

Final report on AOARD grant #074080 ‘Miniature brain decision making in complex visual environments’.

Report author: Adrian Dyer 18 July 2008.

Email: adrian.dyer@med.monash.edu.au

1. Executive summary.

This report covers a 1 year project (AOARD grant #074080) which was an extension of the project #064038 (a separate report on #064038 was provided 23 September 2007). There were five specific aims to #074080 (detailed section 2 below). Aim (i) has had very successful outcomes and produced three original manuscripts published in international peer reviewed journals with credit to AOARD. Aim (ii) (a major focus of the grant) has been completed and the results reported at one major conference and presented at several universities with credit to AOARD. A manuscript has been completed and is currently undergoing colleague peer reviews prior to submission to a major journal. Aim (iii) was initially completed (using -70° freezers) and extraction of RNA from bee brains tested; this was only partially successful and so a variety of alternative storage methods were evaluated and a successful brain storage/RNA extraction technique developed. This work is continuing with funding from the Australian Research Council. Aim (iv) has developed stimulus locked electrophysiological recordings from the bee brain, and a major funding proposal (to Australian Research Council) to continue this pilot work has been submitted (ARC decision due late September; very positive reviews have been received). For aim (v) a preliminary investigation into the possible links between mobile phone (RF) radiation and colony collapse disorder (CCD; a major disruption to honeybee colonies in the USA) has been conducted, and one minor journal article discounting the possibility published with credit to AOARD. There does not appear to be sufficient evidence from my evaluation of the initial pilot work previously done in Germany to warrant recommending funding work on this topic at present (details of further pilot studies being conducted by a different research group in Germany are provided below). Finally, a stated goal of AOARD in initially supporting my research was to assist in helping me bridge to major funding support within Australia; this has been a very successful endeavor as in September 2007 I was awarded a prestigious 5 year ARC QEII fellowship (5 years funding for salary, which began July 2008) to continue work on miniature brain processing of visual information in complex environments, which is work that I hope will continue in collaboration with, or which I hope will at least be of interest to the USAF.

2. Specific aims of the project were:

- (i) **Evaluate the capacity of bees to make complex decisions.** Test complex decision making processes of highly trained bees when deciding whether to choose or reject target and distractor stimuli. Data will be computer modeled using signal detection theory equations to enable comparisons between how miniature and human brains solve complex problems.
- (ii) **Evaluate the ability of miniaturized brains to solve stimulus invariance problems, especially in regard to face recognition.** The ability to recognize stimuli like faces when there is a degree of rotation presents serious computational problems. I will further investigate whether bees can learn to

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14. ABSTRACT The grantee investigated, using the honeybee (Apis mellifera) as a model, how decisions are learnt in complex visual environments. In particular, the grantee investigated the problem of face invariance to understand the role that experience with stimuli can play in permitting a brain to learn how to reliably recognize target stimuli independent of factors including angle of view and contrast variability. The grantee also investigated how signal detection theory (SDT) can be used to model complex behaviors in bees.					
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integrate previously learnt images to solve novel rotational type problems to help understand if there are any biological solutions to this difficult computational problem.

- (iii) **Build a database of bee performance.** At the completion of each experiment all bees from aim (i) and (ii) will be stored at -70°C. This will establish a database of performance of individuals whose genetic code could then, in a separate study, be used to determine the genetic principles underlying high level task performance.
- (iv) **Investigate the neural regions of the brain active during visual task solving.** I will use single-unit and multi-unit electrophysiological recordings to make measurements of the regions of the mini brain that show greatest activity during visual problem solving.
- (v) **Conduct a preliminary investigation into how radiation from mobile phone type devices might affect decision making in bees.** One very recent study suggests that radiation from devices like mobile phones might affect decision making in honeybees, although this finding has yet to be confirmed. I will make preliminary investigations into the feasibility of using the honeybee as a bio-indicator for understanding the effects of radiation on organisms. If preliminary study reveals that bees might be an interesting model, I will prepare and submit a full separate white paper to the USAF to continue work on the topic.

3. Summary of research findings in relation to specific aims.

Aim (i). This has been a very successful part of the project resulting in three journal publications. The major study (Dyer et al. 2008a), published with special coverage in the Journal of Experimental Biology:

<http://jeb.biologists.org/cgi/content/full/211/8/i>

shows that the bee miniature brain learns to make complex decisions following extensive experience (using differential conditioning) with stimuli that require fine discrimination, and that this decision making can be modeled with signal detection theory (with some similarities to a primate brain). A second study was done with the assistance of a student to help understand some of the theory behind how a compound eye can make fine discriminations of complex natural shapes (Williams et al. 2008), this was published in a minor but international peer review journal. A third study investigated the particular colour/green contrast channels involved in target detection by miniature brains and demonstrates how signal to noise limits may have directed how different bee species evolved different imaging solutions for finding targets in complex natural environments (this also partially links to aim iv). This finding was published in a leading neuroethology journal, The Journal of Comparative Physiology A (Dyer et al. 2008b).

Aim (ii). This has been a very exciting part of the overall project. The work clearly indicates that the bee miniature brain can learn to recognize target stimuli at novel rotational angles (horizontal plane) through a process of image interpolation. The findings have been reported at one major conference (Dyer et al 2007a, see poster summary in reference section below), and at several invited seminar presentations (University of Vienna, Max Planck Institute in Frankfurt, University of Toulouse, Macquarie University, Biometrics Institute of Australia and Monash University). The work has thus received a large amount of expert feedback (which resulted in some

extra controls being included), and the final results will be submitted to a major journal following a final round of internal peer reviews at Monash University. A draft of the final manuscript is included as appendix A and notification will be given to AOARD when the final publication of the material occurs.

Aim (iii). Initial investigations on storing bees in a -70° freezer showed that after periods greater than one month that only low quality RNA could be extracted from the bee brain. Subsequent investigations have revealed that if the brains are stored in RNA later then a large amount of high quality RNA can be extracted from the brain. My laboratory is continuing to conduct research on bee speed accuracy for complex decision making (now funded by the ARC) and will use this improved methodology developed during the project to help understand how miniature brains are able to learn to make complex decisions. This is likely to be a several year continuing project now that the initial storage/RNA extraction techniques have been developed.

Aim (iv). I have been able, with the assistance of Dr David Reser, to develop techniques to make recordings from the medulla region of the bee brain for stimulus locked presentation of different colour stimuli (see Figure 1). This has allowed us to make a formal application to the Australian Research Council for major funding to employ a postdoctoral level research to map the brain for different colour stimuli depending upon individual experience. Very positive reviews for this proposal have been received and I will inform the AOARD about what will happen next with this project when funding from the ARC is announced in late September 2008. This is potentially a very interesting avenue of investigation as a research paper (published 18th June 2008 Journal of Neuroscience v28 p 6319) by another research group indicates that the miniature brain has anatomically separated regions for processing (and eventually binding) colour and motion vision. If we receive support from the ARC to continue this work it may be possible to learn how miniature brains learn to process complex visual tasks depending upon experience.

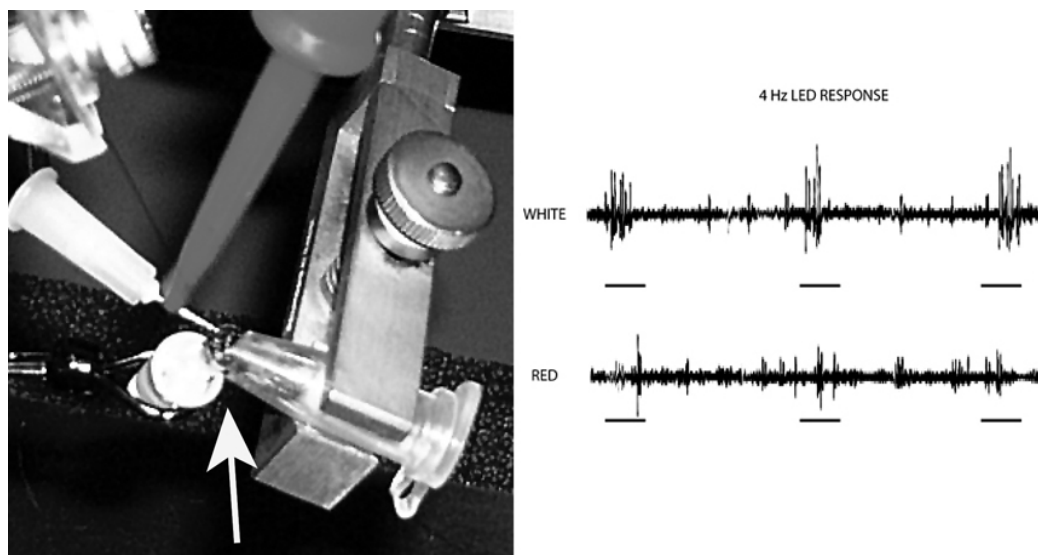


Fig. 1 Photograph of mounted bee during electrophysiological recordings, and responses of a neuron in the medulla region of the bee brain to stimulation from either a 4Hz 'white' LED (strong neural response) or a 4 Hz 'red' LED (little or no response).

Aim (v) The specific initial goal was to understand if RF radiation from mobile phones (or potentially other equipment) might affect the performance of honeybees. This research resulted from reports that colony collapse disorder (CCD; where entire colonies of honeybees fail to return to their colony) in the USA might be caused by mobile phone radiation. After some preliminary background research this possibility seemed very unlikely because in Australia the country has one of the highest mobile phone usage rates in the world, but there are currently no reports of CCD. Since the initial reports of a possible link were widely circulated (including the media in Australia), I prepared and published a short paper (Dyer 2007b; with credit to AOARD) to remove what I thought to be almost panic level response to the unsubstantiated claims. I then visited the authors of the original study at the Institut fuer Mathematik, Universitaet Koblenz-Landau, Landau, Germany on the 4th September 2007 and they made available for me to see some photographs of the original experiments and also they let me see the original data on which the study was based. They were very upfront that the data was pilot study level and they had not expected the intense media interest (generated by Geoffrey Lean of the Independent, UK; see Dyer 2007b) to come from preliminary data. Their data set was very preliminary and inconsistent results were achieved on their one follow up study (not published). There also appeared to be one potentially critical quantitative error as the parameters of bee return time (to hive) and the number of returning bees were combined rather than being analysed independently. It was not clear why this was done but I think it makes the statistical analysis of the data very weak and prone to introducing error; thus I would recommend at this stage that there is insufficient evidence from this pilot work to warrant funding a full scale study. The authors indicated that they have no interest in continuing the work, but have past their data and equipment onto the Bee Group in Wuerzburg in Germany (headed by Professor Tautz). I was formally a postdoctoral fellow at Wuerzburg University and so I was able to secure and invite to visit to find out what they are currently doing. The Bee Group in Wuerzburg plans more controlled follow up experiments in the European summer of 2008 which should allow a better understanding of the initial findings. This group has said that they will keep me informed about their progress and I will advise the USAF if any confirmation of the initial results does become available (preliminary results may be available around November 2008 but this is not directly within my control).

4. Conclusion.

Aim (i) was fully completed and resulted in major publications.

Aim (ii) has been completed with a very important finding that has been reported at a vision conference and a paper will be submitted soon. A draft of this paper is shown in Appendix A.

Aim (iii) has solved several technical issues with extracting RNA from bee brains, and bees for current experiments are being stored using the developed protocols.

Aim (iv) has shown that the medulla region of the brain is suitable for electrophysiological recording during stimulus presentation. A major grant has been

written (to the ARC) to continue this work (positive reviews received; grants announced September 2008).

Aim (v) revealed that the evidence of RF radiation from mobile phone devices affecting bees (and possibly causing CCD) is based on low quality data and there is insufficient evidence at present to warrant funding a full scale study on this topic.

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Published peer reviewed papers with credit to AOARD.

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Published conference proceedings with credit to AOARD.

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Face processing by miniature brains

AG Dyer: adrian.dyer@med.monash.edu.au
Centre for Brain & Behaviour, Physiology Dept, Monash University, Clayton 3800, Vic, Australia. MONASH University

AIM: This study seeks to reveal mechanisms bees use when confronted with complex visual tasks. Recent studies show that invertebrates including wasps^{1,2} and honeybees (*Apis mellifera*)³ are able to recognise either conspecific or human faces (see Fig1st-3). How brains learn to process faces is of considerable interest, but can be confounded by the difficulty of selecting appropriate controls for ontogenetic history. Thus studies of miniature brains in social insects can provide significant insights on what was considered to be a high-order visual task.

Experiment 2: A major problem for object recognition in complex environments is that targets need to be recognised from a variety of different viewpoints⁴. Both humans⁵ (including newborn babies⁶) and pigeons⁷ recognise faces and objects using view dependent representations combined with mechanisms that generalise from familiar to unfamiliar views. This experiment tested how bees learnt to recognise rotated face stimuli. First independent control groups of bees were individually trained to either (5a) faces in a plane (0°) OR (5b) rotated (60°) view, than in non-rewarded tests were evaluated on a novel rotated view of 30°.

Fig 1: BEE INSPECTING FACE. Fig 2: FACE ACQUISITION FOR BEE. Fig 3: FACE RECOGNITION FORMS A LONG TERM MEMORY.

Experiment 1: Individual bees were trained with differential conditioning (promoting whole pattern learning⁸) to achromatic target and distractor face images presented on a rotating screen⁹: bee acquisition reached >70% correct choices after 120 decisions, as confirmed in non-rewarded tests (Fig 4a). Bees were then evaluated in non-rewarded transfer tests using image transformations that have been useful for understanding face processing in primates. Results indicate configural processing independent of stimulus brightness (Fig. 4b-f).

Target	Distractor	Bee choices for target in non-rewarded tests.
Fig. 4a	vs	>70%
Fig. 4b	vs	58.5% (6.3sd), t=7.2, df 9, p<0.001
Fig. 4c	vs	68.5% (4.7sd), t=9.4, df 9 p<0.001
Fig. 4d	vs	51.6% (6.7sd), t=0.6, df 6, p=0.551, ns
Fig. 4e	vs	47.1% (6.4sd), t=1.2, df 6, p=0.281, ns
Fig. 4f	vs	65.4% (9.8sd), t=3.9, df 6, p=0.008

Experiment 2: Acquisition (a) Results for NRT, N=30 bees, 1-sample t-test d.f.29, target/distractor swapped for 15-30 bees.

Condition	Results
Fig. 5a	NRT 73.8%, p=3.16e-07, p=0.001
Fig. 5b	NRT 69.9%, t=9.11e-014, p=0.001
Fig. 6a	70.9% (13.3sd), t=7.795, p=0.001
Fig. 6b	71.5% (12.8sd), t=8.647, p=0.001
Fig. 6c	61.5% (10.9sd), t=5.472, p=0.001

In neither condition could bees recognise the novel face (Fig. 5a,b). Next, an independent group of 30 bees was trained to both plane and 60° rotated faces prior to being evaluated on a novel rotated view of 30°. The bees learnt to interpolate images to solve a novel task (Fig. 6a,b).

CONCLUSION: Miniature brains in bees can use configural processing to recognise complex shapes like faces, and use a mechanism of interpolation to solve complex novel tasks.

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Communications with credit to AOARD.

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Appendix A

Miniature brains recognize rotated faces. [Manuscript to be submitted to a major journal in August 2008 following a final round of internal peer reviews]

Many biologically important objects like flowers for bees (Dafni et al. 1997), and faces for primates (Logothetis et al. 1994), sheep (Kendrick et al. 2001) and wasps (Tibbetts 2002), have to be viewed from different viewpoints. The ability to recognise complex spatial shapes like faces presents significant challenges to visual systems when these stimuli are rotated in depth because of the drastic changes in visible features (Bruce et al. 1999; Turati et al. 2008). Here we show that the honeybee brain can recognise novel views of rotated face stimuli through a process of image interpolation, much like primate brains. The results show that a miniaturised system is able to efficiently recognise objects in complex environments, which has implications for the design of artificial recognition systems based on minimal processing requirements.

Adult humans (Bülthoff and Edelman 1992) and other primates (Logothetis et al. 1994) are able to recognise novel presentations of rotated faces through mechanisms that predominantly rely on image interpolation of a limited number of stored views. For instance, a radial basis function network comprising of an input, output and hidden layer can learn a smooth function which linearly interpolates novel views between stored views (Poggio and Edelman 1990). Interestingly, primate brains perform better with interpolation rather than extrapolation of novel rotated views (Bülthoff and Edelman 1992), but some other animal models like pigeons respond equally well to both interpolated and extrapolated views (Spetch and Friedman 2003). Recent studies on the processing of visual stimuli by honeybees suggests that these animals have a remarkably sophisticated brain for solving tasks efficiently (Giurfa et al. 2001; Stach et al. 2004; Zhang and Srinivasan 2004; Dyer et al. 2008). Furthermore, when provided with differential conditioning, bees show remarkable neural plasticity to learn differences in perceptually similar patterns using global cues (Giurfa et al. 1999; Stach et al. 2004). To understand how miniaturised brains might deal with the problems posed by rotations in depth, we presented bees with a face recognition task that has been successful for understanding face processing in both human adults and infants.

Individual honeybees (*Apis mellifera*) were trained with differential conditioning to vertically presented stimuli (Dyer et al. 2005; Dyer et al. 2008) representing different views (0°, 30° or 60°) of two similar faces (S1, S2; Figure 1) that have been useful for understanding face processing in newborn humans (Turati et al. 2008). Group 1 was trained with face images at 0° view and then given non-rewarded tests with novel images of the faces at 30° view. Group 2 was trained with a 60° view and tested with novel images of the faces at 30° view. Group 3 was provided training with both the 0° and the 60° view before being tested with the novel 30° interpolation view. Finally, Group 4 was trained with both the 0° and the 30° view and tested with a novel non-rewarded test of the 60° extrapolation view. In each group, the target-distractors (S1/S2) were reversed for half the bees to control for potential preference effects (Stach et al. 2004).

After receiving differential conditioning, bees in all four groups were able to recognise the trained target stimuli significantly above (one sample t-test on arcsine square-root transformed proportions) chance performance [G1(0°)= 73.8% (10.2 sd), $t=7.169$, $N=18$, $df=17$, $p<0.001$; G2(60°)= 69.9% (7.9 sd), $t=9.119$, $N=18$, $df=17$, $p<0.001$; G3(0°)= 70.0% (13.3 sd) $t=7.795$, $N=30$, $df=29$, $p<0.001$; G3(60°) = 71.5% (12.8 sd) $t=8.647$, $N=30$, $df=29$, $p<0.001$; G4(0°)=72.7% (10.3 sd), $t=8.667$, $N=18$, $df=17$, $p<0.001$; G4(30°)=69.4% (7.3 sd), $t=10.77$, $N=18$, $df=17$, $p<0.001$]. This new finding shows that the bees can, in addition to storing one complex spatial pattern like a face (Dyer et al. 2005), store and successively retrieve multiple views of an image. However, when these highly trained bees were presented with a novel view of the target and distractor face pair, only bees in Group 3 were able to recognise the correct face significantly above chance (61.4% (10.9 sd), $t=5.472$, $df=29$, $p<0.001$), although performance was also significantly poorer than for the original training stimuli (paired sample t-test, $t=3.419$, $df=29$, $p=0.002$). The target recognition by bees in Group 3 cannot be explained by generalisation principles (Bernard et al. 2007) as neither bees in Group 1 [51.7% (7.9 sd), $t=0.947$, $df=17$, $p=0.357$] nor Group 2 [49.8% (7.4 sd), $t=0.920$, $df=17$, $p=0.928$] were able to recognise a novel view of the target face. The data thus implies that bees in Group 3 were able to extract some relevant information from conditioning with both 0° and 60° face representations, consistent with the idea of image interpolation (Poggio and Edelman 1990; Bülthoff and Edelman 1992).

Another possibility that could explain how bees in Group 3 were able to recognise novel viewpoints is by some form of image extrapolation where learning two different representations of a stimulus permits the bees' miniature brain greater flexibility to solve a novel view (Bülthoff and Edelman 1992; Collett and Baron 1995). However, bees in Group 4, which received conditioning to both 0° and 30°, were not able to extrapolate visual information to recognise a novel view at 60° [50.6% (5.4 sd), $t=0.438$, $df=17$, $p=0.667$]. Thus, the successful recognition of a novel target by the bees in Group 3 must be via a mechanism of image interpolation.

Like many animals that operate in complex visual environments, bees have to be able to reliably find three dimensional objects like flowers when these objects might be viewed from a number of different viewpoints (Dafni et al. 1997). We show that the miniature brain of honeybees, which contains approximately 0.01% the number of neurons in the human brain (Zhang and Srinivasan 2004), manages to reliably recognise objects despite viewpoint changes by using a mechanism of interpolation. The finding may have implications for understanding how computerised systems can solve complex visual problems (Rind 2004).

Supplementary information.

Experiments were conducted outdoors using natural daylight in fine, low-wind weather conditions. Individual honeybees were recruited from a gravity feeder providing 10% sucrose and rewarded with 25% sucrose for making correct choices on designated target stimuli presented vertically using a rotating screen of 50cm diameter (Dyer et al. 2005; Dyer et al. 2008). Incorrect choices (landings on distractor stimuli) were punished with 0.012% quinine hemisulphate (Chittka et al. 2003). Stimuli were 6 x 8cm achromatic photographs supplied by Dr Turati from a study that used these stimuli for investigating face rotation processing in newborn humans (Turati et al. 2008), although in this current study the images were presented on a light grey

background. Two target and two distractor stimuli were presented on the screen promoting differential conditioning (Giurfa et al. 1999; Dyer et al. 2005). Bees were provided with a 10 μ L drop of sucrose for a correct choice, and a second drop was presented on a plexiglas spoon (Dyer et al. 2005) to move the bee 1m away so that stimuli could be exchanged. When the bee became satiated it returned to the colony and all equipment was cleaned with 30% ethanol. For Groups 3 and 4, training was with different viewpoints of the target stimulus in alternative bouts. Each bee was trained until it had successfully chosen the target stimulus with $> 50\%$ accuracy in 6 consecutive bouts, and Figure Sup1 shows the mean acquisition for all 30 bees in Group 3 during initial learning. Once this precondition was met a bee was provided with a non-rewarded test with fresh versions of the training stimuli (to totally exclude olfaction), followed by refresher bouts with training stimuli (for motivation), and a second non-rewarded test with novel transfer stimuli. Finally, a bee was retested with the initial stimuli to confirm possible performance drops were not due to temporal factors. All data used for statistical analysis is from non-rewarded bouts to totally exclude olfaction or positional cues affecting the findings.

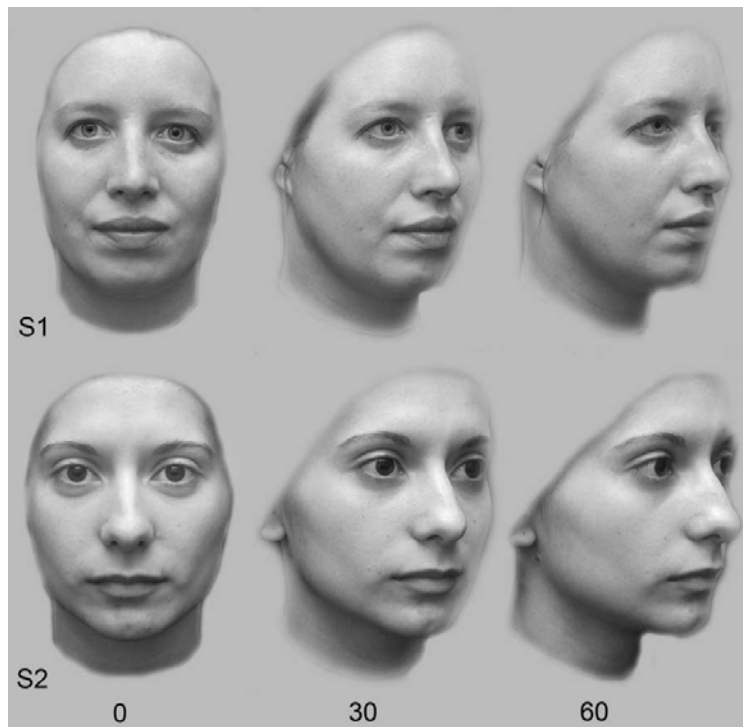


Figure 1. Stimuli representing rotated faces used to train bees with differential conditioning.

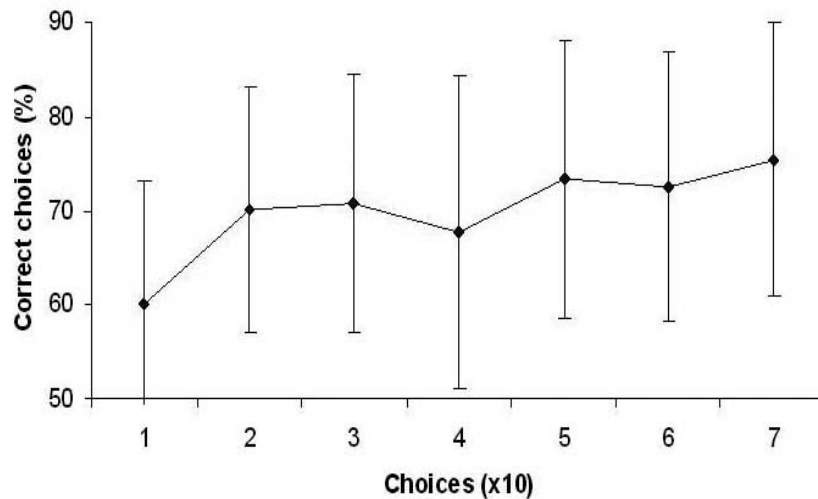


Figure Sup 1. Acquisition (mean +/- SD) for 30 bees (group 3) of the target stimuli.

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CCD and RF radiation

Colony collapse disorder in honeybees and possible effects of RF radiation

By Dr Adrian Dyer

Since August 2006 there have been a number of reports of colony collapse disorder (CCD) in the USA and parts of Europe (see Aust Bee Journal March 2007 p12; The New York Times April 24 2007 pF1).

CCD is an occurrence where honeybees abandon an established colony (including being well stocked with honey).

CCD does not appear to be solely due to factors like the varroa mite because the rate of colony decline progresses too fast (colonies can collapse in as little as two days). Possible other explanations for CCD have included micro organisms weakening bee immune systems, the over use of certain pesticides on plants, and/or over use of bees which are trucked to multiple commercial sites without sufficient opportunity for bees to forage in natural surrounds. Currently there is an absence of conclusive scientific data to prove or disprove these theories, or determine if multiple factors might be responsible.

There has also been a recent suggestion that a contributing factor to CCD could be an influence of Radio Fre-

quency (RF) electromagnetic radiation from mobile phone base stations. This possibility has received worldwide media coverage, including within the Australian media (ABCTV 730 report 7th May 2007; Herald Sun 16th April 2007 p7). The basis of these reports all appear to stem from an initial article by Geoff Lean and Harriet Shawcross published in: (The Independent, April 15, 2007—Are mobile phones wiping out bees? <http://news.independent.co.uk/environment/wildlife/article2449968.ece>).

This newspaper article links CCD to research conducted in Germany in 2005 on the ability of honeybees to act as bio-indicators for RF radiation (Kimmel et al. 2007; reprint at <http://agbi.uni-landau.de/materialien.htm>).

In the Kimmel study DECT mobile phone base stations (1900MHz frequency; average transmitting power 10mW) were placed within eight honeybee colonies, whilst eight control colonies were shielded from radiation. Individual forager bees from each colony were collected and taken 500m away, and frequency of bees finding their way back to a colony was significantly lower for the colonies exposed to RF radiation, indicating bees may have some sensitivity (aversion) to RF radiation.

I have contacted one of the

report authors (Dr Jochen Kuhn) from the Kimmel study, and he emailed (18 April 2007) that their study was specifically not related to CCD.

I have also had an email communication with Geoff Lean (15th May 2007) saying that whilst he did discuss his article with one of the research report authors; he was only able to receive a copy of the full research report after the article for 'The Independent' was published. Thus there may have been a misinterpretation about the apparent connection between CCD and bee sensitivity to RF radiation.

At this stage there would thus appear to be no strong scientific evidence of a link between CCD and mobile phone radiation. In particular, Australia is an interesting case in point as there are currently no reported cases of CCD within Australia (Crop stock and committee reports, April 2007, http://www.honeybee.org.au/april07.html#_Toc165260116), but Australia has high rates of mobile phone use, with base stations providing coverage to 98% of the population and 20% of the landmass

(www.amta.org.au/AMTA/default.asp?ID=365), and much of this coverage is in areas where bees are maintained by beekeepers.

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CCD and RF radiation, cont'd

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Although there are slightly different frequencies of radiation used in mobile phone type devices within Australia, the USA and Europe; the absence of any reports of CCD in Australia, as well as certain states within the USA, are highly suggestive that there is no direct link between radiation from mobile phones and CCD.

However, the evidence from the Kimmel study does suggest that honeybees might be a useful bio-indicator of RF radiation, which could po-

tentially have other important implications for studying radiation and health matters for vertebrates.

Whilst there are currently no reports of CCD within Australia, it would be worthwhile for bee keepers to keep track of any cases of bee disappearances within Australia in the event that this does become a local problem, or indeed if bee populations remain healthy in Australia then having data on this and other factors potentially affecting bees

might help dissect what is currently causing CCD in the USA and parts of Europe.

Adrian Dyer is a Postdoctoral Research Fellow in the Physiology Department at Monash University investigating cognitive processes in honeybees including possible effects of RF radiation (research support USAF AOARD grant 074080).

*Email:
adrian.dyer@med.monash.edu.au*