# Environmental Effects on the Bumble Bee (Apidae: *Bombus impatiens*) Brain: Visual Experience Affects Neuropil Volume Allocation

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**Statement By Author**

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**Abstract**

The environment experienced during development of an animal plays critical roles in the future success of the individual. Many of the effects of environmental stimuli are manifested in changes to the organism's brain, which can lead to changes in the ability to integrate information and thus may have critical implications for learning and memory. In order to better understand the connection between the environment and the developing brain, research must focus on neuronal plasticity and the manner in which neural tissues change over the course of an organism's lifetime. In this study, I investigated the extent to which the environment plays a role in the developing bumble bee (*Bombus impatiens*) brain. First, I compared the brains of newly emerged bees to those of seven-day-old bees that had foraging experience and social interactions within their colony. I discovered significant age and experience-related volume increases in nearly every sensory processing region of the brain. Next, to determine whether these changes were dependent upon certain environmental stimuli, I performed a manipulative experiment: newly emerged bees were housed in one of four chambers, with or without visual stimuli (LED and fluorescent lights) and/or olfactory stimuli (plant-derived odors) for seven days. I found that the presence of olfactory stimuli did not have a significant effect on volumes of the brain regions of interest. Visual stimuli, on the other hand, appear to play a role in the growth and refinement of certain brain regions, including the mushroom bodies, sites of signal integration, learning and memory in the insect brain. These results illustrate that bumble bees can serve as a model system for studies of developmental neuronal plasticity, and can provide opportunities for future studies of environmental effects on brain development.

## Introduction

Early environmental exposure has been shown to be critical for proper development of the brain in many animals. For example, in 1981, the Nobel Prize in Physiology or Medicine was awarded to Wiesel and Hubel for their work on visual processing in mammals. They demonstrated that visual experience in early life is necessary for proper development and functionality of the visual processing systems in cats and monkeys. Wiesel, Hubel and collaborators also found that rodents living in enriched environments showed increased brain size and weight, larger nuclei in the cortex, and enhanced generation of glial cells, neurite branching and synapse formation (Wiesel 1981). This connection between environment and brain function has implications for the treatment of diseases such as Alzheimer's, epilepsy, and Huntington's, as well as development of therapies for stroke, brain trauma, and aging (reviewed in van Praag et al. 2000). In order to better understand environmental effects on the brain, research must focus on neuronal plasticity and the manner in which neural tissues change over the course of an organism's lifetime.

One of the most intensely studied invertebrate models of neuronal plasticity is the honey bee (*Apis mellifera*), a social insect species which shows age polyethism with respect to worker tasks. Over the course of two to three weeks, young honey bees transition from a nurse bee in the hive to a forager who must integrate and remember spatial, visual, and olfactory cues associated with foraging (reviewed by Free 1965; Wilson 1971; Michener 1974). As the honey bee develops, the structure and organization of its brain undergoes dramatic changes. Regions of particular interest are the mushroom bodies (MBs), which are involved in sensory integration and learning (reviewed in Strausfeld et al. 1998; Zars 2000). The MBs receive and process input from olfactory and other sensory regions of the brain (Strausfeld et al. 1998; Mobbs 1982; Gronenberg 2001), and ablation of the MBs or mutations in MB-expressed genes result in learning deficits (de Belle and Heisenberg 1994; Davis 1993). Fahrbach et al. (1998) demonstrated mushroom body volume expansion within the first week after emergence in honey bees deprived of all light and social interaction, a form of development termed experience-independent growth. MB expansion is also observed in male honey bees around the time of flight initiation (Fahrbach et al. 1997). In addition to developmentally pre-programmed MB expansion, honey bee workers also show experience-dependent growth of the MBs, as they expand with sensory experience and foraging (Withers et al. 1993, 1995; Farris et al. 2001).

Other hymenopterans also exhibit plasticity in the mushroom bodies, including the ants *Camponotus floridanus* (Gronenberg et al. 1996), *Pheidole dentata* (Seid et al. 2005), and *Cataglyphis bicolor* (Kühn-Bühlmann and Wehner 2006). In addition to the mushroom bodies, plasticity has been observed in the antennal lobes and medulla of the insect brain. The medulla, which receives visual input from the eyes and is involved in primary visual processing, is affected by visual experience in *Drosophila melanogaster* (Barth et al. 1997; Heisenberg et al. 1995). The antennal lobe, which receives olfactory information from the antennae, exhibits plasticity in the form of volume increases in particular antennal glomeruli, which are activity-dependent and accompanied by improvements in associative learning performance in honey bees (Winnington et al. 1996; Sigg et al. 1997).

In order to elucidate the physiological mechanisms behind neuronal plasticity, a model must be found whose behavior and sensory experience can be manipulated in the laboratory. While honey bees have been a model system in behavioral and learning studies for almost a hundred years, bumble bees *(Bombus* spp.) offer several advantages over honey bees. Bumble bee colonies are commercially available and orders of magnitude smaller than those of honey bees, making them easier to maintain and manipulate. Additionally, the sensory environment of bumble bees can be carefully controlled in the lab. Honey bees require outdoor foraging, while bumble bees exhibit foraging behavior in the lab that is identical in key aspects to foraging in nature (Raine and Chittka 2007, 2008). Finally, the robust nature of the bumble bee make it an ideal candidate for physiological studies (Paulk 2008).

In behavioral respects, honey bees and bumble bees both forage using similar visual and olfactory sensory inputs, yet the two species undergo quite different developmental trajectories. A critical and intriguing difference between the two organisms makes them well-suited for a comparative study of neuronal plasticity: honey bees change tasks with age, while bumble bees engage in the same set of tasks throughout their lives. While it may take three weeks for a honey bee to begin foraging (Winston 1987; Robinson 1992), 1-day-old bumble bees have been shown to learn at the same level as older bees, suggesting their ability to forage soon after emergence (Riveros and Gronenberg 2009).

I first characterized brain volume changes during the first week of life by measuring each brain region in newly-emerged and 7-day-old foraging *Bombus impatiens*. Then, by manipulating the sensory environment experienced by newly-emerged bees, I asked whether environmental inputs affect brain development. Specifically, I manipulated the presence vs. absence of odors and visual stimuli in order to determine whether inputs of these sensory modalities are associated with changes in the brain. Both odor and visual stimuli are known to be relevant to bees foraging for flowers under natural conditions. Since bumble bees may forage soon after emergence, I predicted that they would show a lesser degree of age-related changes in the brain, independent of sensory experience, compared to honey bees. Instead, I expected that bumble bees might show greater effects of sensory experience on brain development.

## Methods

*Animals and Rearing Conditions*

Colonies (N=4) of *Bombus impatiens* were obtained through Koppert Biological Systems, (Romulus, MI, USA). Bees were provided with pollen and BeeHappy® solution from Koppert *ad libitum*. Colonies were housed in plastic boxes (LxWxH: 22x24x12 cm) and fed from a cotton wick feeder within a small foraging box (LxWxH: 15x15x20 cm, constructed of Plexiglas) attached to the colony via tubing. Only workers were used for experiments and all workers were marked using numbered tags (E.H. Thorne Ltd., Wragby, UK) glued to their thorax. Bees used as subjects in the manipulative experiment were taken from colonies maintained in darkness using light-proof fabric enclosing a large arena, ensuring that newly-emerged (callow) bees had no prior exposure to light.

*Assessment of Age-Related Changes in Regional Brain Volumes*

In this experiment, we described brain volume differences between newly-emerged bees and 7-day-old foraging bees. The colony used for this assessment was maintained in the lab and had exposure to fluorescent light (Sylvania Cool White 34W, 60Hz, no. F40CW1SS) for 12 hours each day. This colony was connected to a cubic foraging arena with sides of 30.5 cm equipped with purple and white artificial flowers, BeeHappy® solution delivered via a cotton wick feeder, and floral scents (geraniol and sweet orange, 2 µl undiluted essential oil on cotton swabs in arena). On Day 7, bees were collected and prepared for brain measurements as described below.

*Effects of Exposure to Sensory Stimuli on Regional Brain Volumes*

In this experiment, bees were collected as callows (<12 hours post-emergence, recognizable by features of their external appearance) and placed into one of four Plexiglas boxes (LxWxH: 30.5x20.3x3.8 cm). While bees could walk freely in these shallow boxes, they could not fly. In order to maintain a standard number of bees present per experimental chamber, workers not used in brain volume estimation were added or subtracted such that there were always 12 bees per box. Bees received no pollen to minimize ovarian development (dissection of ovaries revealed no significant development), and received 40% sucrose (wt/wt) solution *ad libitum.* As callows, bees were placed into one of four treatments: 1) visual and olfactory deprivation, 2) visual enrichment and olfactory deprivation, 3) visual deprivation and olfactory enrichment, or 4) visual and olfactory enrichment. Bees in a visual deprivation treatment were placed in boxes (LxWxH: 30.5x20.3x3.8 cm) constructed of black, opaque plastic and kept in darkness at all times. Bees in a visual enrichment treatment were placed in boxes of identical construction but with clear plastic, and were exposed to fluorescent room lighting (Sylvania Cool White 34W, 60Hz, no. F40CW1SS) and LEDs (peak wavelengths of 395, 420, 476, 561, nm and one broad-spectrum emitting LED; see Appendix for emission spectra) for 10 hours each day. Two LEDs of each type (for a total of 10 LEDs) were randomly arranged on the upper surface of each box. The colors of the LEDs were chosen to be representative of colors that bees might encounter when foraging for plants and flowers. Bees in olfactory-enrichment treatments received exposure to one of seven essential oils (lavender, grapefruit, peppermint, geranium, cinnamon, ylang-ylang, or jasmine; obtained from oilshop.com) for 10 hours each day. Three microliters of the respective oils were pipetted onto filter paper placed within the box in a mesh-covered dish, ensuring that bees could not make direct physical contact with the oils. The fragrances used were chosen to be representative of volatiles that a bee might encounter when foraging. Boxes were sealed and a weak air flow was generated by drawing carbon-filtered room air through the box via a vacuum line. On Day 7, bees were collected and their brains prepared for measurement as described below.

*Tissue Preparation*

Bees were decapitated, and heads prepared for fixation by cutting a hole in each eye and removing mandibles to allow fixative to penetrate the head. Brains were fixed within the head capsule in 4% formaldehyde in cacodylate buffer (pH 6.8) overnight on a rotator. After fixation, brains were rinsed with and stored in cacodylate buffer at 4°C until dissection. Brains were dissected from the head capsule, then stained in the dark using 1% aqueous osmium tetroxide for 2 hours on ice, followed by an additional 30 minutes at room temperature. After rinsing with distilled water, brains were dehydrated using 50% ethanol, acidified 2,2-dimethoxypropane (Thorpe and Harvey, 1979) and acetone for 10 minutes each. Next, brains were plastic-embedded in Spurr's low viscosity medium, (RT 14300 Electron Microscopy Sciences). Blocks were polymerized at 65°C for 12 h, sectioned on a sliding microtome at 7-15 μm, mounted and coverslipped.

*Brain Volume Estimations*

A camera lucida attachment to a light microscope was used to trace the outlines of one hemisphere of each brain and individual brain regions within that hemisphere section by section. Specifically, the following brain regions were drawn (see also Fig. 1): optic lobes (medulla and lobula, which are involved in primary processing of visual information), antennal lobe (receiving input from the antenna for primary processing of olfactory information), central body (suggested to function in the coordination of motor control (Strauss 2002)), and the mushroom bodies (involved in learning, memory and multi-sensory integration [reviewed in Heisenberg 1998]; lobes and calyces drawn separately). The remaining neuropil not designated as a region mentioned above is referred to as "Neuropil (rest)," and the volume of cell bodies was also recorded. Drawings of the brain hemispheres were digitized on a flatbed scanner at 300 dpi, and areas of the respective brain components were calculated using the pixel counting routine in Adobe Photoshop v7.0 (Adobe Systems, San Jose CA, USA). Volumes were determined by multiplying the area of each region by the section thickness. To estimate volumes, every other section (15 μm section thickness) or every third section (10 μm section thickness) was drawn and measured. This method leads to an increase in error of measurement of less than 5% compared to measuring each section (see Mares et al. 2005). Relative volume refers to the ratio of the volume of the brain region of interest to the entire brain volume, including cell bodies in total brain volume.

*Statistical Analyses*

JMP 7 (SAS Institute) was used for all analyses. Data were arcsin square root transformed to meet the assumptions of parametric statistical analysis. Data were then confirmed to be normalized and have equal variance for ANOVA. A Student's t-test was used to compare relative volumes of each brain region in callows versus 7-day-old bees. For the sensory manipulation experiment, a factorial ANOVA was used to determine the effects of sensory experience on brain size. In these analyses, the presence of visual and presence of olfactory stimuli were used as factors.

## Results

*Changes in Regional Brain Volumes and Cell Morphology over Time*

Significant relative volume increases were observed in every region of the brain with the exception of cell bodies and the central body during the first week of adult life. Figure 2 summarizes the data comparing brain regions of callow bees to those of 7-day-old foraging bees. Of note, the medulla and lobula (optic lobes), antennal lobe, and mushroom body calyces and lobes were all significantly larger in relative volume in the 7-day-old bees as compared to callows (Fig. 2, N=20; $t_{18}$, $p<0.0001$). The medulla and lobula increased in relative volume by 14% and 17%, respectively, during the first week of adult life. Similarly, the antennal lobe increased in relative volume by 12%. Mushroom body calyces and lobes increased in relative volume by 19% and 14%, respectively. In contrast, cell bodies, including Kenyon cell somata of the mushroom bodies, decreased by 19% in relative volume in 7-day-old bees as compared to callows (N=20; $t_{18}$, $p<0.0001$; see Fig. 3). Relative volume of the central body did not differ between the two groups. See Table 1 in Appendix for complete data and statistics on relative volumes of each subregion in callow and 7-day-old foraging bees.

The decrease in relative volume of the cell bodies observed between callows and 7-day-old bees was associated with changes in the cellular morphology of the brains. Figure 4 shows differences in the appearance of Kenyon cell somata, and Figure 5 shows differences in cell bodies surrounding the antennal lobe in callow and 7-day-old bees.

*Effects of Visual and Olfactory Stimuli on Regional Brain Volumes*

Table 1 summarizes the relative volumes of each region of the brain measured in the four treatment manipulations. Factorial ANOVA analysis reveals a significant effect of the visual treatment on the relative volume of certain brain regions, but no effect of the olfactory treatment or an interaction between visual and olfactory treatments. There was no significant effect of treatment on the relative volume of either component of the optic lobe, the medulla or lobula. The relative volume of the central body was also not significantly different among treatments. The presence of visual stimuli had a negative effect on the relative volume of the antennal lobe (Fig. 6; N=40, $F_{3,36}$ $p<0.0001$). Both the calyces and the lobes of the mushroom body were also significantly affected by exposure to visual stimuli. In bees provided with visual stimuli, the relative volume of MB calyces and lobes was significantly smaller than among bees not exposed to visual stimuli (Fig. 7; N=40, $F_{3,36}$ $p=0.016$ and $p=0.0101$, respectively). Relative volume of cell bodies was significantly larger in bees exposed to visual stimuli (N=40, $F_{3,36}$ $p=0.0044$).

## Discussion

### *Age-Related Changes*

By measuring the volume of each brain region in callow and 7-day-old foraging bees, I characterized a number of changes in the brain that occur during the first week of adult life in *Bombus impatiens*. The relative volumes of many brain regions were significantly larger in 7-day-old bumble bees as compared to callows. Significant growth during adult life has also been documented in the honey bee, with much focus on the mushroom bodies (Fahrbach et al. 1997, 1998, 2003; Withers et al. 1993; Sigg et al. 1997; Maleszka et al. 2009). These studies found both age- and experience-related growth of the mushroom bodies in the honey bee. In my first experiment, effects of age and experience were not considered separately, and the growth observed during the first week of adult life may thus reflect a combination of both factors. However, this growth is likely not associated with a shift in tasks as it is in honey bees. The increase in relative volume of every sensory processing tissue is compensated for by a decrease in relative volume of the cell bodies. These data are consistent with observations that Kenyon cell somata in the honey bee decrease in volume with age and experience (Withers et al. 1995). The decrease in volume of cell bodies is a predictable consequence of the development of individual neurons. During development, cell bodies are first very large, as they provide the material required for building axons, dendrites, synapses and other neuronal processes (Goodman and Bate 1981). Once the neuron becomes established and functional, the cell body is much less active and plays a role in maintaining rather than building the neuron. In older individuals, the cell bodies may not be much larger than their nuclei, since much of the cytoplasm of the cell resides in the processes. In 7-day-old foraging bees, examination of the Kenyon cell body morphology is consistent with these basic developmental patterns, as is the decrease in relative cell body volume over time (Withers et al. 1993; Farris, 2001).

*Sensory Experience-Related Changes*

No significant volume changes were observed in the brain regions of bees with or without olfactory stimuli in the manipulative experiment. This result suggests that plant-derived volatiles do not affect brain volume in *B. impatiens*, at least not as they were administered in this experiment. However, the result does not mean that brain volume is not sensitive to olfactory experience. Short of manually removing the antennae, it is difficult to completely deprive an insect of all olfactory stimuli. Although air entering the chamber was filtered and no outside odorants were introduced, the twelve bees within the chamber undoubtedly contributed olfactory stimuli to their environment. The addition of plant-derived odors in olfactory treatments may have increased synaptic density in some antennal glomeruli; however, previous studies have shown that synaptic proliferation in the antennal lobe does not necessarily correlate with volumetric changes (Brown et al. 2002). As synaptic density or dendritic outgrowth was not measured in this study, I cannot comment conclusively on these potential changes. However, these data suggest that the effect of odors experienced during foraging on antennal lobe volume may be negligible. In honey bees, olfactory glomeruli are significantly larger in nurses (younger bees) than in foragers (older bees) (Withers et al. 1993). Considering the olfactory-rich environment experienced by a nurse bee, this result may be unsurprising. An additional point of consideration is that the antennal lobe of the honey bee requires up to two weeks to become fully active, exhibiting changes in the amplitude of response and the pattern of glomeruli encoding (Shunpeng et al. 2005). In bumble bees, this may not be the case, and the lack of difference in antennal lobe volume in different sensory treatment groups may reflect this possibility.

The effect of visual experience on the relative volume of the antennal lobe and mushroom bodies has, to my knowledge, not previously been documented in *Bombus.* Plasticity of the antennal lobe has been reported in the honey bee numerous times, including changes in the volume of specific olfactory glomeruli (Winnington et al. 1996; Sigg et al. 1997; Brown et al. 2002). A significant decrease in relative antennal lobe volume was observed in bees that received visual experience during the first week of adult life. Since bees in visually-deprived conditions had at least social odors but no visual information to process, the relatively larger antennal lobes in the visually-deprived bees may be functionally relevant. For example, multi-sensory compensation has been documented in cats, where visual deprivation leads to a significant increase in the number of auditory-responsive cells in the superior colliculus (Rauschecker and Harris 1983). In addition, congenitally deaf cats display superior vision in the peripheral field and decreased movement detection thresholds as compared to hearing cats (Lomber et al. 2010). Brain reorganization has also been widely documented in humans, especially when sensory experience is limited in early development, such as with early onset blindness (reviewed in Noppeney 2007). A compensatory response leading to increased olfactory-processing tissue volumes in visually-deprived bees could partially explain my results. Further research is needed to explore the physiological basis of the volume differences observed in this study, as well as potential correlates with performance.

Relative volume decreases in the mushroom body calyces and lobes were observed in bees with visual experience. This result could be a function of a number of different developmental processes. With visual experience, pruning of synapses and dendritic branches in the mushroom bodies may account for the relative decrease in neuropil volume. Pruning and reorganization of the mushroom bodies has been shown in the ants *Cataglyphis fortis* and *Pheidole dentata,* with changes observed due to both visual experience and age (Stieb et al. 2010; Seid et al. 2005). Again, further studies are needed to identify the basis of volume change with visual experience observed in this study. Cytoarchitectural studies of the Kenyon cells could aid in elucidating the effect of visual experience on the mushroom bodies in *Bombus*. The gross measurements taken in this study may give indications for neuronal reorganization, but changes at the cellular and sub-cellular levels may not be captured as they may not affect overall neuropil volume.

The ease with which sensory modalities can be manipulated for bumble bees housed in a laboratory environment makes them an attractive candidate for studies of environment-dependent neuronal plasticity. Results presented here demonstrate that bumble bees, like honey bees, exhibit significant neuropil changes associated with age and experience. These results may provide a starting point for a broader consideration of how differences in the life history of these two species may correlate with the extent and form of neuronal plasticity. In honey bees, neuropil volume increases are associated with developmentally-programmed switches in behavioral tasks, such as the shift from nursing to foraging with age (Withers et al. 1993, 1995; Farris et al. 2001). In bumble bees, brain reorganizations may lead to increased foraging success, faster learning, or more efficient sensory processing. Further research on the functional implications of neuropil volume increases in the bumble bee may elucidate the consequences of the changes associated with age and experience presented in this study.

## Tables

| Vis | Olf | n | Medulla | Lobula | Antennal Lobe | MB Calyces | MB Lobes | Central Body | Neuropil (rest) | Cell Bodies |
|---|---|---|---|---|---|---|---|---|---|---|
| - | - | 10 | 0.1862 ± 0.0122 | 0.0560 ± 0.0027 | 0.0385 ± 0.0050 | 0.1096 ± 0.0080 | 0.0727 ± 0.0044 | 0.0031 ± 0.0003 | 0.2385 ± 0.0147 | 0.2954 ± 0.0240 |
| - | + | 10 | 0.1808 ± 0.0102 | 0.0533 ± 0.0046 | 0.0368 ± 0.0036 | 0.1071 ± 0.0086 | 0.0703 ± 0.0052 | 0.0032 ± 0.0003 | 0.2412 ± 0.0125 | 0.3074 ± 0.0284 |
| + | - | 10 | 0.1789 ± 0.0080 | 0.0545 ± 0.0021 | 0.0318 ± 0.0026 | 0.0995 ± 0.0082 | 0.0683 ± 0.0033 | 0.0030 ± 0.0002 | 0.2388 ± 0.0119 | 0.3251 ± 0.0221 |
| + | + | 10 | 0.1815 ± 0.0088 | 0.0535 ± 0.0020 | 0.0330 ± 0.0034 | 0.1039 ± 0.0062 | 0.0670 ± 0.0048 | 0.0033 ± 0.0003 | 0.2350 ± 0.0149 | 0.3228 ± 0.0193 |
| Visual Effect | | | 1.0607 | 0.4036 | **19.90*** ** | **7.262*** | **7.40*** | 0.7806 | 0.4629 | **9.25**** |
| Olfactory Effect | | | 0.1928 | 3.7043 | 0.0242 | 0.674 | 1.82 | 0.1173 | 0.0195 | 0.4309 |
| Vis*Olf Effect | | | 1.6384 | 0.7141 | 1.4887 | 0.1602 | 0.115 | 0.2584 | 0.592 | 0.9121 |

**Table 1**
Brain subregion volume estimates (mean relative volume ± SD) in sensory treatments.
“Vis” and “Olf” refer to whether the bee received (+) or did not receive (-) visual or olfactory stimuli. Values at bottom of table correspond to $F_{3,36}$ statistic from factorial ANOVA with visual, olfactory, and vis*olf factors. * denotes p<0.05, ** p<0.01, *** p<0.0001

## Figures

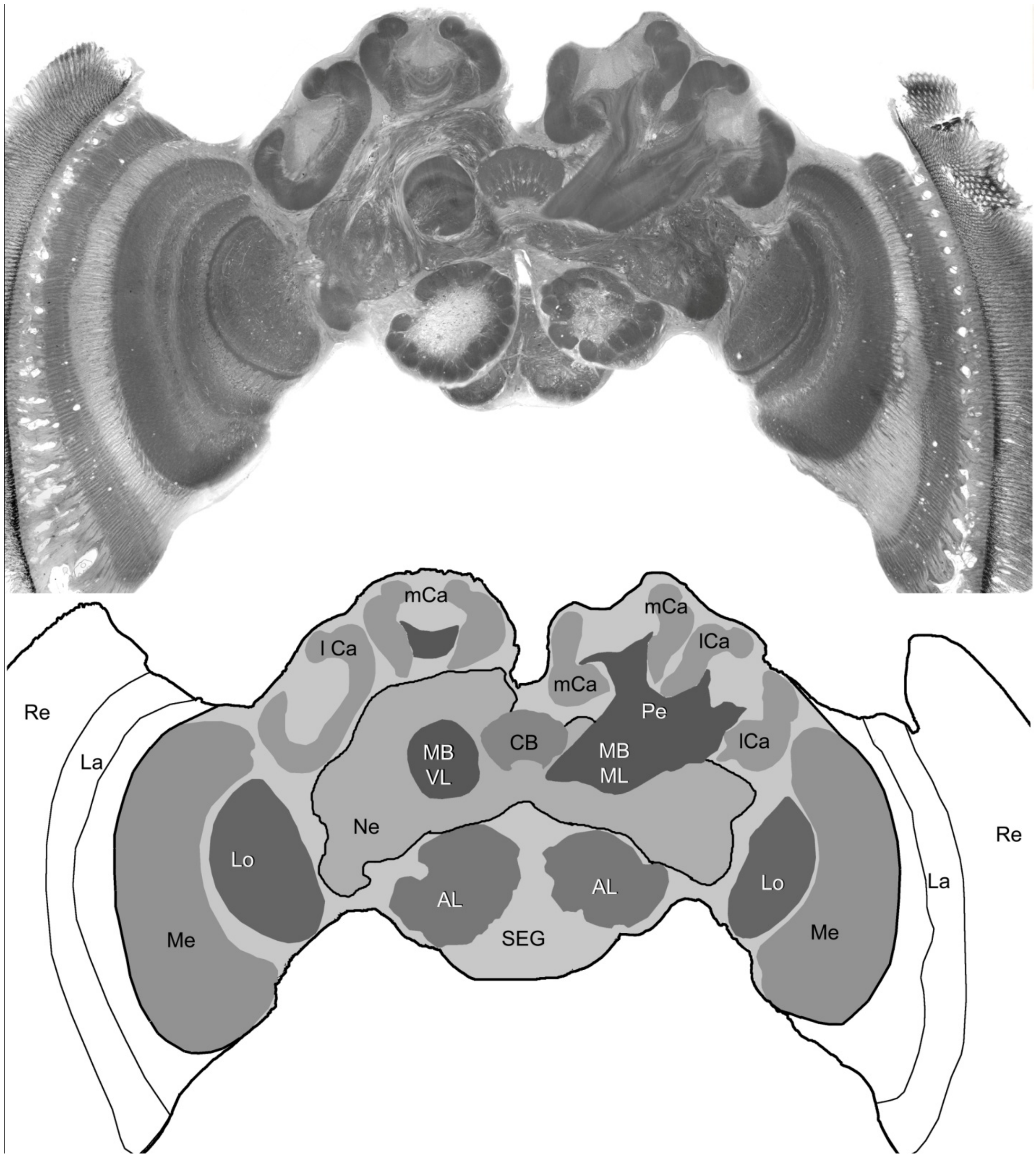


**Figure 1**
Montage of 3 frontal sections through a *Bombus* brain to show major brain components (top) and corresponding outline highlighting regions of interest (bottom).
Volumes of the following regions were measured: medulla (Me), lobula (Lo), antennal lobe (AL), mushroom body lobes (vertical lobe, VL; medial lobe, ML and peduncle, Pe), mushroom body calyces (lateral calyx, lCa; medial calyx, mCa), central body (CB), neuropil (rest) (Ne), and cell bodies (remaining shaded area). Other structures labeled in figure include the retina (Re), lamina (La), and subesophageal ganglion (SEG). (Modified from Riveros and Gronenberg 2010)

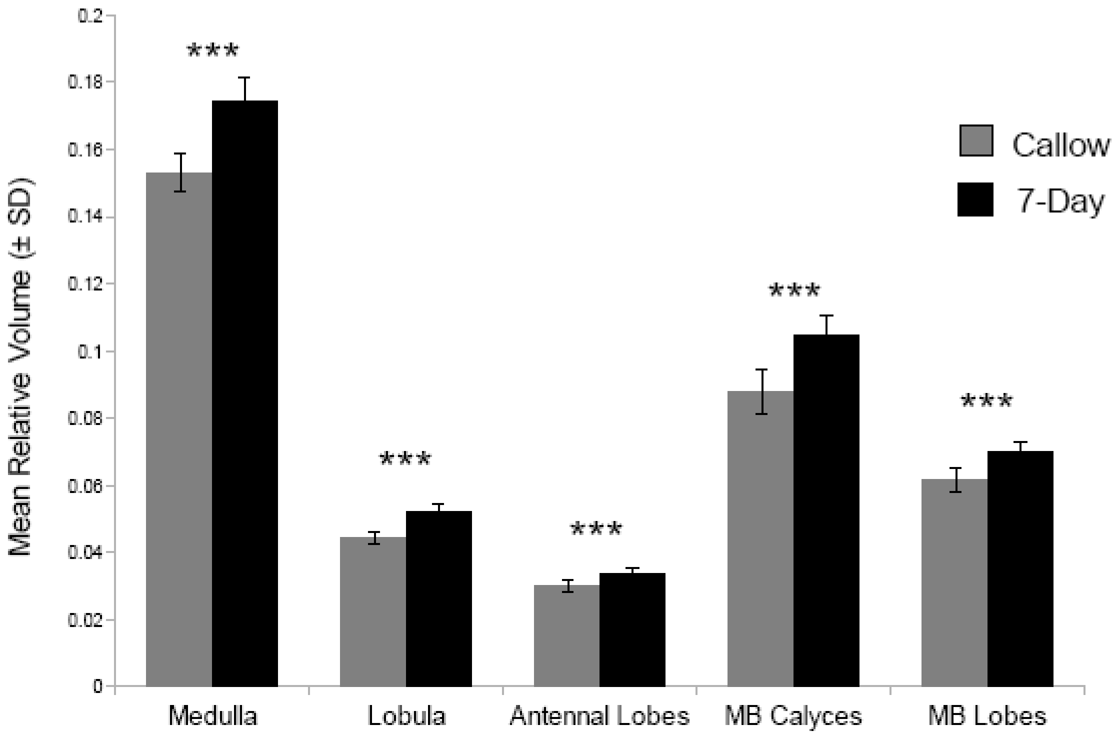


**Figure 2**
Mean relative volume (± SD) of sensory processing tissues in callow and 7-day foraging bees.
*** denotes p<0.0001 obtained from Student's t-test

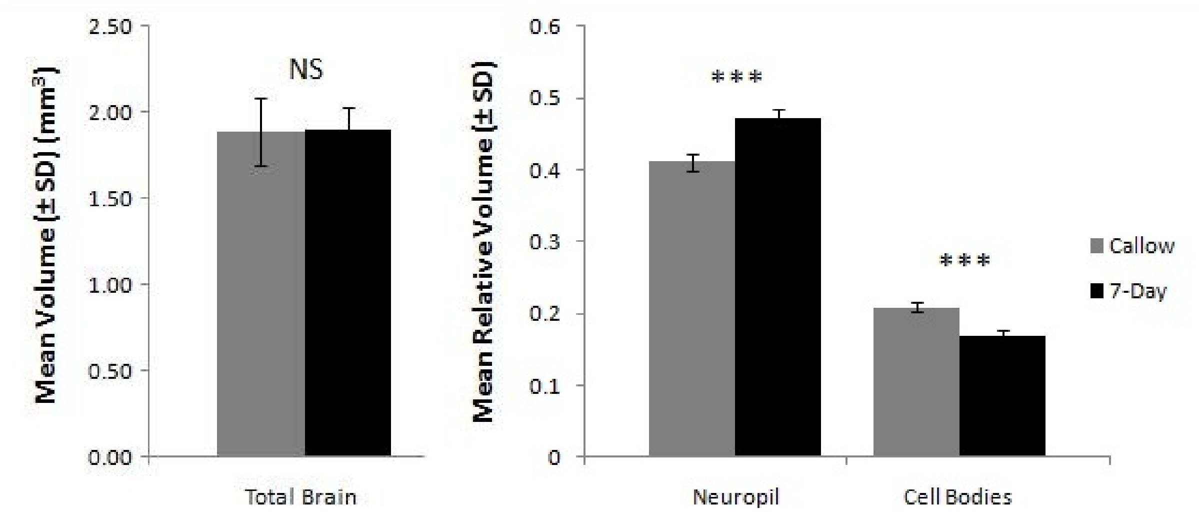


**Figure 3**
Total brain volume and mean relative volumes of total neuropil (all individually measured brain components plus neuropil "rest") and cell bodies in callow and 7-day-foraging bees.
*** denotes p<0.0001 obtained from Student's t-test; NS: not significantly different

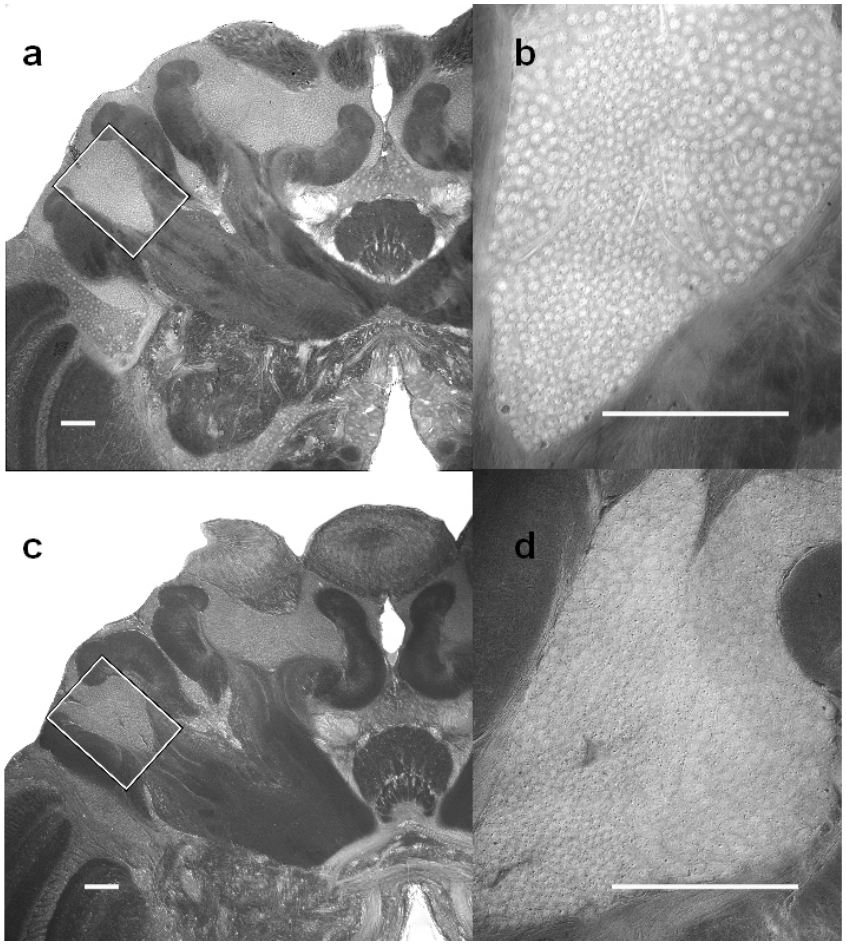


**Figure 4**
Comparison of Kenyon cells in callow and 7-day-foraging bumble bees.
10x magnification of frontal sections of a representative callow (a) and 7-day-foraging bee brain (c). 60x magnification of regions denoted by write frames are shown for the callow brain in (b) and the 7-day-foraging bee brain in (d). White scale bars are 100 μm.

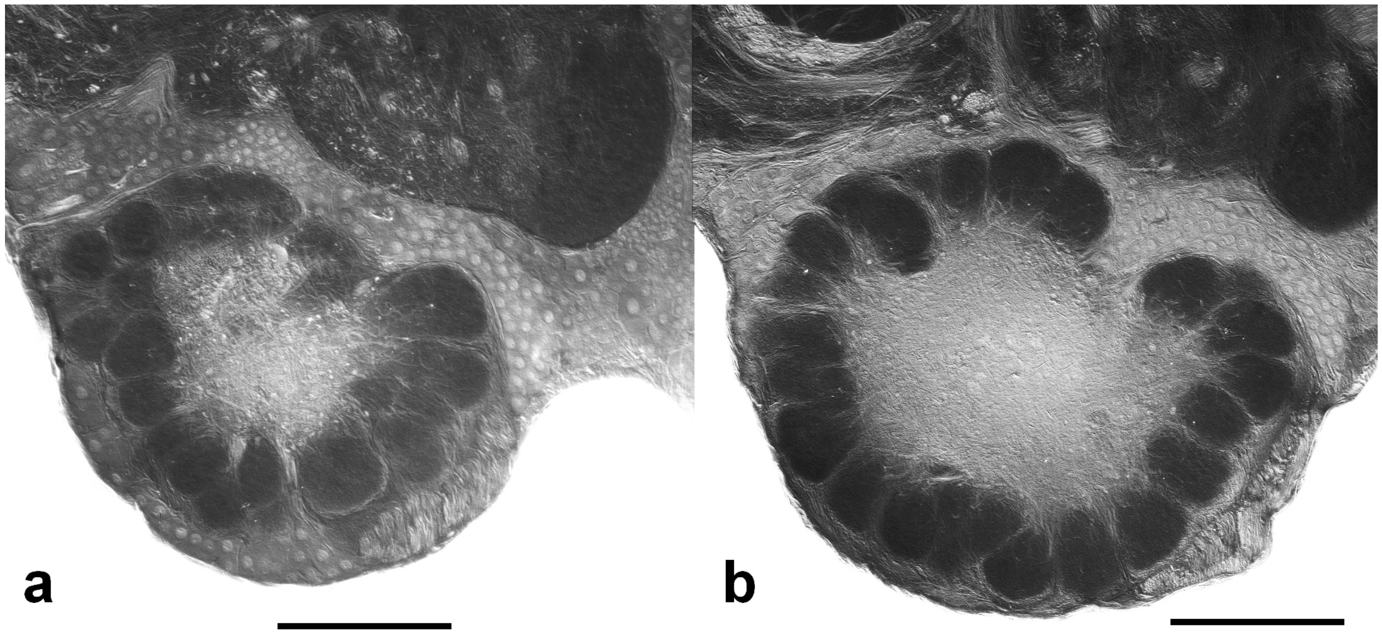


**Figure 5**
Comparison of cell bodies surrounding the antennal lobe in the brains of callow (a) and 7-day-foraging (b) bumble bees. Images are 20x magnifications of frontal sections. Black scale bars are 100 µm.

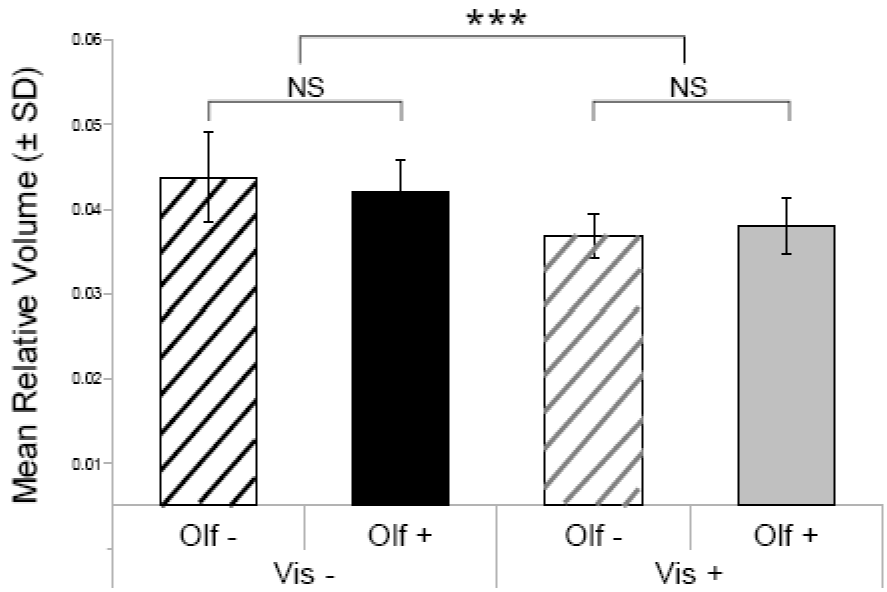


**Figure 6**
Effects of visual and olfactory stimuli on relative volume of antennal lobes.
*** denotes p<0.0001 obtained from Factorial ANOVA, $F_{3,36}$; NS: not significant

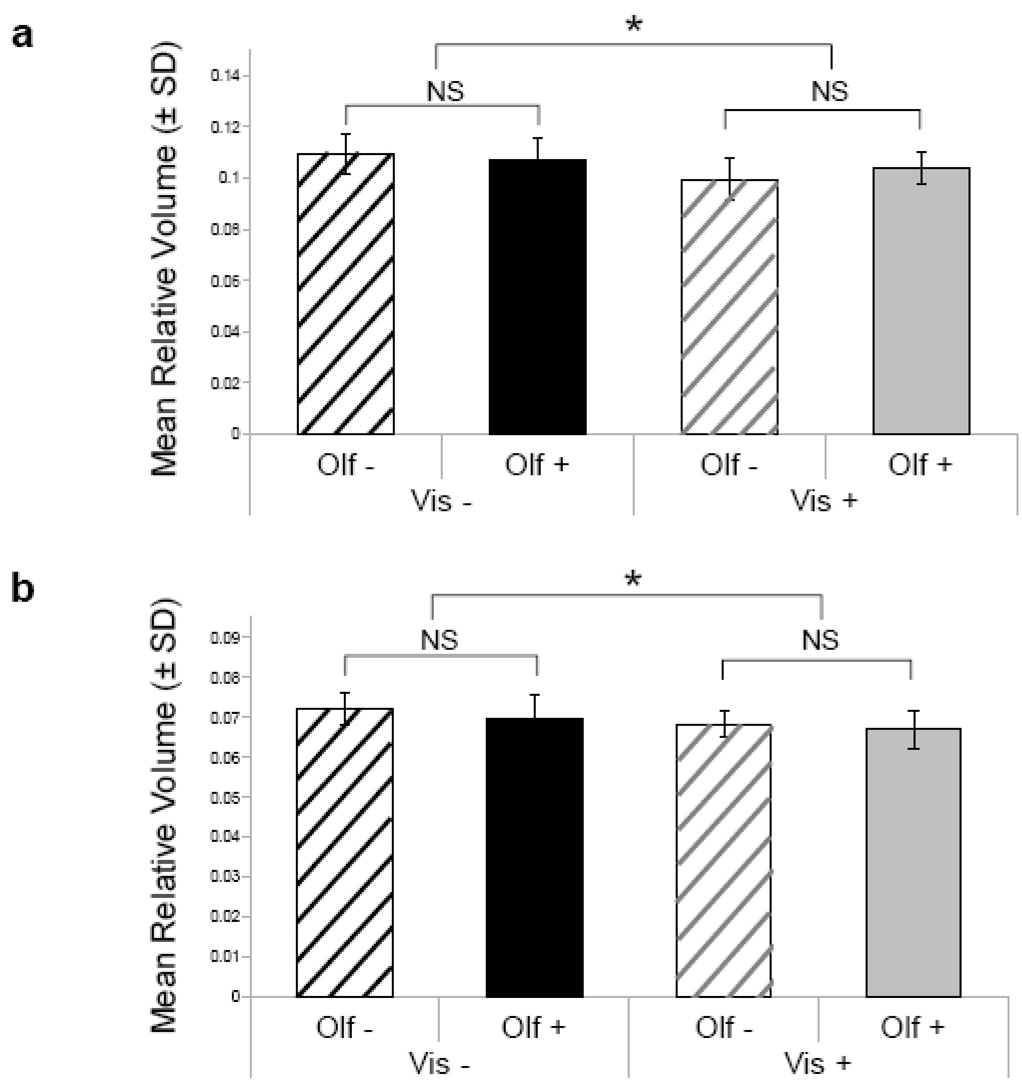


**Figure 7**
Effects of visual and olfactory stimuli on relative volume of MB calyces (a) and MB lobes (b).
* denotes $p<0.05$ obtained from Factorial ANOVA, $F_{3,36}$ ; NS: not significant

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**Appendix**

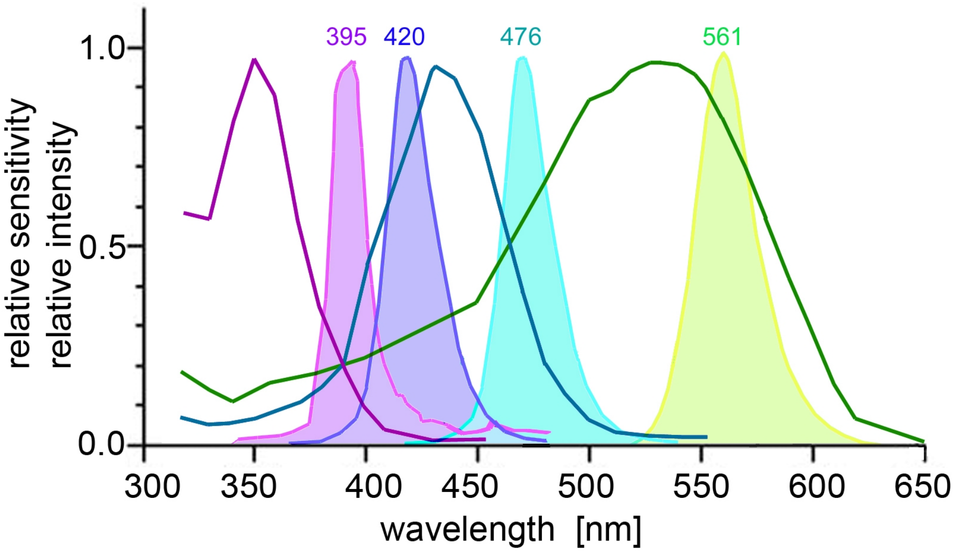


**Figure 1**
Emission spectra of LEDs with peak wavelengths of 395, 420, 476 and 561 nm as shown.
Overlaid are the spectral sensitivities of the three *Bombus* photoreceptors (after Skorupski et al. 2007).

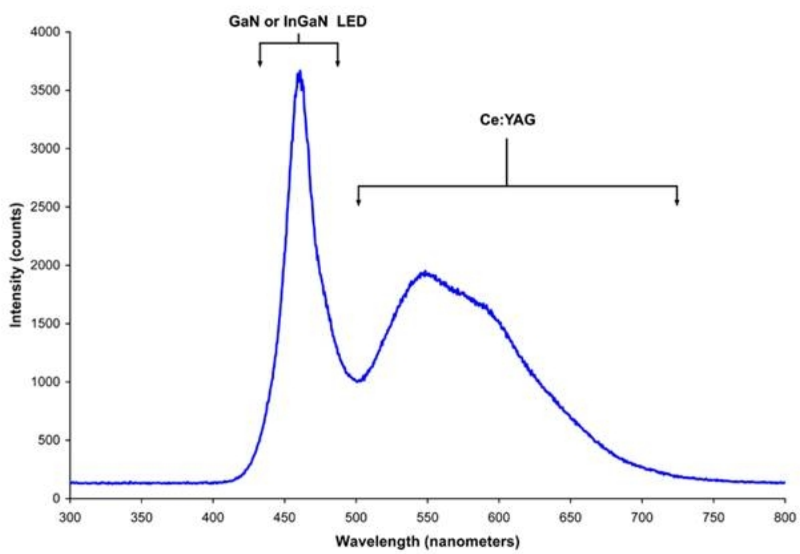


**Figure 2**
Emission spectra of "white" (broad-spectrum emitting) LED used in sensory experience experiment.

| Group | n | Medulla | Lobula | Antennal Lobe | MB Calyces | MB Lobes | Central Body | Neuropil (rest) | Cell Bodies |
|---|---|---|---|---|---|---|---|---|---|
| Callow | 10 | 0.1527 ± 0.0054 | 0.0444 ± 0.0017 | 0.0298 ± 0.0017 | 0.0877 ± 0.0065 | 0.0618 ± 0.0036 | 0.0028 ± 0.0003 | 0.2024 ± 0.0054 | 0.4184 ± 0.0136 |
| 7-day-old | 10 | 0.1742 ± 0.0071 | 0.0520 ±0.0022 | 0.0334 ± 0.0023 | 0.1043 ± 0.0061 | 0.0701 ± 0.0030 | 0.0026 ± 0.0002 | 0.2254 ± 0.0119 | 0.3379 ± 0.0154 |
| | | **-7.309*** ** | **-8.593*** ** | **-5.287*** ** | **-5.833*** ** | **-5.701*** ** | 1.406 | **-5.5702*** ** | **12.192*** ** |

**Table 1**

Neuropil subregion relative volumes (mean ± SD) in callow and 7-day-old foraging bees.

Values at bottom of table correspond to t-ratio from Student's t-test ($t_{18}$). *** denotes p<0.0001