervous system of intact, freely behaving, animals. Various techniques with different time and spatial resolutions were developed to allow accessibility to brain functions, while monitoring behavior. In humans, mostly non-invasive electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) are used, and some medical procedures provide opportunity for more precise methods such as electrocorticography (ECoG). In other organisms, invasive methods, such as extra- and intracellular recordings directly from neuronal tissues are possible and vastly used. Recent advances have also allowed optical recordings for this purpose. However, all of these methods are greatly restricted by the area probed. Moreover, they tend to impact both the repertoire of behaviors demonstrated by the animal, and the behaviors themselves. One additional limitation is that most methods were optimized for leading animal models such as mice, rats, zebrafish and Drosophila, and are much less adopted to other vertebrates and invertebrate preparations. Here we report on overcoming some of these hurdles by the use of the manganese enhanced MRI technique (MEMRI; Massaad and Pautler, 2011; Herberholz et al., 2011) for correlating central nervous system activity and behavior in an invertebrate. We utilized a well-established invertebrate model, the desert locust, *Schistocerca gregaria* (Forskål), and focused on its walking behavior.

Manganese ions (Mn^{2+}) are paramagnetic, and therefore can serve as an MRI contrast agent. Taking advantage of the tendency of the Mn^{2+} ions to differentially accumulate in soft tissues, MEMRI has been developed for various aspects of the study of the nervous system: mostly for tracing of specific neuronal circuits and enhancing neuroanatomical features (review: Silva et al., 2004), but also for activity-dependent labeling of neuronal tissues. The latter takes advantage of the voltage-dependent calcium channels that are also permeable to Mn^{2+} (Herberholz et al., 2011; Narita et al., 1990; Van der Linden et al., 2004). As those open during depolarization of the membrane, Mn^{2+} influx into the neurons is selective to active neurons. Once entering the

* Corresponding author at: School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.
E-mail address: ayali@tauex.tau.ac.il (A. Ayali).

https://doi.org/10.1016/j.jinsphys.2018.05.003
Received 19 March 2018; Received in revised form 10 May 2018; Accepted 11 May 2018
Available online 25 May 2018
0022-1910/

cell, Mn^{2+} accumulates there for hours, due to its slow clearing rate. Thus, neurons which were active during a specific behavior and absorbed Mn^{2+} (outside of the magnet), will show a high signal when imaged hours to days following their activation (Watanabe et al., 2006).

While the use of MEMRI in vertebrates is well established (e.g. de Groof et al., 2010; Jackson et al., 2011; Van der Linden et al., 2004), relatively little research has been conducted in invertebrates utilizing this method. Herberholz et al. (2004) first demonstrated the use of Mn^{2+} for enhancing MRI signals in the study of crayfish neuroanatomy, and later were able to use MEMRI for tracing neuronal pathways, which are active during sensory responses (Herberholz et al., 2011). Jelescu et al. (2013) and Radecki et al. (2014) were able to increase the method's resolution and label active single cells in the buccal neuronal network of another aquatic invertebrate, *Aplysia*. To the best of our knowledge, to date, only one report describes the use of MEMRI in insects: Watanabe et al. (2006) demonstrated the potential of the technique by monitoring neuronal responses to simple sensory stimulation in the moth. The current study was aimed at broadening the scope of MEMRI use in insect models, by comparing the neuronal activity between immobilized locusts, and freely behaving ones, which were forced to walk on a treadmill.

Similar to many motor behaviors, insect walking involves the coordination of different neuronal centers. Each of the six legs is controlled by central pattern generators (CPGs), neuronal oscillators that generate rhythmic alternation between antagonistic leg muscles (Büschges et al., 1995; David et al., 2016; Fuchs et al., 2011; Knebel et al., 2017a; Ryckebusch and Laurent, 1993). Sensory feedback loops modulate the CPGs activity and establish the intra- and inter-leg coordination (Ayali et al., 2015; Borgmann et al., 2009, 2007; Fuchs et al., 2012; Knebel et al., 2018). While these neuronal elements are found locally in the thoracic ganglia, directly innervating the legs, higher motor centers are accommodated in the head ganglia (Kaiser and Libersat, 2015; Knebel et al., 2018, 2017b). The latter are believed to produce the inputs needed for initiating and maintaining walking. Specifically, the central complex (CC) which reside in the brain, was shown to control walking speed changes and turning in cockroaches (e.g. Bender et al., 2010; Ridgel et al., 2007). The subesophageal ganglion (SEG) was found to be instrumental for initiating and maintaining spontaneous walking in semi-intact locust preparations (e.g. Gal and Libersat, 2006; Gal and Libersat, 2008; Kien, 1990a; Kien, 1990b; Kien and Altman, 1984; Kien and Williams, 1983). Subsequent *in vitro* studies further demonstrated the relationship between the SEG and the leg-CPGs, and the leg sensory feedbacks loops (Knebel et al., 2018, 2017b). However, the further the neuronal circuit is from direct innervation of the legs, the more challenging it is to trace its involvement in walking. No previous research has monitored the activity of the SEG in freely walking, fully intact, insects.

In the present study, we show that several neuronal centers are more active in walking locusts than in quiescent ones. They further confirm that SEG circuits are active during walking and establish the use of MEMRI for monitoring neuronal activity simultaneously in several ganglia in a fully-intact behaving insect.

2. Materials and methods

2.1 Animals

All experiments were performed on adult male desert locusts (*Schistocerca gregaria*) from our colony at Tel Aviv University (Ayali and Zilberstein, 2002), within the first two weeks after the final molt. All experiments complied with the Principles of Laboratory Animal Care and the Israeli Law regarding the protection of animals.

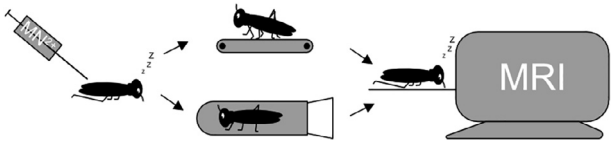

Fig. 1. Experimental procedure. Animals were anesthetized and injected with $MNCl_2$. They were then divided into a walking group and a control group. The former was forced to walk on a treadmill while the latter was placed in a tube where it was immobilized. After 2 h in these conditions, the animals were scanned in an MRI apparatus.

2.2 Experimental procedure

Locusts were injected between the third and fourth abdominal segments with 20 µl of 20 mM $MnCl_2$*$4H_2O$ (Sigma-Aldrich) dissolved in saline containing (in mM): 150 NaCl, 5 KCl, 5 CaCl2, 2 MgCI2, 10 Hepes, 25 sucrose at pH 7.4. They were then divided into two groups: walking and immobilized. At each experiment, three locusts were forced to walk on a treadmill for 2 h, while one animal was placed in 15 ml tube, in which it could not move for the same duration. Out of the three walking locusts, the one that exhibited the most consistent walking behavior throughout the 2 h was chosen for scanning and was placed in a 15 ml tube. Subsequently, both the locust that walked best and the immobilized one were anesthetized in CO_2 and scanned. The experimental procedure is illustrated in Fig. 1.

2.3 MR scan protocol

MRI was performed on 7 T 70/30 Bruker BioSpec MRI scanner (Karlsruhe, Germany). Variable repetition time spin-echo sequence was used to acquire a series of T1 weighted MRIs with the following parameters: TE = 7.9 ms, TRs = 441, 616, 910 and 2205 ms; Spatial resolution of 78 × 78 µm (matrix of 256 × 128 with field of view of 200 × 100 mm^2); 32 coronal slices with thickness of 0.260 µm. The TRs were chosen following on preliminary acquisition and estimation of the locust ganglions T1 (around 400–500 ms). T1 maps were computed using the ParaVision5.1 (©Bruker) tool.

2.4 Analysis

All scans were first co-registered among themselves in order to refer each voxel to its parallel in the scans of other animals, and smoothed (kernel, 400 µm) using SPM12 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/; accessed on 19.3.2018) on MATLAB (MathWorks, USA Inc.). Then, a high-resolution T2 scan of a reference locust (spatial resolution of 75 × 75 µm, matrix of 384 × 192 with field view of 288 × 144 mm^2; 168 coronal slices with thickness of 80 µm) was co-registered to the other scans. After validating the co-registration by visual inspection and by exploring the group STD map, one scan that did not co-registered well was removed. Each voxel in the brain, SEG and prothoracic ganglion of the walking group scans was compared to the corresponding voxel in the immobilized group scans using Student's *t*-test. Only significantly different ($p < 0.05$) voxels were averaged for each group and checked for the intensity of the signal difference. The resulted values were then implemented using a color code in their accurate position on the co-registered T2 anatomical scan (Fig. 2), for the identification of their placement (Fig. 3). No cluster filter was applied.

3. Results and discussion

3.1 Anatomical scans

To identify the areas of interest, i.e. the brain, SEG and prothoracic ganglion, T2 anatomical scans were performed on a locust, which was freezed prior to the scan. The obtained atlas, composed of