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Imaging live bee brains using minimally-invasive

Diagnostic Radioentomology

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Abstract

Sensitivity of the honey bee brain volume and density to behavior (plasticity) makes it a great model for exploring the interactions between experience, behavior and brain structure. Plasticity in the adult bee brain has been demonstrated in previous experiments. This experiment was conducted to identify the potentials and limitations of MicroCT scanning “live” bees as a more comprehensive, non-invasive method for brain morphology and physiology. Bench-top and synchrotron MicroCT were used to scan live bees. For improved tissue differentiation, bees were fed and injected with radiographic contrast. Images of Optic lobes, ocelli, antennal lobes and mushroom bodies were visualized in 2D and 3D rendering modes. Scanning of live bees (for the first time) enabled minimally-invasive imaging of physiological processes such as passage of contrast from gut to haemolymph and preliminary brain perfusion studies. The use of CT for studying insects (collectively termed Diagnostic Radioentomology “DR”) is increasing. Our results indicate that it is feasible to observe plasticity of the honey bee brain “in vivo” using DR and that progressive, real-time observations of these changes can be followed in individual live bees. Limitations of live bee scanning such as movement errors and poor tissue differentiation were identified, however there is great potential for in-vivo, non-invasive DR imaging of the honey bee for brain morphology and physiology.

1

2 Introduction

3 European honey bee (*Apis mellifera*) workers weigh approximately 0.1g, their brain weighs
4 approximately 0.001g, has a volume of approximately 1mm³ and has approximately 1 million
5 neurons (Ribi et al. 2008). The main parts of the brain are the optic lobes, the antennal lobes,
6 the mushroom bodies, and the central complex. The optic and antennal lobes are responsible for
7 processing vision and olfaction respectively. The mushroom bodies and the central complex
8 constitute the most important centers for behavior, instincts and memory (Hourcade et al.
9 2010). Other parts of the brain include the suboesophageal ganglion, tritocerebrum, and ventral
10 cord. It is thought that complex behavior is based on overarching brain networks superimposed
11 on smaller local networks controlling individual responses. Since simple environmental
12 manipulations can both accelerate and delay brain growth in young bees, and since brain

13 volume is sensitive to behavior throughout life, the honey bee has great potential as a model for
14 exploring the interactions between environment, behavior and brain structure. Experience
15 related changes in brain structure are believed to be an important part of the memory engram
16 (Kolb and Whishaw 1998; Kim and Diamond 2002; Mohammed et al. 2002; Gerber et al. 2004;
17 Kim et al. 2006; Liston et al. 2006), and understanding the relationships between experience
18 and brain structure is key to understanding the relationships between brain and behavior (Kolb
19 and Whishaw 1998). A worker honey bee's natural behavioral change is associated with
20 conspicuous growth of the mushroom bodies in the brain (Withers et al. 1993; Farris et al.
21 2001; Ismail et al. 2006). The mushroom body calyx is larger in forager bees than same-aged
22 nurse bees which have not left the hive (Withers et al. 1993; Farris et al. 2001). This structural
23 change may be part of the memory engram for the many foraging-related and navigational tasks
24 learned by a forager bee (Farris et al. 2001; Fahrbach et al. 2003).

25
26 Phenotypic plasticity in the adult bee brain has been demonstrated in previous experiments
27 using various techniques such as the Cavalieri or computer volume segmentation methods
28 (Gundersen & Jenson 1987; Michel & Cruz-Orive 1988; Withers et al. 1993; Brown et al.
29 2000; Ribi et al. 2008; Maleszka et al. 2009). In all cases, dead bees were used to collect data
30 which invariably leads to differences amongst individuals.

31
32 This experiment was conducted to identify limitations and potentials for MicroCT scanning of
33 live bees to be used as a comprehensive, non-invasive method for studying brain plasticity and
34 for teaching morphology and physiology of the brain.

35 36 **Materials and Methods**

37 The SYRMEP beamline facilities at the ELETTRA synchrotron in Trieste and a SCANCO
38 μ CT40 bench-top scanner at the University of Bern and were used to scan the bees. At the
39 beamline, newly emerged, adult bees were scanned once daily over five days to observe
40 differential brain plasticity as a result of asymmetric environmental stimuli. Scans on live bees
41 at the beamline facility were performed using phase contrast with the following parameters:

- 42
- 43 • X-ray energy: 15keV
- 44 • Sample to detector distance: 20cm
- 45 • Number of projection (over 180°): 1800
- 46 • Isotropic Voxel size: 9 μ m
- 47 • Exposure time: 0.9s
- 48 • Measurement time: 1h 48min
- 49

50 To enhance tissue differentiation, bolus injections of radiographic contrast media were
51 delivered directly into the haemolymph, between the dorsal abdominal terga, via a 30G needle
52 (Fig 1). For visual comparisons of gross anatomical features, MicroCT scans of an ancient bee
53 trapped in amber were also performed on the benchtop scanner using absorption techniques
54 with the following parameters:

- 55
- 56 • Tube operating conditions: HV peak was set at 45kV and current was 177 μ A
- 57 • High Resolution mode (1000 Projections/180°)
- 58 • Image Matrix of 2048 \times 2048 pixels
- 59 • Isotropic Voxel size 10 μ m
- 60 • Integration time 3s
- 61 • Total number of 610 slices
- 62 • Measurement time 2h 30min

63
64 Images and brain volume data (Fig 2) were measured using BeeView volume rendering
65 software (DISECT Systems Ltd).
66

67 **Results**

68 Gross brain morphology such as the optic lobes, antennal lobes, aorta, mushroom body calyces
69 and median ocellus were visualized in 2D and 3D projections. Brain volume measurements (Fig
70 2) enabled estimates of plasticity. Scanning of live bees enabled minimally-invasive imaging of
71 physiological processes (for the first time) such as passage of contrast from gut to haemolymph
72 (Fig 3) as well as preliminary brain perfusion and plasticity studies (Fig 4i). The image in (Fig
73 4ii) shows a similar view to (Fig 4i) which was produced by Rybak et al. (2010) using data
74 from two-channel confocal microscopy scans. Comparisons of brain images from live extant
75 bees and the 20 million year old bee *Proplebeia abdita* showed little variation in gross
76 morphological features (Fig 4iii).
77

78 **Discussion**

79 The use of MacroCT and MicroCT imaging for the non-invasive study of insects, collectively
80 termed “Diagnostic Radioentomology” (DR), is increasing (Hornschemeyer et al., 2002;
81 Johnson et al., 2004; Hönnicke et al. 2005; Greco et al., 2005; Greco et al., 2006; Greco et al.,
82 2008; Greco et al., 2009, Greco et al. 2011) Results from this study indicate that it is feasible to
83 observe plasticity of the honey bee brain “in vivo” using DR and that progressive, real-time
84 observations of these changes can be followed in individual live bees in association with
85 environmental stimuli. Plasticity in the adult bee brain has been demonstrated in previous
86 experiments using various techniques such as the Cavalieri or computer volume segmentation
87 methods. In all cases previous to this study, dead bees were used. However, the use of ex-vivo
88 samples increases the chances of fundamental errors in correlation data analyses due to inherent
89 differences among individuals. Movement errors were not a major limitation of this study
90 because it was possible to completely immobilize the head. However, haemolymph flow
91 continued, which caused exposure variations between tomographic slices. The exposure
92 variations were easily corrected by using the “intensity averaging” function during image
93 reconstruction. The greatest challenge for this study was achieving adequate brain tissue
94 differentiation and it was clear that although radiographic contrast showed promise for
95 improving tissue visualization, further improvements on reconstruction algorithms are
96 required to better separate brain structures. Bee brain imaging studies from Ribic et al. 2008 and
97 Rybak et al. 2010 are still of superior quality however, the results in this experiment
98 demonstrate great potential for in-vivo, non-invasive DR imaging of the honey bee for future
99 research in brain plasticity and for teaching brain morphology and physiology.
100

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105

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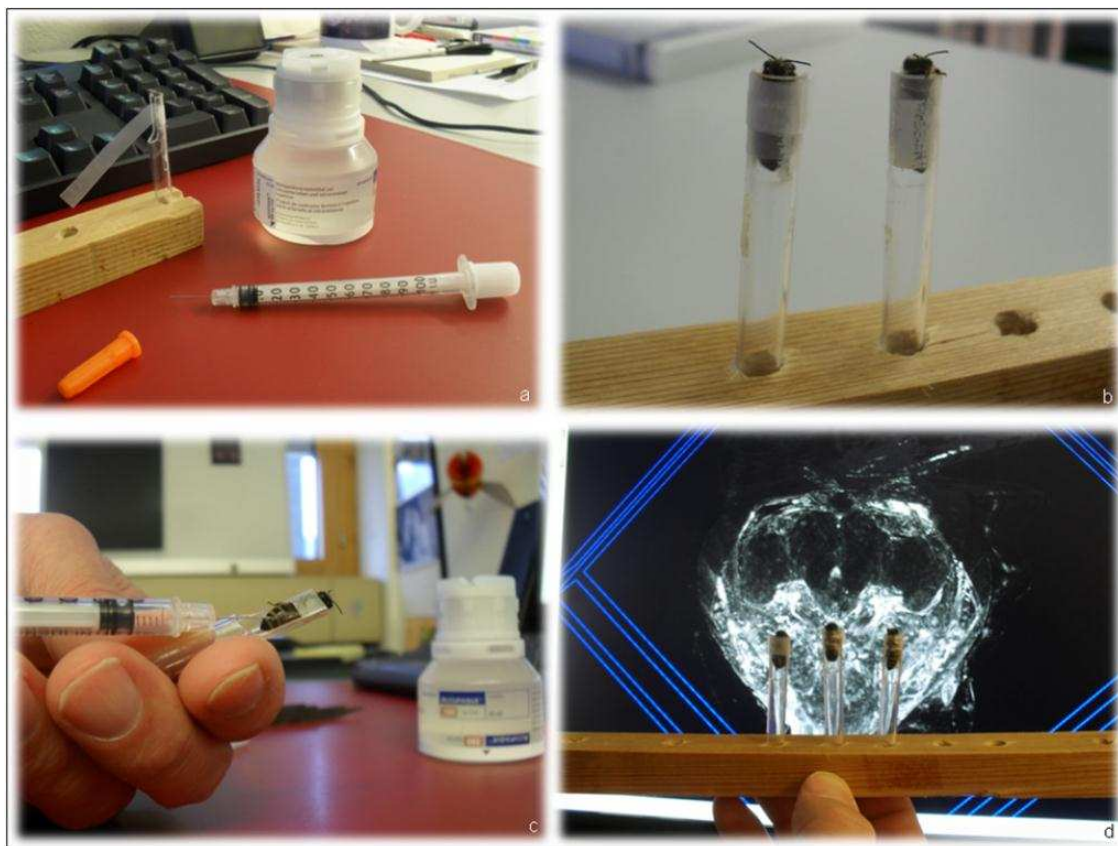
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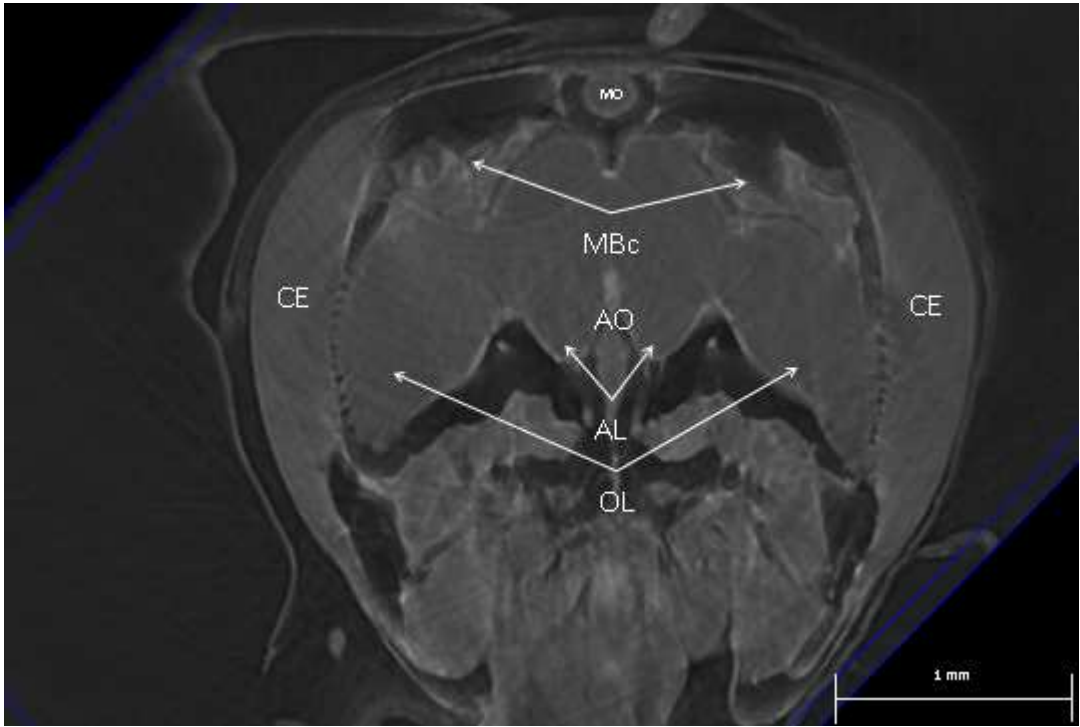
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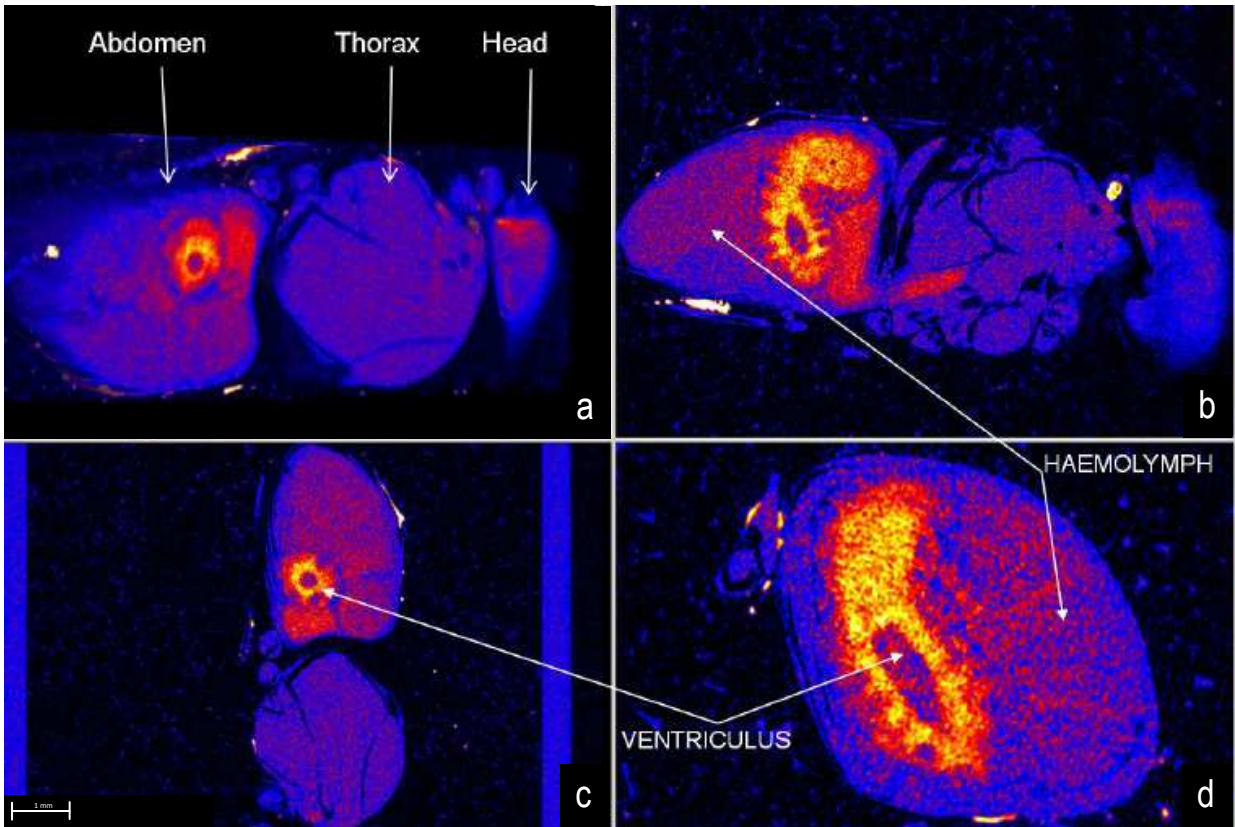


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179 **Figure 1:** To enhance brain tissue differentiation, bolus injections of radiographic contrast
180 media were delivered via a 30G needle (a) directly into the haemolymph, between the dorsal
181 abdominal terga, of live bees that were previously secured for scanning (b) & (c). The 3D
182 rendered brain (d) showed that contrast had perfused into tissue to enable improved structural
183 differentiation.
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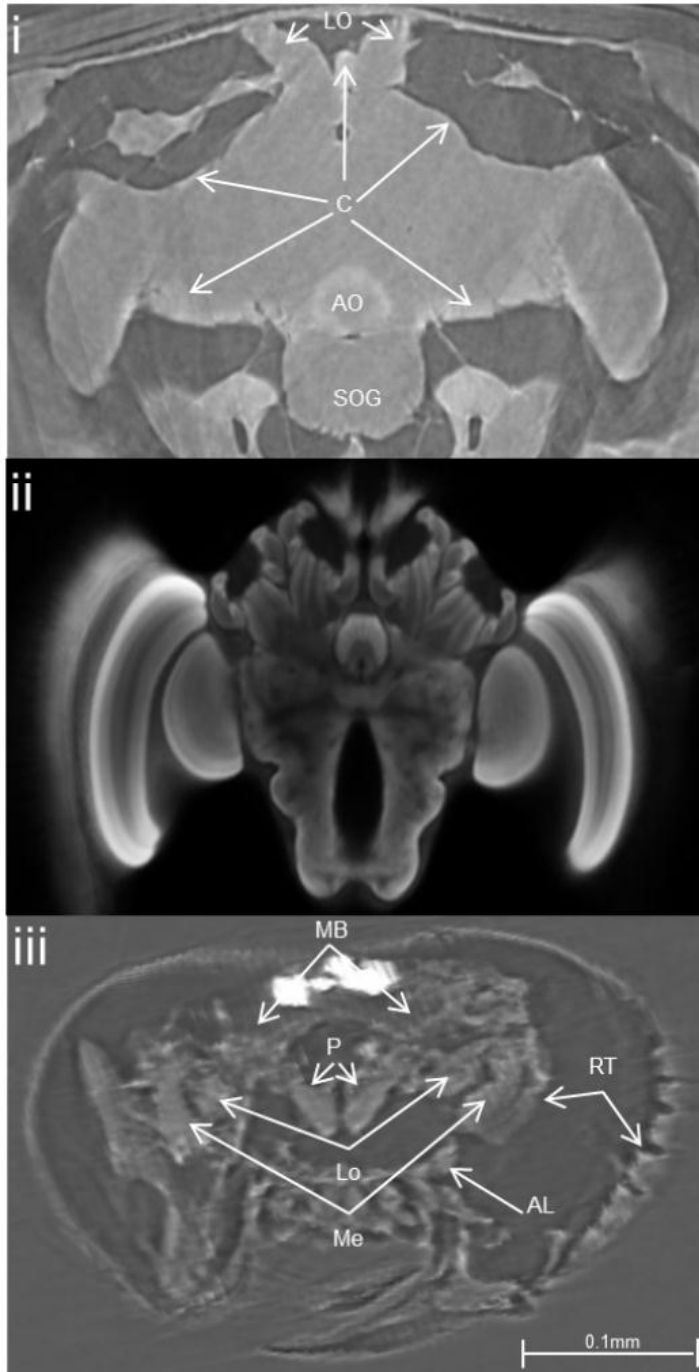
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Figure 2: A 3D volume rendered image of a live honey bee's head capsule showing gross morphological structures such as the optic lobes (OL), antennal lobes (AL), aorta (AO), mushroom body calyces (MBc) and median ocellus (MO). The compound eyes (CE) are visualized immediately adjacent and lateral to the optic lobes.



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196 **Figure 3:** A 3D volume rendered image with BeeView software of a live honey bee
 197 showing the three body segments (a) and orthogonal, 2D images (b), (c) & (d) showing the
 198 passage of radiographic contrast from the ventriculus (true stomach) to the haemolymph in the
 199 coelum. Images were rendered 1.5h after ingestion of contrast.
 200



201
 202
 203 **Figure 4:** (i) A 2D axial view of a live honey bee brain showing perfusion of contrast
 204 medium (C) into peripheral regions. Arrows indicate areas of higher concentration. At 30min
 205 post bolus injection, into the haemolymph, the lateral ocelli (LO) and aorta (AO) contained
 206 more contrast than the sub oesophageal ganglion (SOG). (ii) A comparative 2D axial view
 207 from the bee brain atlas (<http://www.neurobiologie.fu-berlin.de/beebrain/Default.html>) which
 208 was reconstructed from imaging data from two-channel confocal microscopy scans. (iii) An
 209 axial view of the head capsule of an ancient stingless bee *Proplebeia abdita* (Greco et al. 2011)
 210 trapped in amber. The brain of this 20 million year old bee was particularly well preserved as

211 evidenced by the optic lobes including the medullae (Me) and lobulae (Lo), antennal lobes
212 (AL), protocerebral lobes (P) and the mushroom bodies (MB). The retinal zone (RT) was also
213 well preserved.