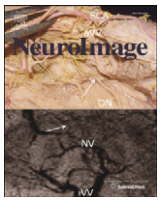
NeuroImage 76 (2013) 264–271

Contents lists available at SciVerse ScienceDirect

NeuroImage

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



Highlighting manganese dynamics in the nervous system of *Aplysia californica* using MEMRI at ultra-high field

Ileana O. Jelescu [a], Romuald Nargeot [b,c], Denis Le Bihan [a], Luisa Ciobanu [a,*]

[a] NeuroSpin, Commissariat à l'Energie Atomique et aux Energies Alternatives, 91191 Gif-sur-Yvette, France
[b] University of Bordeaux, INCIA, UMR 5287, F-33000 Bordeaux, France
[c] CNRS, INCIA, UMR 5287, F-33000 Bordeaux, France

ARTICLE INFO

Article history:
Accepted 9 March 2013
Available online 22 March 2013

Keywords:
Aplysia
Manganese-enhanced MRI
Axonal projections
Neuronal tract-tracing
Dopamine
Ultra-high magnetic field

ABSTRACT

Exploring the pathways of manganese (Mn^{2+}) transport in the nervous system becomes of interest as many recent studies use Mn^{2+} as a neural tract tracer in mammals. In this study, we performed manganese enhanced MRI (MEMRI) at 17.2 T on the buccal ganglia of *Aplysia californica*. The main advantage of this model over mammalian systems is that it contains networks of large identified neurons. Using Mn^{2+} retrograde transport along selected nerves, we first validated the mapping of motor neurons' axonal projections into peripheral nerves, previously obtained from optical imaging (Morton et al., 1991). This protocol was found not to alter the functional properties of the neuronal network. Second, we compared the Mn^{2+} dynamics inside the ganglia in the presence or absence of chemical stimulation. We found that 2 h of stimulation with the modulatory transmitter dopamine increased the extent of areas of intermediate signal enhancement caused by manganese accumulation. In the absence of dopamine, an overall decrease of the enhanced areas in favor of non-enhanced areas was found, as a result of natural Mn^{2+} washout. This supports the hypothesis that, upon activation, Mn^{2+} is released from labeled neurons and captured by other, initially unlabeled, neurons. However, the latter could not be clearly identified due to lack of sensitivity and multiplicity of possible pathways starting from labeled cells. Nonetheless, the *Aplysia* buccal ganglia remain a well-suited model for attempting to visualize Mn^{2+} transport from neuron to neuron upon activation, as well as for studying dopaminergic modulation in a motor network.

Introduction

Magnetic resonance imaging has proven to be a unique tool for *in vivo* tract tracing. Multiple methodologies are now available, based on either iron oxide particles injection (Ghosh et al., 1991), diffusion tensor imaging (Basser et al., 1994) for tracking white matter fibers, or manganese chloride injection (Pautler et al., 1998). The latter takes advantage of two properties of the manganese ion (Mn^{2+}). First, Mn^{2+} is paramagnetic, which translates into local T_1 shortening and therefore signal enhancement on T_1-weighted images. Second, Mn^{2+} is able to enter the neurons via voltage-gated Ca^{2+} channels (Yamashita, 1982), and it is further transported both anterogradely and retrogradely in the nervous system.

Because of the neurotoxicity associated with $MnCl_2$ administration, tract-tracing studies using manganese enhanced MRI (MEMRI) have been mainly performed in rodents (Bearer et al., 2007; Lin et al., 2001; Lindsey et al., 2007; Pautler et al., 1998, 2003), non-human primates (Murayama et al., 2006; Saleem et al., 2002) and birds (Van der Linden et al., 2002). These studies successfully highlighted tracts in the visual and olfactory systems, as well as in several brain regions. The observed transport across multiple regions supports the hypothesis that Mn^{2+} is able to cross synapses, i.e. that it is released in the synaptic cleft and captured by the post-synaptic neurons. However, it has been acknowledged that the high resolution of light microscopy would be needed to bring a definite answer about synapse crossing (Pautler et al., 1998). In the light of these observations, *Aplysia californica* constitutes an excellent model for studying Mn^{2+} transport in the nervous system, using manganese enhanced magnetic resonance microscopy. *Aplysia* has been a valuable model in neuroscience for many years (Kandel, 2001; Kupfermann and Kandel, 1969). Its main advantages over mammalian systems are its large cells which constitute networks of identified neurons, and the cells' tolerance to non-ideal conditions. *Aplysia* neurons became interesting to the MR community when magnetic resonance microscopy (MRM) developed, thanks to high magnetic fields and gradient strengths. Studies have mainly focused on T_2 and diffusion measurements in isolated cells (Grant et al., 2001; Hsu et al., 1996; Schoeniger et al., 1994). The only MEMRI experiments on non-vertebrates have so far been

* Corresponding author at: Neurospin, bat. 145, Commissariat à l'Energie Atomique et aux Energies Alternatives, 91191 Gif sur Yvette, France. Fax: +33 1 69 08 79 80.
E-mail addresses: ileana.jelescu@cea.fr (I.O. Jelescu), romuald.nargeot@u-bordeaux2.fr (R. Nargeot), denis.lebihan@gmail.com (D. Le Bihan), luisa.ciobanu@cea.fr (L. Ciobanu).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.03.022

performed on crayfish. These studies have focused on the enhancement of the anatomy after $MnCl_2$ injection (Brinkley et al., 2005; Herberholz et al., 2004) and more recently on highlighting neural activation through differential Mn^{2+} uptake following electrical shocks (Herberholz et al., 2011).

In this study, we perform, for the first time, MEMRI on the buccal ganglia of *A. californica*. Using retrograde transport of Mn^{2+} along the peripheral nerves of the ganglia, we confirm the mapping of axonal projections from identified neurons into the buccal nerves, previously obtained with Co^{2+} or Ni^{2+} migration and optical techniques (Morton et al., 1991; Quicke and Brace, 1979). We further study the dynamics of Mn^{2+} inside the buccal ganglia following stimulation with dopamine (DA). The identification of individual neurons on MR images, and the *a priori* knowledge of synaptic connections between them (Nargeot and Simmers, 2012) provide a solid starting point for the study of Mn^{2+} transport mechanisms inside the nervous system, as well as for functional MEMRI studies on a DA-modulated neuronal network.

Materials and methods

Fourteen late juvenile *A. californica* (National Resource for Aplysia, Miami, FL) were used in total. The animals were anesthetized by injection of an isotonic magnesium chloride solution ($MgCl_2$: 360 mM; HEPES: 10 mM; pH = 7.5). The bilateral buccal ganglia were isolated and placed in a Sylgard-coated (Dow Corning, Midland, MI) Petri dish, filled with artificial sea water (ASW) (NaCl: 450 mM; KCl: 10 mM; $MgCl_2$: 30 mM; $MgSO_4$: 20 mM; $CaCl_2$: 10 mM; HEPES: 10 mM; pH = 7.5). Salts were obtained from Sigma-Aldrich (Saint-Quentin Fallavier, France).

Confirming axonal projections

Three pairs of buccal ganglia were used for unilateral Mn^{2+} migration along one peripheral nerve: nerve 2 (n.2), 3 (n.3) or esophageal (E.n.). The ending of the selected nerve was isolated in a well of petroleum jelly (Vaseline), as illustrated in Fig. 1. The well was filled with a solution of manganese chloride mimicking intracellular medium ($MnCl_2$: 25 mM; NaCl: 50 mM; KCl: 440 mM; $MgCl_2$: 10 mM; Hepes: 10 mM; pH = 7.2). The ganglia were stored at 4 °C and the manganese was allowed to migrate for 18 h before imaging.

For imaging, the ganglia were inserted into a 1.5 mm inner-diameter glass capillary (VitroCom, Mountain Lakes, NJ) filled with ASW. MR imaging was performed at room temperature, on a 17.2 T system (Bruker BioSpin, Ettlingen, Germany) using a home-built solenoid as RF transceiver. The coil and capillary were immersed in FC-40 (3 M, Cergy-Pontoise, France) for improved B_0 homogeneity. Imaging consisted of a 3D FLASH (TE = 2.4 ms/TR = 150 ms/FA = 40°/25 μm isotropic/NA = 10/TA = 2h40) for T_1 contrast and of a 3D RARE (TE_{Eff} = 18.4 ms/TR = 3 s/25 μm isotropic/TA = 1h20) for T_2 contrast. During the first experiment, the repetition time (TR) of the FLASH sequence was optimized empirically on lower resolution scans ($25 \times 50 \times 50\ \mu m^3$; NA = 1) to provide the best contrast between regions with and without Mn^{2+}. The cell bodies displayed a natural T_2 contrast with respect to ASW, making them all visible on T_2-weighted (T2w) images, but hardly any T_1 contrast on FLASH

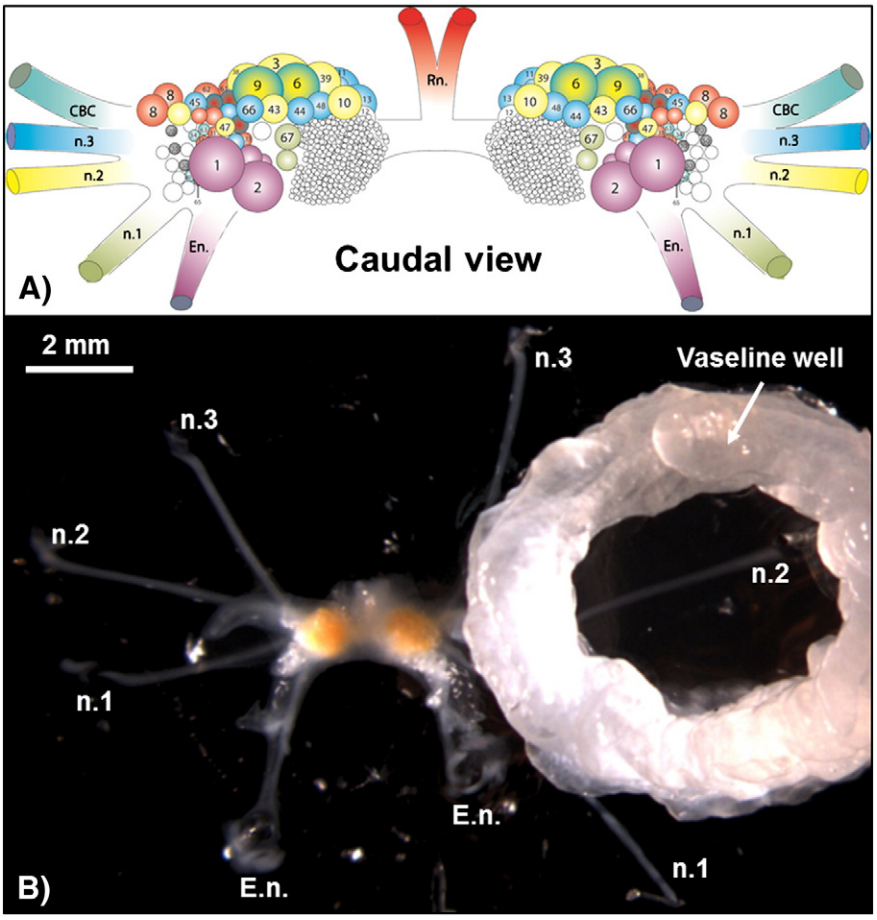


Fig. 1. Caudal views of the buccal ganglia in *Aplysia*. A. Color-coded schematic of neurons' axonal projections into peripheral nerves, established using Co^{2+} migration and optical imaging. Colored circles indicate the cell bodies identified by individual numbers. B. Isolation of a single nerve 2 (n.2) into a Vaseline well. The well is filled with a $MnCl_2$ solution, while the rest of the ganglia bathe in ASW. Digital microscope camera (World Precision Instruments, Hitchin, UK) image.