



Université
de Toulouse

THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par *Université Toulouse III - Paul Sabatier*
Discipline ou spécialité : *Neurosciences cognitives*

Présentée et soutenue par *Aurore Avarguès-Weber*
Le 13 décembre 2010

Titre : *Cognition visuelle chez l'abeille *Apis mellifera*: Catégorisation par extraction de configurations spatiales et de concepts relationnels*

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UNIVERSITE PAUL SABATIER – TOULOUSE III

U.F.R. Sciences de la Vie et de la Terre

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**« Tout accomplissement est
une servitude, il oblige à un
accomplissement plus haut. »**

Albert Camus

Remerciements

Je remercie évidemment en tout premier lieu ceux qui m'ont permis de faire cette thèse : les responsables de l'Ecole Normale Supérieure de Cachan pour s'être finalement laissé convaincre de financer une thèse dans un domaine des sciences « molles » et mon directeur Martin Giurfa pour m'avoir acceptée dans son équipe et donner les moyens de mener à bien cette thèse. Je le remercie aussi particulièrement pour m'avoir poussée à développer une rigueur scientifique associée à une recherche d'excellence dans le domaine scientifique. Je souhaite aussi le remercier pour sa compréhension et son soutien pour la conciliation de mon travail de thèse et de la maternité. Je tiens aussi à remercier ici Adrian Dyer qui a fait infiniment plus que de m'avoir donné les moyens nécessaires à la réalisation de mes expériences pendant l'été australien. Il s'est toujours montré d'une immense disponibilité pour répondre à toutes mes interrogations scientifiques et réfléchir aux divers problèmes rencontrés lors de mes expériences. Il a aussi toujours réussi à garder ma motivation au plus haut en me rappelant à quel point ce métier est passionnant. Nous sommes par ailleurs devenus de vrais amis au fil de mes séjours à Melbourne. J'en profite pour embrasser affectueusement sa femme et ses adorables fillettes rousses pour m'avoir fait partager de si bons moments familiaux et m'avoir permis de découvrir leur magnifique région en très bonne compagnie. Je voudrais aussi remercier Dave Reser, le texan célibataire, avec tout ce que ça implique, pour m'avoir ouvert son appartement lors d'un de mes séjours, son aide logistique et son soutien dans les débuts difficiles de ma grossesse. Je garde un souvenir amusé de nos échanges culturels.

Retour sur Toulouse, où je souhaite remercier mille fois mes collègues de bureau sans qui ces années auraient été bien tristounettes ! On a partagé de nombreux fous rires, une grande complicité, une écoute, des conseils et un soutien sans faille. Merci donc à Edith, qui est maintenant pour moi une véritable amie, car l'on partage bien plus que des discussions de bureau. Bien sûr, on aurait toutes les deux été certainement beaucoup plus efficaces dans notre travail sans la proximité de nos bureaux, mais ça aurait été bien dommage ! Merci aussi à Théo, notre cher brésilien, qui nous a fait découvrir la véritable Caipirinha, pour l'invention de son « je désiste », expression qui prend tout son sens en thèse et pour tous nos partages de soucis... J'ai réussi à finir la thèse sans cachet, tu y arriveras aussi ;)o). Et merci bien sûr à Julie et Esther, qui ont elles-aussi apporté beaucoup de vie à notre bureau. Enfin, un grand merci au 'phénomène' Julie (Bénard), qui est partie bien trop vite, pour m'avoir tout transmis sur l'art de dresser des abeilles et quel plaisir par la suite d'avoir une collègue qui comprenait mes galères !

Merci à tous les membres de l'équipe abeille, des services techniques et administratifs et au laboratoire en général pour leur aide et conseils aussi souvent que nécessaire.

Merci à Jean marc Devaud et Christian Jost pour leur aide dans le parcours du combattant du moniteur qu'est la recherche d'heures d'enseignement pour enfin réussir à atteindre son quota. Merci aussi à tous les enseignants (en particulier, Sylvie Berkès, Kerstin Bystricky et Dominique Larrouy) avec qui j'ai collaboré pour préparer et donner mes cours, une belle bouffée d'air en dehors de ma discipline.

Un grand merci à Michel Garcia, chargé de l'enseignement supérieur au rectorat, pour sa disponibilité et grande aide dans tous mes problèmes et questionnements administratifs issus de ma situation particulière de normalienne, agrégée, souhaitant continuer en post-doc et ayant bénéficié d'un congé maternité lors de ma thèse. Que d'impasses contournées grâce à vous !

Je souhaite remercier aussi particulièrement les gens du rucher-école de Pechbusque dans la campagne sud-toulousaine, et tout particulièrement Christian Pech, pour leur enthousiaste accueil et mise à disposition de leur ruche pour mes expériences. De bien longues journées de manips répétitives égayées par le paysage magnifique du haut de la colline, l'apparition d'un renard ou d'écureuils et le foisonnement d'insectes de toutes les tailles et de toutes les couleurs à observer. Des moments très agréables pour une naturaliste dans l'âme !

Je remercie aussi la bande des physiciens, Peyo, Stéphane, Nico, FX, Thierry, Elsa... pour tous ces bons moments partagés autour d'un bon repas le midi. Beaucoup d'échanges, de discussions, de rires, rien de tel pour se mettre dans de bonnes dispositions pour le travail de l'après-midi !

Un merci plein de tendresse et d'affection à mon mari Sébastien, pour son écoute patiente de tous mes soucis, son exemple et pour avoir comblé ma vie avec autre chose que le travail pendant ces années. Je le remercie aussi pour avoir réussi à me convaincre à tenter l'aventure d'un enfant pendant la thèse. C'est une belle victoire ! Et puis comment regretter d'avoir un bébé si sage et attachant... Merci p'tit Léon pour ces beaux moments d'émotion, de rire et de complicité lors de la rédaction de ma thèse (à côté tes chagrins dus à l'éloignement de ton père et la difficulté à tout gérer sont déjà oubliés).

Mes parents ont aussi toute leur place ici pour leur soutien téléphonique sans faille à chaque fois que j'avais besoin de râler, d'être conseillée ou tout simplement de parler. Merci infiniment et particulièrement à ma maman avec qui on a battu des records de durée de conservation téléphonique. Ils ont su, ce qui s'est parfois avéré essentiel, me rappeler dans les moments difficiles pourquoi je faisais cette thèse et relativiser l'importance des problèmes rencontrés.

Et pour finir, merci aux abeilles, qui se sont pliées librement et sans broncher et avec détermination à tous les casse-têtes que je leur proposais. Une petite mention spéciales aux abeilles australiennes, bien plus dociles, peut-être grâce à mon accent 'frenchy' lorsque je les encourageais dans leur langue natale, bien sûr !

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Introduction

Afin de pouvoir réagir efficacement face à notre environnement, il est nécessaire de pouvoir analyser les informations en provenance de nos systèmes sensoriels non seulement pour reconnaître un objet rencontré auparavant mais également pour savoir comment se comporter face à un nouvel objet. Notre système visuel est donc capable de transformer un flux de photons en reconnaissance précise des objets qui nous entourent malgré des changements de point de vue, d'éclairage ou encore une obstruction partielle. Le traitement des informations visuelles au niveau du cerveau permet aussi de déterminer la catégorie d'appartenance d'un nouvel objet grâce aux connaissances récoltées par l'expérience. Pour ce faire, notre perception du monde repose notamment sur l'analyse de la configuration des objets qui nous entourent ainsi que sur la capacité de classer les objets sur la base de caractéristiques physiques, fonctionnelles ou relationnelles communes.

Nous nous sommes intéressés dans ce travail de thèse à l'existence de telles capacités chez l'abeille *Apis mellifera* afin de mieux comprendre comment cet insecte perçoit le monde qui l'entoure mais aussi et surtout dans une perspective comparative. Nous avons voulu ainsi questionner la possibilité pour un mini-cerveau d'insecte d'effectuer des analyses à un tel niveau cognitif. L'abeille est un modèle particulièrement approprié pour les études de cognition car il est possible de tirer parti de son comportement naturel de butinage pour étudier ses capacités perceptives, d'apprentissage et de mémorisation. L'abeille a déjà fait preuve de capacités étonnantes sous forme de catégorisation, apprentissage de règles ou encore résolution de labyrinthes (Zhang et Srinivasan, 2004; Giurfa, 2007; Avarguès-Weber et al., 2011). De plus la relative simplicité de son système nerveux ainsi que son accès relativement aisé permettent d'espérer une caractérisation des bases neurales responsables de tels comportements complexes. Enfin, une fois décortiquées, les stratégies utilisées par l'abeille peuvent éventuellement être appliquées en robotique ou programmation pour effectuer des tâches ou comportements sophistiqués à l'aide de capacités computationnelles réduites.

L'abeille *Apis mellifera*, un modèle invertébré classique en cognition visuelle

Richesse comportementale

Les abeilles font preuve d'une richesse comportementale étonnante (Seeley, 1995; Giurfa, 2007; Chittka et Niven, 2009) de la part d'un insecte. C'est une espèce eusociale

(Wilson, 1971) ce qui implique une stricte répartition des tâches, avec une coopération dans l'élevage des jeunes, au sein de colonies qui peuvent contenir plusieurs dizaines de milliers d'individus (Winston, 1991). On y trouve la reine, la mère de toutes les abeilles de la ruche. Le développement complet de ses ovaires et sa fertilité dépendent d'une nourriture particulièrement riche, la gelée royale, produite par les ouvrières à partir de nectar et de pollen. Elle est donc seule à pondre et ce, jusqu'à 2000 œufs par jour, dans des loges hexagonales construites à cet effet par les ouvrières. Les mâles, issus d'œufs non fécondés, ne représentent, au sein de chaque colonie, que quelques centaines d'individus en période de reproduction. Leur seul rôle est de féconder les futures reines. Celles-ci stockent leurs spermatozoïdes plusieurs années dans des organes spécialisés appelés spermathèques pour les utiliser durant toute leur vie. L'immense majorité de la colonie est donc composée d'ouvrières, femelles aux ovaires atrophiés, chimiquement castrées par la phéromone royale. Ces ouvrières se répartissent l'ensemble des tâches à effectuer selon les besoins de la colonie mais aussi un polythéisme d'âge issu d'une maturation physiologique séquentielle. Ainsi, de façon schématique, les plus jeunes ouvrières se consacrent à l'entretien de la ruche (nettoyage, construction) puis au soin du couvain (œufs et larves) et ne quittent donc jamais l'intérieur de la ruche. Elles vivent dans le noir et utilisent l'olfaction et les stimuli mécaniques dans leurs comportements au détriment de la vision. Seules les ouvrières les plus âgées sont amenées à sortir de la ruche, tout d'abord en tant que gardienne, restant à l'entrée pour la défendre, puis, suite à des vols de reconnaissance à proximité de la ruche, elles deviennent des butineuses, chargées de l'approvisionnement en nectar (matière première à l'origine du miel produit par mélange avec des enzymes présentes dans l'estomac des abeilles), pollen et eau. Ainsi chaque ouvrière aura donc effectué de nombreux types de comportements différents durant sa vie (Winston, 1991). Nous nous intéresserons dans ce travail uniquement aux butineuses dont les facultés visuelles d'apprentissage s'avèrent étonnantes.

Les abeilles butineuses doivent constamment effectuer des allers-retours à la ruche et peuvent néanmoins s'en éloigner de plus de 10 km pour rejoindre une source de nourriture ; elles possèdent par conséquent des capacités de navigation exceptionnelles allant jusqu'à la création de l'équivalent de cartes cognitives (Menzel et al., 2005): elles utilisent de façon combinée, la position du soleil, qu'elles peuvent extraire de la polarisation de sa lumière lorsqu'il est voilé (Rossel et Wehner, 1986; Collett et Zeil, 1998), le champ magnétique terrestre (Collett et Baron, 1994) ainsi que des repères visuels (Collett, 1996; Collett et Zeil, 1998) pour se repérer. Lorsqu'elles localisent une nouvelle source de nourriture, elles indiquent sa position aux autres grâce à une forme de langage: la danse des abeilles dont la découverte a valu à l'autrichien Karl von Frisch de partager le prix nobel de physiologie et médecine en 1973. Il est, en effet, le premier à consacrer sa vie à l'étude des abeilles et a ouvert ainsi la voie d'une étude intensive du comportement et des capacités visuelles des abeilles. Les abeilles butineuses font, de plus, preuve d'une constance florale dans leur recherche de nourriture, les amenant à exploiter une même espèce de fleurs jusqu'à son épuisement (Grant, 1950; Chittka et al., 1999), ce qui implique un apprentissage et une mémorisation des caractéristiques florales (von Frisch, 1967). Cette caractéristique est écologiquement adaptée dans le sens où les floraisons dans les biotopes exploitées par les

abeilles européennes sont prévisibles, se succèdent dans le temps, et sont de longue durée (plusieurs jours).

Ces capacités naturelles, associées à une facilité d'étude expérimentale (voir ci-dessous), en font donc le modèle invertébré privilégié pour l'étude des caractéristiques comportementales et neurobiologiques sous-tendant les phénomènes cognitifs (Menzel et Giurfa, 2006; Giurfa, 2007).

Capacités visuelles de l'abeille

L'abeille voit par l'intermédiaire de ses yeux composés, recouvrant une grande partie de la circonférence de la tête, ce qui lui donne accès à un champ visuel de près de 360° malgré des mouvements de tête fortement limités. Elle possède, tout comme nous, une vision trichromatique (Daumer, 1956; Helversen, 1972; Menzel et Backhaus, 1991) reposant sur trois types de photorécepteurs dont les spectres d'absorption vont de l'ultraviolet (300 nm) à la limite du rouge (650 nm). Pour comparaison, la vision humaine s'étend de 400 à 700 nm. Elles perçoivent les formes et les motifs géométriques (Wehner, 1981; Srinivasan, 1994; Lehrer et Campan, 2005) et détectent des mouvements à haute résolution temporelle (Srinivasan et Lehrer, 1984). Leur résolution spatiale est néanmoins 100 fois inférieure à la nôtre (Srinivasan et Lehrer, 1988).

Les yeux sont composés de 5000 à 6000 ommatidies, petites structures hexagonales, constituant l'unité fonctionnelle de l'œil. Chaque ommatidie est composée d'un cône cristallin concentrant les faisceaux lumineux sur la rétine contenant 9 cellules photoréceptrices concentriques (Strausfeld, 1976; Wehner, 1981). En réaction à la lumière, ces cellules libèrent de l'histamine (Bornhauser et Meyer, 1997), neurotransmetteur stimulant les neurones visuels. Les photorécepteurs se divisent en trois types : les récepteurs 'UV' dont le maximum d'excitation correspond aux courtes longueurs d'ondes (nommés S pour Short, pic de sensibilité 344 nm), les récepteurs 'bleus' dont le maximum d'excitation correspond aux longueurs d'ondes moyennes (M pour Medium, 436 nm) et les récepteurs 'verts' dont le maximum d'excitation correspond aux ondes longues (L pour Long, 544 nm) (Figure 1 ; Menzel et Backhaus, 1991; Peitsch et al., 1992).

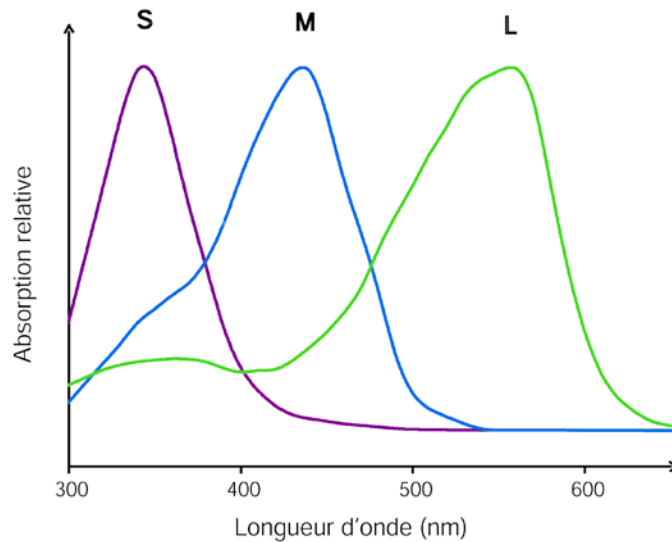


Figure 1: Sensitivité des 3 types de photorécepteurs présents dans la rétine de l'abeille.

Les photorécepteurs L et M projettent alors l'information dans la lamina, premier lobe optique (Menzel, 1974; Strausfeld, 1976) par des fibres visuelles courtes. Elle est ensuite relayée au sein de la medulla et la lobula (Ribi, 1975a; b; Strausfeld, 1976; Ribi et Scheel, 1981) pour ensuite atteindre les corps pédonculés (Hertel et Maronde, 1987). Les photorécepteurs S projettent directement l'information au sein de la medulla grâce à des fibres visuelles longues (Figure 2). Les voies visuelles de l'abeille sont organisées de façon rétinotopique (Figure 2). Chaque ommatidie de l'œil composé contient neuf cellules photoréceptrices (Strausfeld, 1976; Wakakuwa et al., 2005). Les axones de ces photorécepteurs sont connectés dans la lamina, premier lobe optique de l'abeille, à des interneurones, appelés « lamina monopolar cells » (LMC) (Menzel, 1974; Strausfeld, 1976). La lamina est composée de milliers de « cartouches » optiques, chacune regroupant l'information en provenance des neuf photorécepteurs de chaque ommatidie. L'organisation spatiale de l'image sur l'œil est donc conservée à ce stade (Ribi, 1975a; Strausfeld, 1976). Ensuite, l'organisation est inversée car les fibres en provenance de la partie antérieure de la lamina projettent sur la partie postérieure de la medulla, second lobe optique, et inversement (Ribi, 1975b; Strausfeld, 1976). Ainsi, la rétinotopie est bien maintenue malgré son inversion. La medulla est elle-même constituée de cartouches distinctes. Enfin, le troisième lobe optique conserve l'organisation rétinotopique dans sa partie distale et la projette entre autre aux corps pédonculés des deux hémisphères (Strausfeld, 1976; Ribi et Scheel, 1981; Hertel et Maronde, 1987; Ehmer et Gronenberg, 2002). Nous pouvons donc constater que l'organisation du système visuel de l'abeille permet une conservation des informations relatives à la position des différents éléments d'une image.

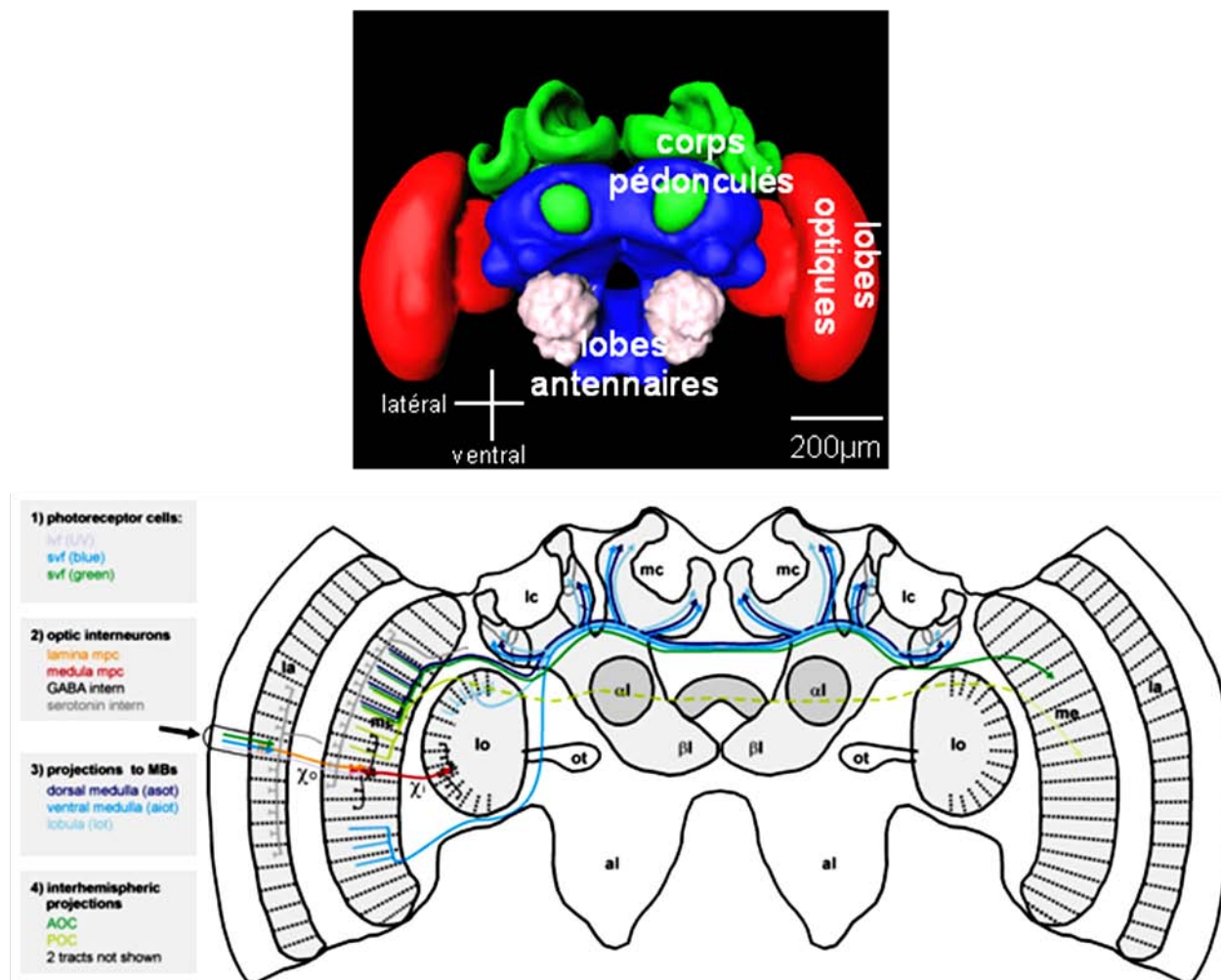


Figure 2: Vue frontale du cerveau de l'abeille. Reconstruction 3D et coupe permettant de visualiser les principaux tractus et l'organisation des structures impliquées dans le traitement visuel.

la= lamina, χ_o = chiasma extérieur, me = medulla, χ_i = chiasma intérieur, lo = lobula, lc = calyx latéral des corps pédonculés, mc = calyx médian, α = lobe alpha, β = lobe beta, al = lobe antennaire, ot = tubercule optique.

Deux modèles différents ont été développés dans le but de représenter l'espace perceptuel de l'abeille et donc de modéliser ses capacités de perception et discrimination des couleurs. Le premier (Color Opponent Coding (COC) ; Backhaus, 1991) repose à la fois sur les études physiologiques ayant mis en évidence certains neurones antagonistes chez l'abeille (Kien et Menzel, 1977) et sur des résultats comportementaux de discrimination des couleurs par l'abeille (Backhaus, 1991) ; le second (Hexagone) (Chittka, 1992) se veut plus général et applicable à différentes espèces d'hyménoptères car il repose sur un traitement antagoniste non spécifique. Ces deux modèles, bien que présentant chacun des avantages et des limites, donnent des résultats similaires (Vorobyev et al., 1997). La vision des formes et des motifs achromatiques, en plus de l'analyse de la fréquence spatiale et du contraste, serait sous-tendue au niveau central par des neurones spécialisés répondant spécifiquement à certaines

caractéristiques comme l'orientation (Srinivasan et al., 1994; Yang et Maddess, 1997), les patron radiaires, concentriques, etc.

Protocoles d'études expérimentales

Depuis le pionnier Karl von Frisch (1914), les chercheurs tirent parti du fait que les abeilles peuvent être individuellement marquées avec un point de couleur sur le thorax et entraînées à associer une variété de stimuli sensoriels avec une récompense sucrée, pour étudier leurs capacités d'apprentissage. En effet, dans le contexte naturel de butinage, les abeilles mémorisent les caractéristiques des fleurs associées au nectar ou au pollen pour les exploiter tant que la source reste profitable. De plus les abeilles effectuent naturellement de nombreux voyages par jour de la ruche à une source de nourriture et peuvent donc être entraînées durant plusieurs heures.

Les butineuses, individuellement marquées peuvent donc être conditionnées en libre vol à se diriger vers un stimulus visuel préalablement associé à une solution sucrée. Après l'apprentissage, l'abeille est alors mise en situation de choix (situation de test sans récompense) ce qui permet de révéler ses capacités de discrimination, de généralisation ou d'apprentissage.

Karl von Frisch utilisait divers stimuli présentés horizontalement sur une surface plane. Durant l'entraînement, une seule image était associée à une solution sucrée. Il pouvait ainsi étudier les capacités visuelles des abeilles en proposant des alternatives au stimulus récompensé afin de tester leur capacité de discrimination. Il a ainsi pu, par exemple, mettre en évidence une vision chromatique chez l'abeille en observant leur choix entre une couleur récompensée et des stimuli achromatiques de même luminosité (Figure 3). Par des méthodes similaires, il a aussi pu caractériser leur perception des formes (Figure 3 ; von Frisch, 1914). Depuis, la méthode a légèrement évolué, bien que conservant les mêmes principes de bases. Depuis Wehner (1967), les stimuli sont présentés verticalement, ce qui permet de garder constante l'orientation du stimulus visuel par rapport à la direction d'approche de l'abeille et donc de mettre à jour des capacités plus précises de reconnaissance de formes. Celles-ci étaient auparavant considérées comme très limitées, dépendant uniquement de caractéristiques simples, traitées au niveau périphérique, comme la périodicité spatiale, disruption du motif ou le pourcentage de surface noire (Hertz, 1933; 1935). En effet, lorsque les stimuli sont présentés horizontalement, l'abeille les perçoit différemment selon son angle d'approche, ce qui limite ses possibilités d'apprentissage. Enfin, l'utilisation de labyrinthes en Y (Figure 4) a permis un contrôle de l'angle visuel sous-tendu par les stimuli au moment du choix (mesure prenant en compte la taille du stimulus et sa distance) en restreignant celui-ci à la chambre de décision du labyrinthe. Des études sur l'acuité visuelle de l'abeille ont donc pu être entreprises (Srinivasan et Lehrer, 1988; Giurfa et al., 1996b), connaissances nécessaires pour estimer ce que l'abeille perçoit d'un stimulus visuel. La forme en Y est conçue pour des conditionnements différentiels (c'est-à-dire des procédures d'entraînements dans lesquels un stimulus, associé à la récompense, est présenté dans un des bras du labyrinthe, tandis qu'un

stimulus différent, non récompensé, est présenté dans l'autre bras). Cette procédure de conditionnement permet de mettre en évidence une perception plus fine en augmentant le niveau d'attention de l'abeille (Giurfa et al., 1999). Ce paradigme expérimental s'oppose au conditionnement absolu dans lequel seul un stimulus récompensé est présenté dans un des bras ; l'autre bras ne présentant aucun stimulus. L'apprentissage des caractéristiques du stimulus est alors moins précis et conduit à une apparente faible capacité de discrimination lors de tests de choix réalisés à l'issue de ce conditionnement absolu (Giurfa et al., 1999).

Dans notre travail, nous avons encore cherché à améliorer le protocole de conditionnement différentiel chez l'abeille en libre vol afin de dévoiler les performances fines de discrimination visuelle des abeilles. Concrètement, nous avons voulu améliorer la méthode traditionnelle en introduisant l'utilisation d'une solution de quinine (substance amère) comme renforçateur négatif des choix incorrects. Nous avons donc testé l'influence de l'utilisation de quinine sur les performances d'apprentissage et de discrimination visuelle de l'abeille (Article I).

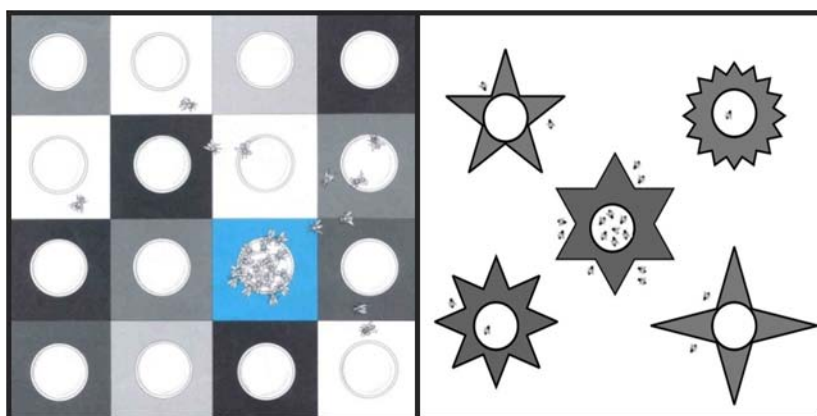


Figure 3: Exemples de tests effectués par Karl von Frisch pour tester la perception des couleurs (à gauche) et des formes (à droite). Après avoir été préalablement entraînées à associer une solution sucrée à la couleur bleue (à gauche) ou à l'étoile à 6 branches (à droite), il est possible de vérifier leur capacité à les reconnaître en analysant leur choix face à différents stimuli alternatifs. Source : von Frisch, 1984.

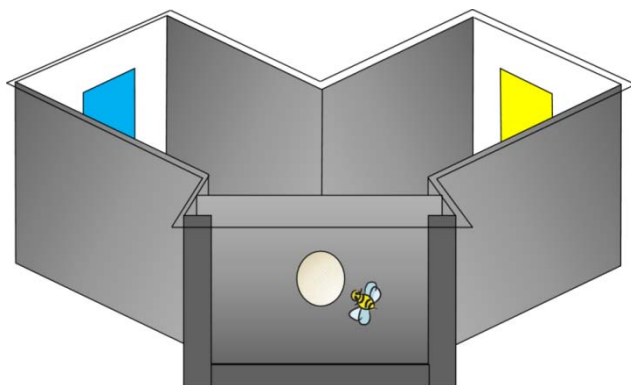


Figure 4: Exemple d'un labyrinthe en Y utilisé pour conditionner des abeilles en libre vol. Une porte coulissante limite l'accès à l'abeille testée seule, reconnaissable par un marquage coloré. Une fois entrée, l'abeille passe par un orifice circulaire au niveau duquel les 2 stimuli sont visibles. Elle fait alors son choix au niveau de la chambre de décision (intersection entre les deux branches du Y) avant de se diriger dans la branche contenant le stimulus choisi. Le labyrinthe est recouvert d'une feuille de plexiglas transparent aux rayons ultraviolets afin de ne pas perturber la vision de l'abeille au sein du dispositif.

Parallèlement, l'abeille a été amenée au laboratoire, où ses facultés d'apprentissage et de mémorisation ainsi que leurs mécanismes sous-jacents peuvent être étudiés de façon plus précise. Ces études sont possibles grâce au conditionnement d'extension du proboscis (Figure 5A ; Kuwabara, 1957; Menzel et Giurfa, 2001; Giurfa, 2007). L'abeille est fixée dans un tube en métal et conditionnée à étendre le proboscis lors de la présentation d'un stimulus conditionnel (conditionnement pavlovien). L'abeille possède en effet le réflexe d'étendre son proboscis lorsque l'on touche ses antennes avec une solution sucrée. Lors de la phase d'apprentissage, l'abeille apprend à associer la présentation d'une odeur par exemple avec une récompense sucrée présentée au niveau des antennes puis du proboscis une fois étendu. L'abeille est alors conditionnée à étendre son proboscis en présence de l'odeur renforcée seule, celle-ci étant devenue annonciatrice de la récompense. Un conditionnement différentiel est aussi possible en intercalant, lors de l'apprentissage, des présentations d'une autre odeur, celle-ci étant non renforcée. Une méthode de conditionnement aversif a aussi été développée (Figure 5B) dans lequel la solution sucrée est remplacée par un faible choc électrique déclenchant un réflexe d'extension du dard (Vergoz et al., 2007; Carcaud et al., 2009). Grâce à l'immobilisation de l'abeille lors de l'apprentissage, il est alors possible d'étudier les bases physiologiques et neurales de l'apprentissage et de la mémorisation. En effet, il est possible de mesurer l'activité des neurones (électrophysiologie : Denker et al., 2010), d'observer l'effet de l'injection de divers antagonistes (neuropharmacologie : Farooqui et al., 2003; Vergoz et al., 2007), ou inhibiteur de certaines synthèses protéiques (RNAi : Farooqui et al., 2004). On peut en particulier observer en direct l'activité du cerveau de l'abeille selon un protocole d'imagerie cérébrale (Joerges et al., 1997; Sachse et al., 1999; Roussel et al., 2010) grâce à une ouverture dans la cuticule recouvrant le cerveau et à un colorant sensible à la libération de calcium par les neurones activés.

Malheureusement, à ce jour, la perspective d'une application de ces méthodes invasives à la cognition visuelle reste éloignée. En effet, même s'il s'avère possible de conditionner une extension du proboscis avec des stimuli visuels (Figure 5C), cela implique jusqu'alors de conditionner des abeilles sans antennes (Figure 5C ; Hori et al., 2006; Hori et al., 2007; Niggebrügge et al., 2009). De plus, les abeilles montrent dans ces conditions des capacités de discrimination très limitées comparées aux abeilles en libre-vol (Helversen, 1972; Niggebrügge et al., 2009). Ainsi, les comportements de l'abeille pouvant être altérés par le stress de la contention et des manipulations, les protocoles de laboratoire actuels ne permettent pas d'observer la richesse cognitive de l'abeille, contrairement aux conditions de libre vol. Cette méthode reste donc toujours d'actualité pour étudier les procédés de cognition visuelle mis en jeu par ce mini-cerveau.

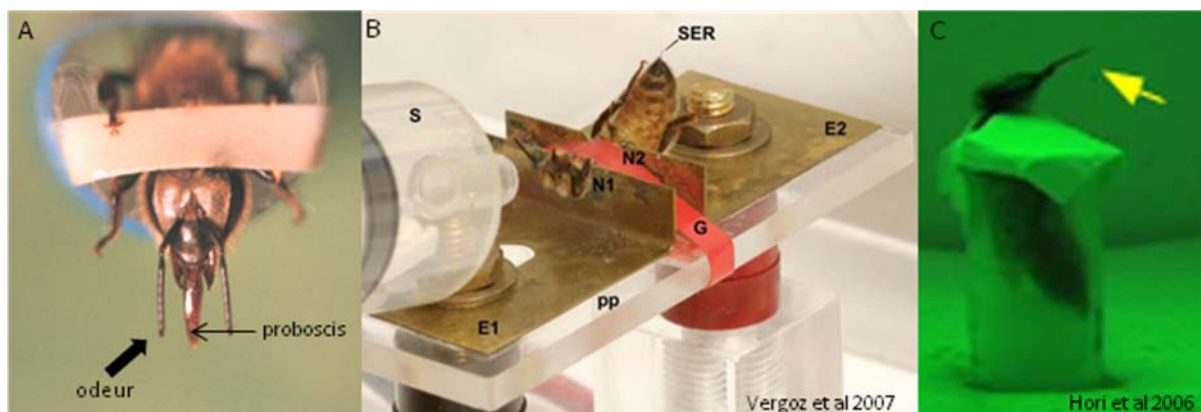


Figure 5: Dispositifs de conditionnement utilisés en laboratoire avec fixation de l'abeille. (A) Dispositif de conditionnement appétitif d'extension du proboscis (Proboscis Extension Reflexe PER) en présence d'une odeur préalablement associée à un renforcement de solution sucrée. (B) Dispositif de conditionnement aversif d'extension du dard (Sting Extension Reflexe SER) en présence d'une odeur préalablement associée à un choc électrique. (C) Dispositif de conditionnement appétitif d'extension du proboscis (PER) en présence d'une couleur préalablement associée à la présence d'une solution sucrée.

Performances cognitives dans le domaine de la classification visuelle

Catégorisation perceptive

La capacité de regrouper des objets ou des événements discriminables sur la base de fonctionnalités ou de caractéristiques communes est un processus cognitif connu sous le nom de « catégorisation » (Zayan et Vauclair, 1998). Cette faculté implique l'analyse de son environnement par détection et extraction de propriétés communes parmi un ensemble de stimuli uniques. Elle permet ensuite de transférer à de nouveaux stimuli les connaissances accumulées sur leur catégorie d'appartenance, comportement adaptatif, évitant ainsi un coûteux apprentissage par essai-erreur de chaque situation nouvelle tout en limitant la quantité d'informations à mémoriser.

La catégorisation repose donc sur le traitement équivalent de tous les items de la catégorie. Elle implique aussi un transfert positif de réponse face à chaque nouvel élément rencontré répondant aux caractéristiques de la catégorie (Herrnstein, 1990; Zayan et Vauclair, 1998). Elle se distingue d'une autre stratégie de réponse face à des stimuli nouveaux, la généralisation (Spence, 1937; Shepard, 1987). Dans le cas de la généralisation, le sujet apprend un comportement adapté face à un objet-cible et diminue progressivement sa réponse face à des objets dont la similarité par rapport à l'objet appris diminue aussi progressivement

tout au long d'une échelle perceptives (Ghirlanda et Enquist, 2003). Dans le cas d'une catégorisation par contre, la réponse reste identique pour tous stimuli remplissant les critères d'appartenance à la catégorie même s'ils diffèrent fortement du stimulus appris.

La première étude montrant une capacité de catégorisation chez l'animal date de 1964. Herrnstein et Loveland (1964) démontrent alors que des pigeons sont capables de classer des photographies en deux catégories selon la présence ou non d'êtres humains sur la photographie. Ils utilisèrent un protocole classique de type « go/no-go », dont le principe sera par la suite majoritairement utilisé dans les études de catégorisation chez le pigeon ou les primates. Lors d'une phase d'entraînement, un ensemble de 40 photographies fut utilisé. Les pigeons recevaient une récompense alimentaire lorsqu'ils picorait l'écran en présence d'une photographie contenant un être humain (cas de la moitié des photographies). Ils apprirent conjointement à ne pas répondre en présence des 20 photographies ne contenant pas d'être humain. Une fois que les pigeons obtinrent des résultats satisfaisants, leurs choix fut observé en présence d'un nouveau ensemble de stimuli afin d'écarter l'hypothèse d'une association élémentaire entre chaque photographie et la récompense. Les individus testés furent capables d'un tel transfert positif de la catégorie apprise. Suite à ces travaux pionniers, de nombreuses études ont confirmé cette capacité chez le pigeon mais aussi chez le primate en utilisant de nombreux critères de catégorisation différents (arbre; feuille de chêne; poisson; chaises; voitures; lettres de l'alphabet; visages; patterns schématiques; peintures de Monet et Picasso; etc.; voir Huber, 2001 pour une revue).

Toutes ces études tendent à montrer la présence, chez des animaux, de catégories naturelles. Cependant, la prudence s'impose concernant l'existence de telles catégories dans le cadre d'expériences où des photographies de situations ou objets réels sont utilisées (Fagot et al., 2004). En effet, l'utilisation de photographies ne permet pas toujours un contrôle suffisant des indices utilisés. Ainsi, même si les expérimentateurs tachent de varier de nombreux critères perceptifs tels que, par exemple dans le cas de l'étude d'Herrnstein et Loveland de 1964, la position, le nombre, l'apparence (homme ou femme, enfant ou adulte, nus ou habillés, etc...), il est néanmoins impossible de contrôler tous les paramètres dans de tels stimuli complexes. Ainsi, des pigeons entraînés à classer des visages humains entre deux catégories: hommes et femmes, se sont avérés utiliser en réalité des indices de bas niveau comme la légère différence d'intensité des images entre les photographies d'hommes et de femmes (Troje et al., 1999). De même, l'analyse des erreurs effectuées par des singes lors d'une tâche de catégorisation basée sur la présence d'êtres humains d'« Homme » a permis de conclure que le choix des individus reposait sur la présence de tâches roses à l'image (D'Amato et van Sant, 1988). Il est donc souvent préférable, dans les études de cognition visuelle chez l'animal, d'avoir recours à des stimuli artificiels dont les caractéristiques physiques sont contrôlables avec plus de précision. Cela est d'autant plus vrai que l'animal est capable d'utiliser une combinaison d'indices perceptifs pour définir une catégorie. Une illustration de cette affirmation peut se trouver dans les travaux de von Fersen et Léa (1990) dans laquelle des pigeons ont été entraînés à former deux catégories à partir de photographies de scènes naturelles. Les stimuli ont été choisis de façon à présenter toutes les combinaisons possibles de critères binaires parmi les cinq suivants: le bâtiment pris en photographie, le

niveau d'ensoleillement, la distance, la hauteur de prise de vue ainsi que l'inclinaison de l'image. Une valeur de chacune de ces dimensions a été choisie arbitrairement comme positive avant le début de l'expérience. Durant la phase d'apprentissage, les stimuli qui comportaient au minimum trois critères de valeur positive sur les cinq étaient récompensés de façon à ce qu'aucun des critères seuls ne permette de prédire avec certitude la présence de récompense, c'est-à-dire la catégorie d'appartenance (+ ou -) des stimuli. Les pigeons se sont avérés utiliser les cinq caractéristiques dans les tests de transfert et ce avec un effet cumulatif (réponse d'autant plus importante que les stimuli présentaient un plus grand nombre de critères positifs).

Une autre précaution à prendre dans l'interprétation des résultats est la vérification de la capacité des animaux testés à discriminer les différents stimuli au sein de chaque catégorie (Thompson, 1995; Zayan et Vauclair, 1998). Cette discrimination intra-catégorie, malheureusement trop souvent manquante dans les études (voir cependant Vauclair et Fagot, 1996 par exemple) est tout aussi nécessaire que la discrimination inter-catégorie pour pouvoir parler de catégorisation.

Néanmoins, il n'y plus aucun doute depuis les années 1990 quant à la capacité des modèles vertébrés classiques en cognition visuelle tels que les pigeons et primates de former des catégories visuelles (basées sur des critères perceptifs) suite à un apprentissage.

Les facultés d'apprentissage des insectes et des invertébrés en général sont, à l'opposé, majoritairement considérées comme se limitant à des associations élémentaires de type stimulus-stimulus (apprentissage pavlovien) ou réponse-stimulus (apprentissage opérant). Cependant, les abeilles se sont, elles aussi, avérées capables d'association non-élémentaires de type catégorisation. Elles peuvent en effet extraire au fil de l'entraînement les caractéristiques communes des stimuli récompensés définissant la catégorie à apprendre et transférer l'association apprise envers de nouveaux stimuli (Benard et al., 2006)

Van Hateren et coll. (1990) démontrèrent les premiers une telle capacité, bien qu'ils n'ont pas interprété leurs résultats de cette façon à l'époque. Ils ont entraîné des abeilles en libre-vol à choisir des stimuli visuels en fonction de l'orientation de motifs rayés. Lors de la phase d'entraînement, les abeilles devaient discriminer deux orientations (par exemple 45° versus 135°) dans un protocole de conditionnement différentiel (association d'une des orientations à une récompense sucrée). D'un essai à l'autre, le type de rayures utilisé variait mais les orientations récompensées et non récompensées restaient constantes afin de faciliter l'extraction de l'orientation comme critère de récompense plutôt qu'une image donnée. Une fois l'orientation apprise, les abeilles ont pu transférer leur choix à des stimuli rayés d'apparence très différente, faisant ainsi preuve d'une faculté de catégorisation de stimuli selon leur orientation (Figure 6A).

Des travaux postérieurs ont montré que les abeilles peuvent traiter des images comme équivalentes selon la présence ou non d'un axe de symétrie bilatérale (Figure 6B ; Giurfa et al., 1996a), d'une organisation radiale ou concentrique (Figure 6C ; Horridge et Zhang, 1995)

ou encore d'une périodicité spatiale particulière (Figure 6D ; Horridge, 1997). Les abeilles sont donc capables de classer des stimuli visuels sur la base d'une propriété physique commune et de répondre de façon appropriée à de nouveaux stimuli présentant cette caractéristique. Mais peuvent-elles définir des catégories basées sur de multiples caractéristiques ? En d'autres termes, les abeilles extraient-elles de leur environnement visuel les éléments qui se répètent pour pouvoir établir des correspondances entre les éléments corrélés définissant ainsi des catégories ? Un premier élément de réponse peut se trouver dans les travaux de Zhang et coll. (2004) dans lesquels les abeilles se sont avérées capables de transférer leur choix au sein de catégories « naturelles » : fleurs à symétrie radiale, fleurs circulaires, plantes verticales et paysages (Figure 7A). Dans ce travail, des photographies réelles et des dessins réalistes de ces objets ont été utilisées avec les difficultés que cette utilisation implique (voir ci-dessus). Les auteurs se sont néanmoins attachés à contrôler la possibilité d'utiliser des indices de bas niveau pour classer les images comme la couleur ou encore la fréquence spatiale. Ces résultats semblent suggérer l'utilisation par les abeilles d'une combinaison de traits visuels définissant les catégories apprises. Par exemple, la catégorisation de fleurs de type radial pourrait reposer sur une configuration simplifiée consistant en cinq ou plus rayons divergeant d'un centre commun. Cependant, les images ayant été utilisées sont complexes et il est donc difficile de s'assurer que des tels critères ont été réellement utilisés par les abeilles.

Stach et coll. (2004), ont alors vérifié la capacité des abeilles à combiner différentes informations communes à un ensemble de stimuli pour répondre de façon appropriée face à de nouveaux stimuli. Pour cela, les chercheurs ont entraîné des abeilles avec des stimuli divisés en quatre quadrants, chacun présentant des rayures d'une certaine orientation (Figure 7B). D'un essai à l'autre, la forme des rayures variaient mais l'orientation représentée au sein de chaque quadrant était conservée. Les abeilles ont ensuite choisi d'autant plus fortement de nouveaux stimuli que le nombre de quadrants présentant une orientation correcte augmentait. Ainsi, l'utilisation de ces stimuli très simples et de caractéristiques connues comme facilement extraites par les abeilles (des orientations) a permis de mettre en évidence que ces insectes peuvent extraire et combiner différentes informations pour définir une catégorie visuelle.

Nous avons voulu, quant à nous, tester la capacité des abeilles à catégoriser des stimuli visuels à partir de configurations ou topologies spécifiques. Nous nous sommes demandé si, au-delà de leur capacité plusieurs fois démontrées de catégoriser des stimuli sur la base de traits uniques, les abeilles peuvent extraire et combiner les relations entre les différents traits dans une configuration pour définir une catégorie visuelle. Afin de tester cette hypothèse nous avons choisi des configurations simplifiées de visages humains (deux ronds à la place des yeux, situés au-dessus d'une barre verticale pour le nez, elle-même située au-dessus d'une barre horizontale pour la bouche (de type smiley)) (Article II).

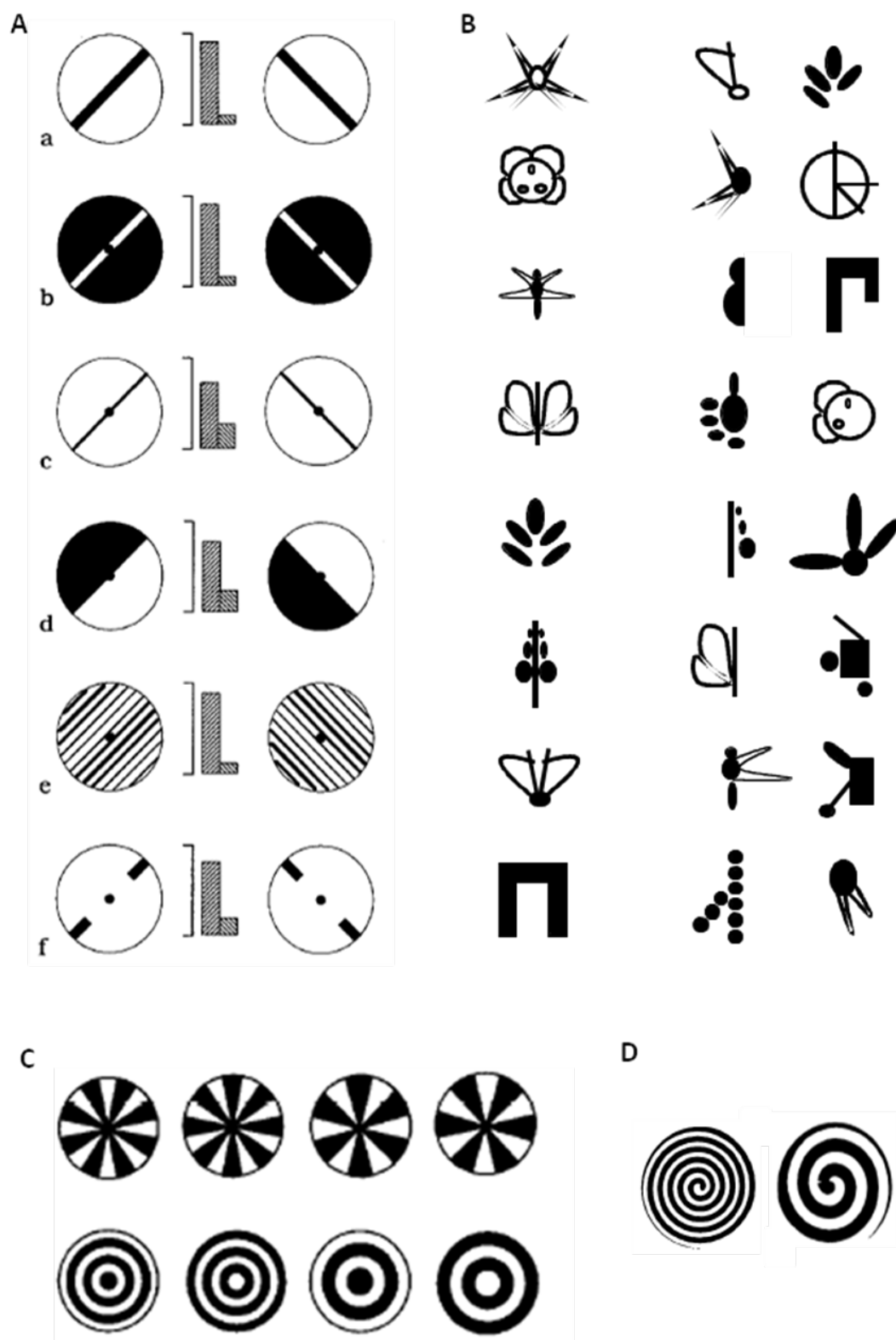


Figure 6: Exemples de stimuli utilisés dans les études sur la catégorisation basée sur une propriété physique commune chez l'abeille. (A) Orientation (van Hateren et al., 1990). (B) Symétrie bilatérale (Giurfa et al., 1996b). (C) Patterns concentriques ou radiaux (Horridge et Zhang, 1995). (D) Périodicité spatiale (Horridge, 1997).

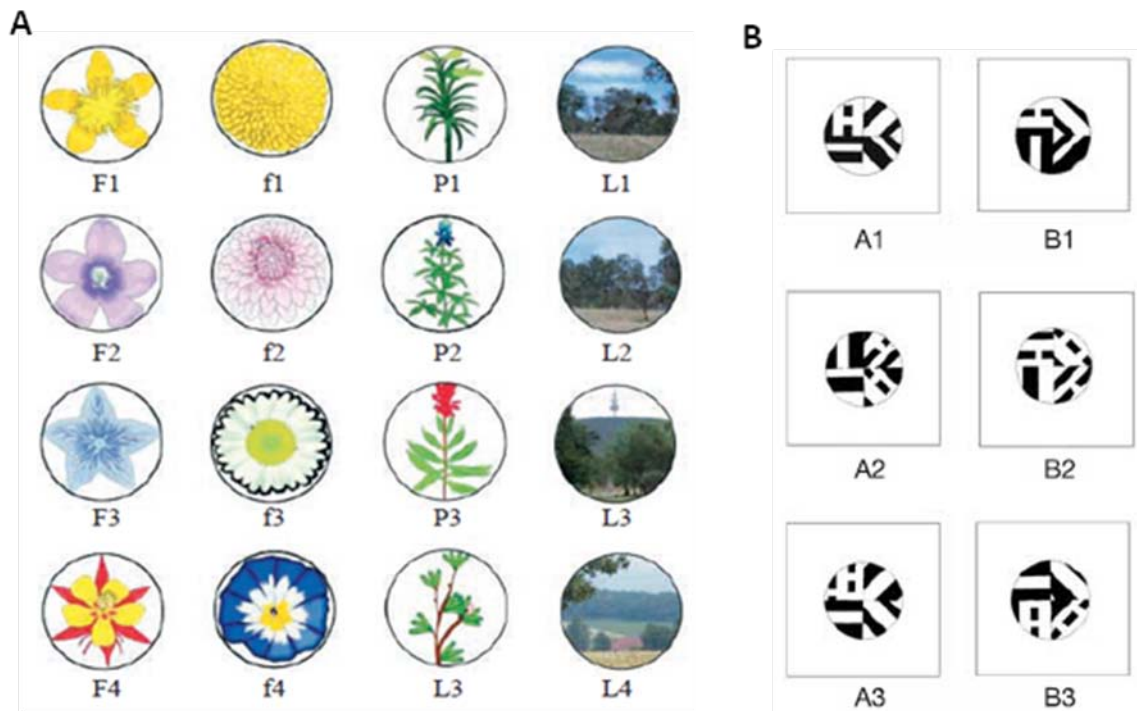


Figure 7: Exemples de stimuli utilisés dans les études de Zhang et al., 2004 (A) et Stach et al., 2004 (B) sur la catégorisation basée sur la combinaison de plusieurs propriétés physiques chez l'abeille.

Perception des configurations spatiales

La configuration spatiale d'un objet ou d'une scène correspond à la combinaison des éléments de cet objet ou de cette scène et de leurs inter-relations. Ainsi une configuration de type « visage » se définit par la disposition relative des yeux l'un à côté de l'autre, situés au-dessus du nez, lui-même au-dessus de la bouche. La perception et la mémorisation des configurations est particulièrement efficace pour reconnaître un nouvel objet (cf. Discussion). Ainsi, nous pouvons reconnaître une maison dans n'importe quelle construction comportant des murs verticaux surmontés d'un toit, et ce, quel que soit le style architectural, la matière ou les couleurs utilisés. Par exemple, aussi différentes soient-elles, nous pouvons en un regard classer une pagode chinoise et une hutte de paille dans la catégorie habitation même si nous n'en avons jamais rencontré auparavant. De même, il suffit qu'une image présente une configuration de type visage pour être reconnue aisément en tant que visage même si les éléments de détail de l'image ne sont en rien des éléments de visages humains (Figure 8 ; Maurer et al., 2002).



Figure 8: Sensibilité à la configuration de type visage. On peut reconnaître aisément un visage à partir d'une peinture d'Archimbaldo représentant des légumes (d'autant plus si on la retourne) (A) ou à partir de simples patch lumineux s'ils représentent la bonne configuration (B).

Une autre façon d'aborder le problème de la perception de la configuration est la question de la hiérarchie d'analyse par notre système visuel. Ainsi, perçoit-on d'abord les objets dans leur globalité avant de se focaliser sur les détails les composant ou perçoit-on d'abord les détails avant d'appréhender leur configuration lorsque l'on analyse une image ou un objet de notre environnement ? Cette question a nourri une abondante littérature depuis les années 70. Depuis les travaux de Navon (1977; 1981; 1983; 1991), l'utilisation de stimuli structurés hiérarchiquement (l'agencement de symboles compose lui-même un symbole ; exemple Figure 9) s'est imposée pour tous les travaux relatifs à cette question. En effet, de tels stimuli sont particulièrement adaptés car les informations locale (détail) et globale (agencement) ont un sens équivalent pour les sujets de l'étude. Ce paramètre est essentiel lorsque l'on compare la vitesse et la réussite des sujets dans des tâches de reconnaissance de l'une ou l'autre des informations. Dans de telles tâches, les résultats montrent une prédominance de la vision dite globale sur la vision locale. Cette conclusion repose notamment sur un avantage temporel de l'information globale qui est perçue en premier et l'existence d'une interférence des informations globales sur les informations locales. Par exemple, le taux d'erreur est beaucoup plus important lorsqu'il est demandé de nommer la lettre présentée au niveau local si la lettre globale est différente. Une telle interférence ne se retrouve pas si on doit nommer la lettre composée (information globale) même si les lettres la composant sont différentes. Ainsi, selon Navon, ces deux types d'informations seraient analysés par deux voies parallèles avec une plus grande rapidité de la voie concernant l'information globale. Il est néanmoins essentiel de préciser que, même si cette prédominance d'un système sur l'autre semble très robuste, elle peut être altérée par des variations importantes de l'angle visuel, la densité des stimuli (nombre d'éléments composant la forme globale) ou encore par une importante durée d'exposition faisant perdre l'avantage de l'information globale (Kimchi, 1992).

Dans le contexte où la configuration semble primordiale pour la reconnaissance des objets de notre environnement, une telle caractéristique de notre système visuel apparaît comme particulièrement adaptée pour notre espèce dont la perception du monde qui l'entoure repose essentiellement sur la vision. Une rapidité de reconnaissance des objets visuels est ainsi indispensable. Il semble donc naturel que cette propriété de notre perception soit partagée par les autres primates, d'autant plus qu'ils possèdent un système physiologique visuel très similaire (De Lillo et al., 2005). Ce n'est pourtant pas ce qu'on observe. Les

macaques, babouins ou chimpanzés montrent plutôt une prédominance de la vision locale ou tout au mieux une absence de prédominance d'une information sur l'autre (Fagot et Deruelle, 1997; Fagot et Tomonaga, 1999; Fagot et Tomonaga, 2001; Hopkins et Washburn, 2002; Spinozzi et al., 2003; De Lillo et al., 2005). Nous pouvons donc faire l'hypothèse d'une explication cognitive plutôt que purement perceptives pour ce qui apparaît être une particularité humaine au sein des primates acquise au cours de son évolution. Cependant, la question de l'explication de cette exception humaine reste ouverte car une hypothèse alternative serait l'influence de l'expérience chez l'Homme par l'apprentissage de l'écriture notamment (Fagot et Tomonaga, 1999) même si cette propriété de notre perception visuelle semble être présente avant la scolarisation chez l'enfant (De Lillo et al., 2005).

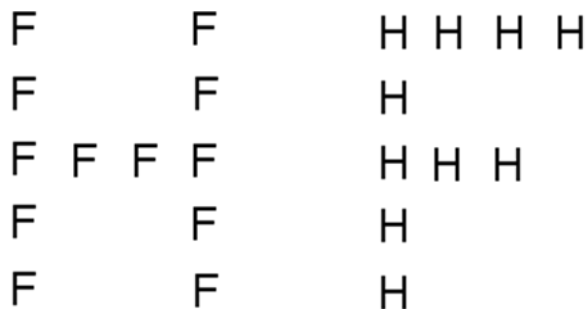


Figure 9: Exemple de stimuli composés utilisés pour tester la prédominance d'un type de vision (globale ou locale) sur l'autre.

Pour mieux comprendre ce phénomène, il serait intéressant d'étudier la perception d'animaux appartenant à des branches évolutives différentes mais dont la perception de l'environnement repose aussi en grande partie sur la vision. Chez les Vertébrés, le pigeon semble présenter une prédominance de l'analyse des informations locales (Cavoto et Cook, 2001). Chez les Invertébrés, la question n'est pas totalement résolue pour l'abeille. Zhang et coll. (1992) ont mis en évidence l'existence des deux types de perception chez l'abeille en utilisant des stimuli représentant des rectangles dans une certaine orientation (information globale) composés de nombreux petits rectangles dans une autre orientation (information locale) (Figure 10). Leurs résultats semblent pencher en faveur d'une préférence pour l'information globale bien que l'analyse, statistique notamment, des résultats est à revoir. Il est néanmoins nécessaire, avant de conclure, de confirmer et préciser ces résultats en utilisant des stimuli plus complexes. En effet, la détection de l'orientation étant le fait de neurones spécialisés chez l'abeille (Yang et Maddess, 1997), ces résultats peuvent ne pas être généralisables à d'autres stimuli car dépendants de la sensibilité en terme d'angle visuel de ces neurones.

Nous avons donc voulu étudier l'interaction entre système de perception visuelle locale et globale chez l'abeille en utilisant des stimuli plus complexes que ceux de la Figure 10. Nous avons donc utilisés des formes géométriques hiérarchisées dans le but de tester l'existence d'une prédominance d'un niveau de traitement sur l'autre en conditions où les information globale et locale sont toutes deux accessibles pour le système visuel de l'abeille (Article III).

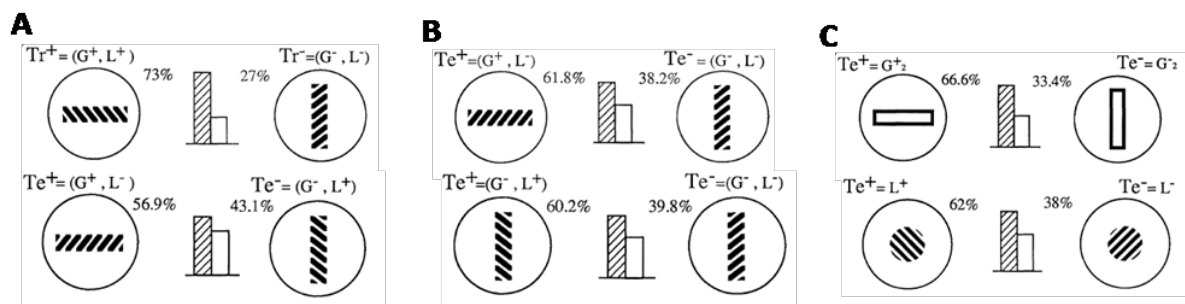


Figure 10: Stimuli et résultats obtenus chez l'abeille concernant l'existence d'une prédominance d'un type de vision (local ou global) sur l'autre à l'aide de stimuli hiérarchiques (information locale: orientation des rayures; information globale: orientation du rectangle) (Zhang et al., 1992). (A) Test d'apprentissage montrant les stimuli utilisés durant l'apprentissage et test de conflit montrant la priorité de l'information globale sur l'information locale lorsque les informations sont conflictuelles (Global + Local – vs. Global – Local +). (B) Tests de contrôle permettant de vérifier que les deux niveaux d'information ont été mémorisés. (C) Tests de contrôle confirmant l'apprentissage des deux niveaux d'information dans une situation où le stimulus récompensé lors de l'entraînement n'est pas présent.

Formation de concepts

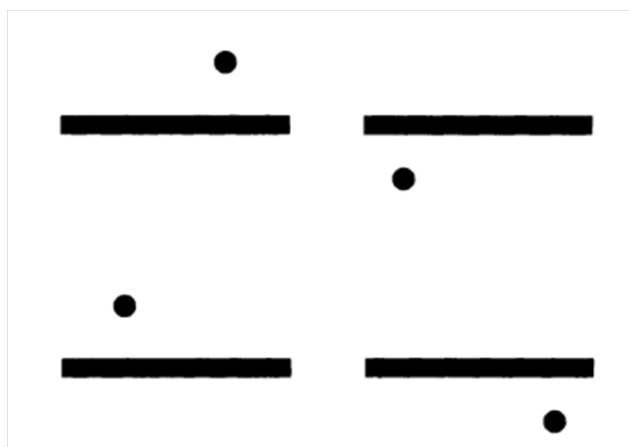
Au-delà des catégories perceptives basées sur une ou plusieurs caractéristiques physiques communes, les concepts ne sont pas définis par des similarités perceptives et impliquent donc l'utilisation de règles abstraites (Herrnstein, 1990; Zayan et Vauclair, 1998; Fagot et al., 2004; Zentall et al., 2008). La formation de concepts est d'ailleurs souvent considérée comme spécifique de l'Homme et des primates dans le sens où les concepts abstraits peuvent paraître intuitivement dépendre de l'existence d'un langage permettant de les nommer. En absence de langage, les pigeons ont aussi, dans certaines conditions expérimentales, fait preuve de maîtrise de concepts (Zentall et Hogan, 1976; Herrnstein et al., 1989; Kirkpatrick-Steger et Wasserman, 1996; Wright et Katz, 2006). En dehors de ces animaux, rares sont les cas où une telle capacité a été prouvée.

Au sein des catégories conceptuelles, on peut distinguer les concepts reposant sur la fonction ou la nature de l'objet (exemple : outil – animal – véhicule...) dont il a été en partie question plus haut et ceux définis par les relations entre des objets (exemple : identité – quantité – relations spatiales...). Les premiers reposent néanmoins souvent sur des similarités perceptives. En effet, des objets partageant une même fonction, partagent souvent des traits distinctifs qui peuvent être utilisés pour la classification (par exemple, la présence de roues sur un véhicule). Dans le deuxième cas, au contraire, les relations définissent à elles seules l'appartenance au concept sans incidence des aspects perceptifs des objets. C'est cette dernière classe de problèmes que nous avons voulu étudier chez l'abeille.

Nous nous intéresserons donc plus particulièrement ici aux concepts relationnels. La méthode classique d'étude de la présence de ces concepts chez des animaux est de tester leur habilité dans l'apprentissage d'une règle permettant d'accéder à une récompense (par exemple : « choisir le stimulus identique à celui que l'on vient de te présenter » et à l'appliquer à de nouveaux stimuli, différents des stimuli utilisés dans l'entraînement. Cette capacité de transfert montre l'indépendance de la règle par rapport à la nature physique des stimuli. L'un des premiers concepts recherché chez l'animal est celui d'identité ou équivalence. Le protocole le plus souvent employé consiste à présenter à chaque essai à l'animal un stimulus-échantillon puis après un court délai à lui demander de faire un choix entre deux stimuli-cibles, dont un est identique au stimulus-échantillon qui vient d'être présenté. L'animal reçoit alors une récompense lorsqu'il choisit le stimulus identique (protocole dit de « delayed-matching-to-sample » (DMTS = appariement retardé à la cible). Une variante consiste à entraîner l'animal à maîtriser la règle opposée, c'est-à-dire à choisir le stimulus-cible différent de l'échantillon proposé (delayed-non-matching-to-sample (DNMTS)). Primates (D'Amato et al., 1985), dauphins (Herman et Gordon, 1974) et pigeons (Zentall et Hogan, 1976; Wright et al., 1988) se sont montrés capable d'une telle tâche. Par ailleurs, l'étude de Wright et coll. (1988) chez le pigeon démontre l'importance, lors de l'entraînement, d'utiliser plusieurs stimuli différents afin de faciliter l'extraction de la règle (ou des critères définissant la catégorie récompensée) au détriment d'une association élémentaire stimulus-récompense. En effet, suivant un protocole de type DMTS, les pigeons ont été entraînés soit avec 152 stimuli-cibles différents soit avec seulement 2. Dans le deuxième cas, les pigeons se sont avérés incapables de transférer leur réponse à de nouveaux stimuli (voir aussi Oden et al., 1990; Thompson et Oden, 1996).

Dans le but de comprendre le lien entre le langage et la formation de concepts, Quinn (2007) s'est intéressé au cours de nombreuses études, à la capacité des bébés à manipuler des concepts basés sur des relations spatiales et à la maturation de cette faculté au cours du développement (Quinn, 2007). Il observe ainsi que des bébés de 3-4 mois peuvent apprendre à discriminer des images composées d'un point au-dessous ou en dessous d'une barre horizontale (Figure 11) suivant la position du point (Quinn, 1994). Ils peuvent ensuite transférer la règle apprise ('en dessous de' ou 'au dessus de') à de nouveaux stimuli dans lesquels le point est plus ou moins écarté de la barre tout en étant déplacé latéralement. Les stimuli restent cependant très similaires d'un point de vue perceptif. A cet âge, les bébés échouent à transférer leur choix lorsque l'on change le symbole utilisé (le point devenant un carré, un losange ou un triangle...) (Quinn, 1996). Par contre, des bébés de 6-7 mois se sont montrés capables de réussir un tel transfert et peuvent donc construire des catégories conceptuelles basées sur une relation spatiale ('au-dessus'/'en dessous'). Néanmoins, il faut noter que les bébés n'ont pas été testés dans la condition où la barre horizontale servant de référent serait aussi modifiée. Ce résultat est aussi applicable à la relation 'entre' (Quinn et al., 1999; Quinn et al., 2003) et généralisable à tout type de concepts relationnels (Doumas et al., 2008). La possibilité d'une existence de ces concepts avant la mise en place d'un langage (même si l'expérience du langage des parents peut influencer l'évolution des résultats chez les bébés de 3 à 6 mois), est confirmée par les études chez l'animal. Ces études, utilisant le même

type de stimuli (symbole au-dessous ou en dessous d'une barre horizontale) démontrent une capacité similaire chez le babouin (Dépy et al., 1999) ou encore le capucin (Spinozzi et al., 2004). Se poser la question de la présence de concepts spatiaux chez l'animal est d'autant plus justifié de part l'utilité potentielle d'une telle capacité pour l'orientation et la navigation (Gallistel, 1990; Bennett, 1996; Thinus-Blanc, 1996) ou encore la reconnaissance des objets grâce à la configuration des éléments les composant (Biederman, 1987; Cave et Kosslyn, 1993; Maurer et al., 2002).



Quinn 1994

Figure 11: Exemples de stimuli utilisés dans les études sur les concepts « au-dessus » et « en dessous » chez l'enfant et les primates.

La capacité des abeilles à maîtriser des concepts a été aussi étudiée en libre vol dans des protocoles de type DMTS et DNMTS (Giurfa et al., 2001; Zhang et al., 2005). Cet insecte s'est avéré capable d'un tel niveau d'abstraction et a ainsi maîtrisé une règle d'équivalence (DMTS) ou de différence (DNMTS). Les abeilles ont été entraînées à choisir la branche d'un labyrinthe en Y contenant un stimulus identique à celui présenté à l'entrée du labyrinthe (couleur jaune ou bleue), l'autre branche contenant la couleur alternative (Figure 12). Elles peuvent alors appliquer la règle non seulement à d'autres types de stimuli visuels (par exemples des rayures achromatiques de différentes orientations ; Figure 12) mais aussi à des stimuli d'une autre modalité sensorielle : des odeurs (Giurfa et al., 2001). Des protocoles similaires ont pu démontrer aussi la capacité des abeilles à apprendre une règle basée la quantité d'éléments présents dans une image (indépendamment bien entendu, de la position de ces éléments dans l'image ainsi que de leurs caractéristiques perceptives (couleur, forme...)). Ce dernier travail suggère que le sens de numérosité chez l'abeille pourrait aller jusqu'à quatre (Gross et al., 2009).

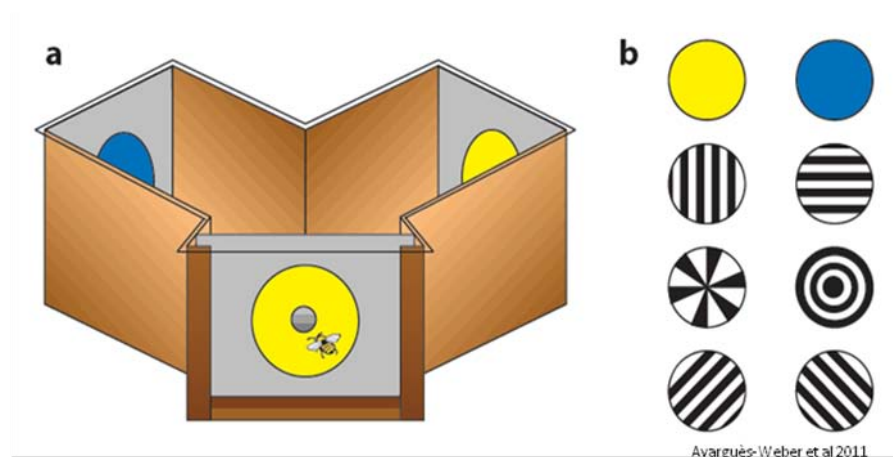


Figure 12:
Dispositif (A) et stimuli (B) utilisés pour mettre en évidence le concept d'identité chez l'abeille (Giurfa et al., 2001).

Au-delà des concepts d'équivalence ou de différence, et dans le contexte d'une capacité chez l'abeille de combiner des relations spatiales entre éléments d'une image en une configuration, nous avons voulu étudier la capacité des abeilles à maîtriser des concepts abstraits de nature spatiale. Nous avons ainsi testé la capacité des abeilles à discriminer des stimuli visuels en fonction de relations de type 'au-dessus de' vs. 'en-dessous de'. Ces relations étant définies en fonction d'un stimulus de référence ('réfèrent') (Article IV).

Enfin, dans un dernier chapitre, nous avons souhaité aller plus loin dans l'analyse des facultés d'abstraction de l'abeille en testant leur capacité à maîtriser les relations 'au-dessus / en dessous' vs. 'droite / gauche' pour lesquelles il n'y a pas conservation d'un 'réfèrent' d'un stimulus à l'autre. De plus, nous avons évalué la possibilité pour l'abeille de combiner le concept spatial appris avec une autre caractéristique partagée par les stimuli, la différence des éléments impliqués dans la relation. Cette tâche nécessite donc l'utilisation simultanée de deux concepts différents (relation spatiale et différence entre les éléments composant le stimulus) (Article V).

Objectifs

Les études que nous avons réalisées ont eu pour but de mieux comprendre le système de traitement des images complexes chez l'abeille ainsi que d'étudier dans une perspective comparative, l'existence chez un Invertébré, de traitements cognitifs visuels, connus pour leur efficacité dans la reconnaissance des objets, mais considérés jusque alors comme réservés aux Vertébrés du fait de leur complexité. Plus précisément, notre travail s'est articulé autour de cinq objectifs résumés ci-dessous :

Objectif 1 : Mise au point d'un protocole de conditionnement différentiel chez l'abeille en libre vol avec utilisation de quinine comme renforçateur négatif afin d'améliorer l'efficacité d'apprentissages complexes à la base des études de cognition visuelle.

Objectif 2 : Etudier la capacité des abeilles à catégoriser des stimuli sur la base d'une configuration spatiale commune de type « visage », ce qui implique l'extraction et la combinaison des relations entre les éléments d'une image

Objectif 3 : Etudier l'existence potentielle d'une prédominance d'un niveau de perception visuel (traitement global de stimuli composés ou traitement des éléments de détails locaux) dans la perception des configurations.

Objectif 4 : Tester la capacité des abeilles à extraire et utiliser les concepts relationnels des dispositions spatiales 'au-dessous' vs. 'en dessous'.

Objectif 5 : Tester la faculté des abeilles à combiner et maîtriser simultanément deux concepts différents, un concept spatial ('au-dessus' / 'en dessous' vs. 'droite / gauche') et un concept de différence entre les objets engagés dans la relation spatiale.

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Article I

Aversive reinforcement improves visual discrimination learning in free-flying honeybees

Avarguès-Weber A, de Brito Sanchez MG, Giurfa M, Dyer AG (2010)

PLoS ONE 5, e15370

Dans cette étude, nous avons voulu mettre en place une méthode de conditionnement visuel chez des abeilles en libre vol qui permettrait de dévoiler des performances fines de discrimination visuelle. Nous avons ainsi testé l'influence de l'introduction de quinine (substance amère) comme renforçateur négatif sur les performances d'apprentissage et de discrimination visuelle de l'abeille.

L'utilisation de procédures de conditionnement différentiel (présentation d'une alternative non récompensée au stimulus récompensé) permet de mettre en évidence chez l'abeille des performances de discrimination plus élevées qu'en conditionnement absolu (présentation d'un stimulus récompensé seul). Dans ce contexte, il nous est apparu qu'une amélioration des capacités d'apprentissage et de discrimination pourrait découler de l'association du stimulus alternatif avec une solution aversive. En effet, la force de l'attraction pour le SC+ (Stimulus Conditionnel récompensé) serait ainsi renforcée par l'introduction d'une aversion pour le stimulus alternatif SC-. De plus, l'utilisation d'une punition peut induire chez l'abeille une augmentation de son degré d'attention envers les stimuli et au moment de la prise de décision.

Pour tester une éventuelle amélioration des performances de l'abeille, nous avons utilisé deux groupes indépendants d'abeilles, individuellement entraînées et testées dans un labyrinthe en Y, dans une tâche de discrimination de couleurs. Au cours d'essais de conditionnement (15 essais), une couleur était récompensée par une solution sucrée tandis qu'une autre couleur présentée simultanément était associée à une solution de quinine fortement concentrée (groupe quinine) ou à de l'eau (groupe eau). Les capacités d'apprentissage (et donc de discrimination) de la couleur récompensée pour les deux groupes n'ont pas présenté de différence significative. Ainsi, la présence de quinine sur la couleur 'négative' n'a pas amélioré les performances d'apprentissage discriminatif par rapport à la présence de l'eau, au pouvoir renforçateur neutre. Néanmoins, lorsque l'on teste ces abeilles avec le SC- (associé préalablement à la quinine ou à de l'eau) versus une couleur neutre (nouvelle couleur), les abeilles du groupe quinine montrent un évitement du SC- à la

différence des abeilles du groupe eau. Ce dernier résultat suggère une aversion due à la quinine malgré l'absence observée d'amélioration des performances du groupe testée (quinine) par rapport au groupe contrôle (eau). Nous avons donc fait l'hypothèse que les résultats du groupe contrôle étant déjà très élevés, l'absence d'amélioration significative des performances avec la quinine serait due à un plafonnement des résultats.

Afin de tester cette hypothèse, nous avons soumis deux nouveaux groupes d'abeilles (quinine et eau) à une même tâche de discrimination de couleurs mais cette fois en utilisant des couleurs très proches pour le système perceptif de l'abeille, ce qui rendait la tâche de discrimination plus difficile. Dans ce cas là, on observe des performances plus élevées à la fois lors de l'entraînement et lors du test d'apprentissage pour le groupe quinine par rapport au groupe eau. Ce dernier ne montre d'ailleurs aucun signe d'apprentissage à la suite des 15 essais de conditionnement. Dans ce cas-ci, la pénalisation par présence de quinine sur le stimulus 'négatif' a bien induit une amélioration significative de l'apprentissage discriminatif.

Enfin, dans une troisième série d'expériences, nous avons pu préciser le mode de perception de la quinine par l'abeille. En effet, sa faculté de percevoir le goût amer faisait jusqu'alors l'objet d'études contradictoires. Une explication alternative à la perception gustative serait l'apparition d'une sensation de malaise après absorption de la quinine. Nous avons pu montrer que dans notre situation expérimentale, l'abeille ne consomme pas de quinine et présente un comportement de rejet presque immédiat, ce qui implique l'existence d'une perception gustative amère, d'éventuels facteurs olfactifs ayant été exclus.

Ainsi, nous proposons, grâce à cette étude, un protocole de conditionnement de l'abeille permettant un apprentissage plus rapide et des facultés de discrimination plus élevées dans le cas d'apprentissages complexes, notamment lorsque les stimuli utilisés présentent une forte similarité. C'est ce protocole qui sera employé dans les chapitres suivants et qui nous a permis d'appréhender des phénomènes cognitifs élaborés. De plus, ces résultats mettent en évidence un effet de l'expérience dans la perception de l'abeille dont l'acuité ne peut pas être simplement déduite de la physiologie de ses systèmes sensoriels.

Aversive reinforcement improves visual discrimination learning in free flying honeybees

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Keywords: Aversive conditioning – Aversive taste perception – Honeybee – Visual learning - Color

Published on PLoS ONE 5(10): e15370 (2010)

Abstract

Background

Learning and perception of visual stimuli by free-flying honeybees has been shown to vary dramatically depending on the way insects are trained. Fine color discrimination is achieved when both a target and a distractor are present during training (differential conditioning), whilst if the same target is learnt in isolation (absolute conditioning), discrimination is coarse and limited to perceptually dissimilar alternatives. Another way to potentially enhance discrimination is to increase the penalty associated with the distractor. Here we studied whether coupling the distractor with a highly concentrated quinine solution improves color discrimination of both similar and dissimilar colors by free-flying honeybees. As we assumed that quinine acts as an aversive stimulus, we analyzed whether aversion, if any, is based on an aversive sensory input at the gustatory level or on a post-ingestional malaise following quinine feeding.

Methodology/Principal Findings

We show that the presence of a highly concentrated quinine solution (60mM) acts as an aversive reinforcer promoting rejection of the target associated with it, and improving discrimination of perceptually similar stimuli but not of dissimilar stimuli. Free-flying bees did not use remote cues to detect the presence of quinine solution; the aversive effect exerted by this substance was mediated via a gustatory input, i.e. via a distasteful sensory experience, rather than via a post-ingestional malaise.

Conclusion

The present study supports the hypothesis that aversion conditioning is important for understanding how and what animals perceive and learn. By using this form of conditioning coupled with appetitive conditioning in the framework of a differential conditioning procedure, it is possible to uncover discrimination capabilities that may remain otherwise unsuspected. We show, therefore, that visual discrimination is not an absolute phenomenon but can be modulated by experience.

Introduction

The honeybee is a useful model for the study of complex visual-problem solving by a miniature brain [1-3]. Despite their relative small brain, honeybees learn to navigate mazes [4], use top-down processing to break camouflage [5], solve delayed matching to sample tasks and thus demonstrate rule learning [6], process rotated complex objects like faces [7], categorize complex spatial information [8-10] and exhibit performances akin to numerical counting [11-12].

A crucial aspect to reveal the cognitive capacities of honeybees, and other animals, is the training procedure employed by the experimenter. For instance, in several cases of complex problem solving by bees, learning sets have been used in which insects were confronted with a random succession of changing stimuli in which a specific feature remained constant and associated with reward. In this way, it was possible to ask whether bees are able to extract this feature *per se* and solve a problem on its basis (e.g. symmetry categorization [8]; orientation categorization [13]; configurational categorization [9-10]). Recent work has also demonstrated that the learning of color stimuli for both bumblebees [14] and honeybees [15] is dependent on the type of conditioning procedure. Specifically, fine color discrimination is achieved when both a target (rewarded conditioned stimulus or CS+) and a distractor (non-rewarded conditioned stimulus or CS-) are present during training (differential conditioning), whilst if the target is learnt in isolation (absolute conditioning), discrimination is coarse and limited to perceptually dissimilar alternatives. Thus, differential conditioning procedures seem to promote high levels of visual discrimination. An explanation provided to account for differences in discrimination resulting from absolute and differential conditioning is the hypothesis that the former, contrary to the latter, recruits attentional processes that are necessary to learn the difference between a target and a distractor [15].

Different procedures allow enhancing attention levels during differential conditioning. Manipulation of reinforcer intensity and/or hedonic value is a possible strategy. In any differential conditioning two specific memory traces are established, an excitatory one derived from CS+ experiences, and an inhibitory one derived from CS- experiences; choice following conditioning with a CS+ and a CS- results from the interaction between these two traces [16]. Thus, increasing the penalty associated with a CS- could enhance discrimination performances. In the case of free-flying bees subjected to visual discrimination problems,

traditional differential conditioning procedures reward the CS+ with sucrose solution (usually in a range between 30% and 50% weight/weight) and do not reward the CS-. In this experimental framework, one could ask whether coupling the CS- with an aversive reinforcement (instead of presenting it without reinforcement) could further improve learning performance in honeybees. To increase the penalty associated with a CS-, and to potentiate learning abilities of free-flying bees, we decided to pair the visual stimulus acting as CS- with a highly concentrated quinine solution.

The choice of quinine was based on recent studies reporting that quinine promotes accurate learning of stimuli in bumblebees [17-20]. These studies, however, contrast with the fact that naturally occurring concentrations of secondary compounds in nectar that taste bitter to human do not have a deterring effect in free-flying honeybees but can even elicit a feeding preference [21]. Moreover, to date there has been no evidence of bitter gustatory receptors being present in the honeybee (electrophysiology [22] or genomic study [23]; see [24] for review). Additionally, experiments in the laboratory with harnessed bees could not find clear evidence supporting that bitter compounds are aversive to bees in contention [22,25].

Given this apparent lack of agreement on the aversive nature of bitter compounds for honeybees, we decided to explicitly test whether a highly concentrated quinine solution would confer an enhanced aversive associative strength to a visual CS-, thus facilitating its discrimination from a CS+. In asking this question we took into account the perceptual similarity of the visual stimuli to be discriminated and analyzed whether the negative reinforcement would indistinctly favor discrimination both for dissimilar and similar stimuli. To understand the mechanisms underlying behavioral performances, we analyzed whether quinine aversion, if any, is based on an aversive sensory input at the gustatory level or on a post-ingestional malaise following quinine feeding.

Results

Experiment 1: Does quinine improve visual discrimination of perceptually dissimilar colors when used as negative reinforcer?

The potential aversive effect of quinine leading to an improvement of visual discrimination was investigated using a differential conditioning protocol in which one color

stimulus (CS+) was associated with sucrose solution 1M and another color (CS-) with either a highly concentrated 60 mM quinine solution (quinine group) or tap water (water group). Colors were presented in a Y-maze (Fig. 1a) to which honeybees were individually trained. Only one marked bee was present at a time in the Y-maze.

Color stimuli were cut from broadband HKS-N colored papers. Four colors were used in this experiment (HKS 8N, 26N, 44N and 54N, which appeared orange, pink, blue and green to humans respectively; see Fig. 2a). All six dual combinations were used as conditioning stimuli (8N vs. 26N, 8N vs. 44N, 8N vs. 54N, 26N vs. 44N, 26N vs. 54N, 44N vs. 54N). Bees in the quinine group and in the water group were matched with respect to its training combination. All colors were easily distinguishable for bees as they were well separated from each other irrespective of the color space used to represent them (*color opponent coding space*: mean perceptual distance between stimuli \pm s.e.m. = 6.15 ± 1.10 COC units; *hexagon color space*: 0.31 ± 0.04 hexagon units).

Each bee was trained with its particular CS+ vs. CS- combination for 15 trials. Afterwards, it was subjected to three different non-rewarded tests: a learning test presenting the CS+ vs. the CS-; a test presenting the CS+ vs. a novel stimulus (NS) ('appetitive' test), and a test presenting the CS- vs. NS ('aversive' test). Whilst the learning test allows verifying whether or not bees learned the discrimination task, the CS+ vs. NS test verified that the CS+ gained an excitatory associative strength leading the bees to choose it preferentially; the CS- vs. NS test, on the contrary, assesses whether or not the CS- has gained an inhibitory associative strength, leading the bees to avoid it and to prefer the NS. For each bee trained with a particular combination of colors, one of the two remaining colors not used during the training was assigned randomly as NS for the tests.

Both groups of bees (quinine group and water group) learned the task as their acquisition curves significantly increased during the three blocks of 5 trials (Fig. 2b; ANOVA for repeated measurements; N=18 bees; block effect: $F_{2,32}=12.7$, $p<0.001$). There was no group effect ($F_{1,16}=1.8$, $p=0.20$) thus showing that, at least at the level of acquisition, having quinine or water associated with the CS- did not significantly affect discrimination learning.

In the learning test, bees of both groups preferred the CS+ to the CS- (quinine group: N=9 bees; $82.3 \pm 2.7\%$ correct choices; mean \pm s.e.m., black bar in Fig.2c 'learning test');

water group: $N=9$ bees; $75.9 \pm 2.8\%$, white bar in Fig.2c 'learning test'). In both cases, the percentage of correct choices differed significantly from a random choice (quinine group: one-sample t-test against 50%; $t_8=9.7$, $p<0.001$; water group: $t_8=7.7$, $p<0.001$), thus confirming that both groups learned the discrimination between CS+ and CS-. There were no significant differences between groups (two-sample t-test: $t_{16}=1.6$, $p=0.14$).

In the test comparing the CS+ and the novel stimulus (NS), both groups of bees preferred the CS+ to the NS (quinine group: $76.6 \pm 2.2\%$ correct choices, $t_8=11.2$, $p<0.001$, black bar in Fig.2c 'appetitive test'; water group: $76.3 \pm 4.5\%$, $t_8=5.4$, $p<0.005$, white bar in Fig.2c 'appetitive test'). There were no significant differences between groups (two-sample t-test: $t_{16}=0.1$, $p=0.90$), which shows that the CS+ gained a similar excitatory strength in both cases, a result that was expected given that the same appetitive US was paired with the CS+ for both groups of bees.

Differences between groups were evident in the test comparing the CS- and the NS. The quinine group avoided the CS- and chose therefore the NS ($34.8 \pm 4.0\%$ of choices for CS-; $t_8=3.8$, $p<0.01$; black bar in Fig.2c 'aversive test') while the water group did not avoid the CS- ($56.4 \pm 4.0\%$ of choices for CS-; $t_8=1.6$, $p=0.16$, white bar in Fig. 2c 'aversive test'). The performance of both groups was significantly different ($t_{16}=3.8$, $p<0.005$), indicating that only quinine induced an aversion of the CS- in free-flying honeybees.

We analyzed whether performance in the tests was not affected by color-specific effects so that choices were independent of the particular color distance separating the test stimuli presented to each bee. This analysis is important because the basic assumption of this experiment is that given the clear dissimilarity between the colors used, the performance of the bees would be similar in all cases. To test this assumption we performed Pearson correlation analyses between the percentage of choices for a CS (CS+ in the learning and appetitive tests, and CS- in the aversive test) and the color distance between the stimuli presented in each test. For the tests in which there was no difference between the performance of the quinine and the water groups (learning and appetitive tests; see above), data from both groups were pooled. For the aversive test, both groups were treated separately as performances in the aversive test were significantly different. Distances used in the correlation analyses were derived from the COC and the hexagon colour models (independent analyses).

When considering all color combinations used, performance in the learning test confronting the CS+ and the CS- was independent of the color distance separating these two stimuli (COC: $r=0.1578$, $p=0.53$; hexagon: $r=-0.0337$, $p=0.89$). The same result was obtained for the appetitive test confronting the CS+ and the NS (COC: $r=-0.0911$, $p=0.72$; hexagon: $r=-0.2872$, $p=0.25$). Finally, in the aversive test confronting the CS- and the NS, choices were independent of the distance separating these stimuli both for the quinine (COC: $r=-0.1852$, $p=0.63$; hexagon: $r=-0.2272$, $p=0.56$) and the water group (COC: $r=-0.5619$, $p=0.12$; hexagon: $r=-0.6434$, $p=0.06$). These results show, therefore, that performance was not affected by differences in color distances, which were all large enough as to facilitate discrimination.

Based on these results, the large perceptual distance between color stimuli is a potential explanation why we did not observe significant differences in acquisition between the quinine and the water groups (see Fig. 2b and 2c, ‘learning test’). Specifically, as discrimination was facilitated by the dissimilarity of colors, using quinine may not have led to an improvement in performance due to a ceiling effect in the water group. Hence in experiment 2 we trained bees in a differential conditioning protocol using colors that were perceptually closer and thus more difficult to discriminate.

Experiment 2: Does quinine improve visual discrimination of perceptually similar colors when used as negative reinforcer?

We used the method described for experiment 1, but using perceptually similar visual stimuli as CS+ and CS-. Our goal was to determine if with increased task difficulty quinine would improve visual discrimination due to its potential aversive nature. In this experiment, only the learning test was performed after conditioning, as we aimed at detecting potential differences in learning of a difficult visual task.

Four colors were used in this experiment (HKS 3N, 43N, 47N and 68N), two of which (3N and 68N) appeared yellow to humans, and the other two blue (43N and 47N) (see Fig. 3a). Bees were trained to discriminate the similar colors (i.e. either 3N vs. 68N or 43N vs. 47N), which, irrespective of the color space used to represent them, were perceptually close (*color opponent coding space*: mean perceptual distance between stimuli \pm s.e.m. = 1.47 ± 0.11 COC units; *hexagon color space*: 0.08 ± 0.003 hexagon units).

A clear difference between the quinine and the water group was apparent during acquisition (Fig. 3b). While bees from the quinine group learned the discrimination (N=8 bees; $F_{2,12}=4.1$, $p<0.05$, black dots in Fig.3b), bees from the water group did not learn to discriminate the CS+ from the CS- (N=8 bees; $F_{2,12}=0.3$, $p=0.78$, white dots in Fig.3b). The difference between groups was significant (group effect, $F_{1,14}=10.1$, $p<0.01$; group \times trial interaction, $F_{2,28}=3.6$, $p<0.05$), thus showing that for a perceptually difficult color discrimination, associating quinine with the CS- facilitates discrimination by free-flying honeybees. This conclusion was confirmed by the results of the learning test, in which bees of both groups were again confronted with the CS+ and the CS- in the absence of reinforcement. Whilst bees in the quinine group significantly preferred the CS+ ($70.8 \pm 2.3\%$ of correct choices, $t_7=8.6$, $p<0.001$; black bar in Fig.3c), bees in the water group did not discriminate between CS+ and CS- ($45.1 \pm 2.7\%$ of correct choices, $t_7=1.8$, $p=0.12$; white bar in Fig.3c). The difference between groups was highly significant ($t_{14}=7.1$, $p<0.001$).

We analyzed again whether color specific effects affected test performance of bees. Two color distances were used for the Pearson correlation analyses (those between 3N and 68N and between 43N vs. 47N). A significant correlation between color distance and correct choices for the CS+ was neither found for the quinine group (COC: $r=-0.3965$, $p=0.33$; hexagon: $r=0.3965$, $p=0.33$) nor for the water group (COC: $r=0.3696$, $p=0.37$; hexagon: $r=0.3696$, $p=0.37$). This result thus shows that no color specific effects affected the bees' performance. When the visual discrimination that has to be achieved by freely-flying bees is perceptually difficult, a highly concentrated quinine solution (60 mM) facilitates discrimination acting as an effective negative reinforcer.

Experiment 3: Possible mechanisms accounting for the aversive nature of quinine solution.

The highly concentrated quinine solution could exert its aversive effect via different physiological processes. Two plausible options would locate the aversive effect either at the periphery, i.e. at the level of gustatory receptors, or at a general internal, physiological level. While the former option would consist in a distasteful gustatory experience elicited by quinine solution, the latter option would consist of a post-ingestional malaise induced by the quinine

solution, which would not necessarily taste bad to bees but which would be toxic once ingested [25].

To determine the process by which quinine solution exerts its aversive effect, we designed an experiment to compare the quantities of quinine solution and water imbibed by bees in similar experimental conditions. While gustatory aversion would be consistent with bees imbibing significantly less quinine solution than water, post-ingestional malaise would be consistent with bees imbibing comparable volumes of water and quinine solution. In the latter scenario, only after ingestion would bees experience the malaise effect and thus the aversive nature of quinine solution.

Bees were trained to collect sucrose solution in the Y-maze. They faced an impossible task as the same visual stimulus was presented in both arms of the maze during 15 trials. Half of the bees were trained with HKS 8N (orange to humans) and the other half with HKS 54N (green to humans). During the first 5 trials the color chosen for training was rewarded with 35 μ L of sucrose 1M ('target') whilst the same stimulus on the other side contained no reinforcement ('distractor'). After the first 5 trials, for one group of bees the 'distractor' was associated with 35 μ L of quinine solution 60 mM from trials 6 to 10 and with 35 μ L of water from trials 11 to 15; for a second group of bees the sequence was inverted, so that water was obtained on the distractor from trials 6 to 10 and quinine solution from trials 11 to 15.

In order to estimate the volume imbibed by each bee, we established a standard curve relating drinking duration (sec) and volume of 1M sucrose solution imbibed (μ L). The relationship between both variables was almost linear (0.7 μ L/sec; Pearson linear correlation analysis: $r=0.9997$, $p<0.001$; $N=8$ bees; Fig.4a), and is consistent with previous estimations obtained by Núñez [26].

During the three blocks, bees made random choices between the 'target' and the 'distractor', a result that was expected given that the same stimulus was used for both categories. There was no block effect ($F_{2,12}=2.7$, $p=0.11$), showing that irrespective of 'distractor' reinforcement (quinine, water or nothing), the percentage of choices of the 'target' remained the same. Furthermore, the sequence of presentation of negative reinforcements (nothing, water and quinine vs. nothing, quinine and water; see above) did not affect the percentage of choices of the 'target' ($F_{1,6}=0.6$, $p=0.46$). These results show that the sequence

of presentation of quinine and water did not affect performance. The results also show that the different solutions were not remotely detected by olfactory cues. A similar analysis was performed on the time spent by bees drinking the negative reinforcer associated with the 'distractor' (quinine or water). As the experiment included a block of trials in which there was no reinforcer on the 'distractor', drinking time values assigned to this block was zero in all cases. As in the previous analysis, the sequence of presentation of negative reinforcements (nothing, water and quinine vs. nothing, quinine and water; see above) did not affect drinking time ($F_{1,6}=0.74$, $p=0.42$). However, the block effect was significant ($F_{2,12}=59.4$, $p<0.001$), showing that drinking time varied with the type of reinforcement provided. In fact, post hoc Tukey tests showed that bees spent significantly less time drinking quinine than water (mean of 5 trials: water: 1.7 ± 0.3 sec; quinine: 0.7 ± 0.2 sec; $p<0.001$; see inset in Fig. 4a). This result shows that bees only consumed very low volumes of quinine, if any. Indeed, such a short time may be just enough for the bee to extend the proboscis and taste the solution, and then reject it without significant drinking. Hence drinking time values indicate that the aversive effect of quinine was not determined by a post-ingestional malaise but rather by a distasteful gustatory experience.

As the mean drinking times for quinine solution and water correspond to theoretical volumes ($0.7 \mu\text{L}$ and $1.7 \mu\text{L}$) that are below the initial point ($2 \mu\text{L}$) of our time-volume curve (see inset in Fig. 4a), we performed an additional experiment to more precisely quantify the imbibing activity of free-flying bees confronted with 16 drops of 1 and $2 \mu\text{L}$ of 1M sucrose solution, water or highly concentrated quinine solution (60 mM). Drops were randomly placed on a Plexiglas sheet housed within box with a UV transparent Plexiglas cover. During 6 visits to the setup, each lasting 180 sec, we quantified for each substance the number of drops that were consumed. In visits 1, 3 and 6, bees were presented with 8 drops of sucrose and 8 of water while in visits 2, 4 and 5, they were presented with 8 drops of sucrose and 8 of quinine solution.

Figure 4b shows that bees imbibed nearly all presented sucrose droplets ($N=8$ bees, $96.9 \pm 1.7\%$ of $1 \mu\text{L}$ droplets, $97.4 \pm 1.1\%$ of $2 \mu\text{L}$ droplets), and a reasonable number of the water droplets ($36.5 \pm 3.5\%$ of $1 \mu\text{L}$ droplets, $17.7 \pm 1.9\%$ of $2 \mu\text{L}$ droplets). However, they almost never imbibed the quinine droplets ($3.1 \pm 1.5\%$ of $1 \mu\text{L}$ droplets, $0.0 \pm 0.0\%$ of $2 \mu\text{L}$ droplets). Both for the $1 \mu\text{L}$ and the $2 \mu\text{L}$ droplets, there was a significant difference between

the quantity of water and quinine droplets imbibed (1 μ L droplets: Wilcoxon sign ranks test; $Z=2.52$, $p<0.05$; 2 μ L droplets: $Z=2.52$, $p<0.05$).

One possibility for the very low frequency of bees imbibing quinine could be that they used olfaction to detect and avoid quinine droplets at a very close range, even if we showed that this was not the case in the Y-maze experiment (see above). To test this possibility the proportion of droplets tasted by bees (droplets for which proboscis extension was observed) was evaluated (number of droplets tasted/total number of droplets). The proportion of tasted droplets was similar for each solution ($98.7 \pm 0.5\%$ of sucrose droplets, $97.9 \pm 1.2\%$ of water droplets and $97.9 \pm 0.9\%$ of quinine droplets). A similar result was obtained even if data from the first trial were not taken into account to exclude presentation order effects. Thus, consistent with the preceding experiment in the Y-maze, bees showed no evidence of sensing the presence of quinine prior to tasting it via their proboscis.

These data show that in a foraging context, free-flying honeybees do not imbibe quinine, even on their first encounter, which suggests an ability to taste quinine and to label it as an aversive substance via a gustatory input. The aversive effect induced by this substance in visual discrimination experiments would be based on a distasteful gustatory experience rather than on a post-ingestional malaise.

Discussion

The present work shows that visual discrimination by free-flying bees is not an absolute phenomenon but a process that can be modulated in a significant way by the nature of reinforcers associated with the visual stimuli that have to be discriminated. In our work we show that the presence of a highly concentrated quinine solution (60mM) acts as an aversive reinforcer promoting rejection of the target associated with it, and improving discrimination in perceptually difficult tasks (discrimination of perceptually similar stimuli). We show that bees do not use remote cues to detect the presence of the quinine solution and that the aversive effect exerted by this substance, in the case of free-flying bees, is mediated via a gustatory input, i.e. via a distasteful sensory experience, rather than via a post-ingestional malaise.

Improving visual discrimination by means of a negative reinforcer

Colour discrimination was usually described as a fast form of learning [27], compared, for instance, with learning of visual patterns, which usually takes longer (twenty or more trials). Recent studies on bumblebee and honeybee color and pattern learning [14-15,28] have introduced a new view of visual learning by free-flying bees, by showing that what the insects learn and discriminate in a given visual task depends on the training procedure which may or not inculcate the use of specific cues for solving a discrimination problem. It was previously thought that what an animal sees and visually learns is constrained by its perceptual machinery with little or no place for experience-dependent modulations of perception. The studies mentioned above and our work show that this idea is wrong: in some cases, learning a particular color may occur after few trials, but in other cases with perceptually similar stimuli it may need many more trials incorporating aversive conditioning.

Previous work has shown that bees [14-15] and ants [29] exhibit different visual discrimination powers after absolute conditioning (promoting less discrimination power) and differential conditioning (promoting more discrimination power) of color stimuli. Here we show that a negative reinforcer associated with a distractor in differential conditioning significantly enhances visual discrimination power. Visual discrimination in free-flying bees is usually studied in protocols in which one target is rewarded with concentrated sucrose solution (usually 1M) and one or various distractors are presented without any reinforcement. In the current study when we associated the distractor with a highly concentrated quinine solution, the discrimination of perceptually similar stimuli was possible, while it was not when the distractor was associated with water.

In the context of the hypothesis that differential conditioning to color stimuli improves performance by recruiting attentional processes [15], the difference in performance reported in our study suggests that attentional processes are enhanced by the penalizing effect of the aversive quinine solution. This may result in an improvement of the bees' discrimination performance, when compared to a situation where the distractor penalty is low or non-existent. Thus our new finding is likely to be of value for testing the hypothesis of attentional processes in insect brains [15]. In any case, our results go against the idea that the difference between two colors is an immutable property constrained by the visual machinery of the

honeybee. Rather, they indicate that aversive reinforcements may modulate discrimination by acting on attentional processes.

Previous work, mainly on vertebrate models, has shown that pairing an aversive reinforcer with a distractor promotes an increase of the appetitive associative strength of the rewarded stimulus [16]. This interpretation is not mutually exclusive with that provided above. In differential conditioning, experience with a CS+ and a CS- leads to the formation of an appetitive (excitatory) and an aversive (inhibitory) memory trace, respectively. Choice results from the interaction between these two traces so that if the subjective intensity of one of the reinforcers, the appetitive or the aversive, overcomes that of the alternative reinforcer, choice would be biased towards the dominant CS. In our study, although the appetitive reinforce (1M sucrose) remained constant on the CS+ for experiments, the presence of quinine on the CS- (distractor) may have enhanced the relative value of the appetitive reinforcer, thus promoting not only avoidance of the distractor, but also enhanced preference of the target CS+.

Our findings have several important implications for research on cognition using free-flying honeybees as a model. In conceptual terms, they underline the necessity to study attentional processes in the honeybee and to relate them to specific neural structures in the bee brain. Moreover, they raise the fundamental question of the real limits of the bees' visual capacities. Several studies that have reported negative results in terms of visual discrimination capabilities in bees [30-31] may be missing the fact that bees were not paying attention to the cues that they were supposed to learn in a given task. Thus, before concluding that bees are not capable of solving a certain visual discrimination, researchers should address the critical question of whether their experimental designs are able to push the cognitive capacities of bees to their limits. The example provided by the perceptual similar pair of colors in Fig. 3, which cannot be discriminated if the distractor is paired with water but which can be distinguished if it is paired with concentrated quinine solution underlines this point. Besides, using such a more efficient training may allow a more robust analysis of the cognitive mechanisms contributing to perception, by decreasing the amount of training necessary to tackle specific complex experimental questions. For example, recent studies which had use quinine had been successful in using bees to study processing of complex pictures such as human faces [7,10,32].

Interestingly, the enhancing effect of the aversive reinforcement was not evident when stimuli were perceptually distinguishable (Fig. 2). This lack of effect can be interpreted as the negative reinforcement not being necessary to solve an easy problem, i.e. performance without quinine already reached a high level of correct choices (Fig. 2). An alternative, although not mutually exclusive, explanation can be raised in term of speed-accuracy trade-off. For the discrimination of large color distances (a simple discrimination task) bees making relatively fast decisions easily choose the correct color with a very low rate of error. Thus, even if bees made decisions slower and more carefully when the cost of making an error was increased by adding quinine, it didn't significantly change accuracy. For example, a significant difference in accuracy between fast and slow bumblebees was consistently found only when colors were perceptually close [18]. In this case, bumblebees were fast when they solved simple tasks but when the task became more difficult some individuals decreased the speed by which they chose, thus leading to an increase of performance [18]. Such modulation of response time based on the perceptual difficulty of the task is well-known in humans [33]. Equivalent data are still missing for honeybees. Moreover, there may be differences in visual processing between different bee species [34]. Thus, measuring the time allocated for decision making by free-flying honeybees confronted with tasks of different difficulty may constitute an interesting research perspective for the future [35-36].

Quinine solution as a negative reinforcement

The concentrations of quinine used in our work are far from being ecologically relevant, as they were highly concentrated. The use of these highly concentrated solutions is justified by the fact that we wanted to associate an intense negative experience with the CS- and that free-flying bees seem to be more tolerant than humans to intermediate concentration levels of bitter substances [37]. Deterrent secondary compounds such as alkaloids or phenolic compounds are naturally present in nectar or pollen flowers as a defense against herbivores, but their concentration levels never reach those used in our experiments. It has even been reported that at natural concentration levels in the nectar, these substances may be neutral or even attractive for honeybees [21]. However, when bees have the choice between two nectars or pollens, one with secondary compounds and another with less secondary compounds, they usually prefer the latter [38-40]. Thus, the use of unnatural, higher concentrations allows establishing an effective aversive reinforcement for our studies and uncovering in this way the real visual discrimination abilities of honeybees.

In concluding that the use of quinine improves learning performance, we need to specify that the experimental conditions in which quinine acts as a negative reinforcement are those provided by our work, i.e., free-flying bees subjected to visual discriminations. It therefore appears that the critical aspect of our experimental procedure is the fact that honeybees could free move and, more importantly, free express their avoidance of the quinine solution as a negative reinforcer.

This aspect may explain apparent contradictory results on the effect of substances like quinine on the behavior of bees in the laboratory (see [24] for review). In the laboratory, contrary to the experimental results described above, bees are harnessed in individual metal tubes, which is the common procedure to test their sucrose responsiveness and/or learning in olfactory conditioning using the proboscis extension reflex (see [41] for review). In these experimental conditions, harnessed bees do not show an aversion for even higher concentrations of quinine solution than that used in the current study [22,25]. The same lack of aversion applied to a variety of substances that also taste extremely bitter to humans (salicine, amygdalin, caffeine, etc.) [22,25]. Furthermore, harnessed bees imbibe large amounts (20 μ L, one third of their crop capacity) of aversive solutions, including quinine solution, even if the imbibed solutions turn to be toxic and induce post-ingestional mortality [25]. In the case of studies on honeybee gustation in the laboratory (harnessed bees), recent results have suggested that the main effect produced by bitter substances is not a distasteful gustatory one, as suggested in our case, but rather a post-ingestional one, given that in all cases bees drank the aversive compounds without reluctance [25]. This difference with the current study may be due to the fact that in both experimental contexts, the capacity to express an active avoidance of the aversive reinforcement varies dramatically. When bees are in contention, the impossibility of movement may induce important changes in acceptance or rejection thresholds for gustatory compounds, making the bees more tolerant to substances that they would otherwise reject, even at the cost of their own death.

This hypothesis is plausible given that harnessed and free-flying bees exhibit striking differences in performances of other tasks such as color learning and discrimination. Experiments with free-flying bees have shown that the $\Delta\lambda$ discrimination function (i.e. the function accounting for the bees' wavelength discrimination along their visual spectrum) varies depending on the region of the spectrum. It reaches extremely fine discrimination

values of 4.5 nm for wavelengths at the intersection of photoreceptor sensitivity curves [42]. On the contrary, harnessed bees in the laboratory, which can be trained to associate a color with sucrose reward and which extend their proboscis to the learned color [43], have difficulties in learning this association and show very poor color discrimination abilities [44]. This difference may be motivational, as to learn colors in harnessed conditions it is necessary to cut the bees' antennae [43,45]. This procedure substantially decreases the subjective value of sucrose as a reward [46], thus impairing learning. Therefore our data confirm that studying free-flying bees remains essential to approach the natural behavior and learning capabilities of this animal as a model for neuroscience.

Whilst our study shows that honeybees do have an ability to detect quinine solution, the physiological mechanisms by which they do this are still unclear. Our results support a peripheral detection via gustatory receptors (see Experiment 3), but so far, bitter receptors have not been found in electrophysiological experiments [22] nor in genomic analyses comparing honeybee gustatory receptor genes with those of the fruit fly *Drosophila melanogaster*. In the fruit fly, 68 gustatory receptor genes have been identified [47-50]. Two of these genes have been associated with bitter taste as they both respond to caffeine and are coexpressed in the same gustatory receptor neurons [51-52]. In the honeybee, the picture seems to be drastically different: only ten gustatory receptor genes were found [23] suggesting that the gustatory world of a bee might be considered as relatively poor. Among these receptors, two are similar to the trehalose ('sweet') receptor of flies, but none is similar to their 'bitter' receptors. In our experiments, the bees extended the proboscis before being repelled by the quinine solution, which leads us to hypothesise that they detect the presence of the aversive quinine solution via a gustatory input. The concrete mechanism by which this is achieved remains unknown, and thus warrants further investigation.

Conclusion

The present study supports the hypothesis that aversion conditioning is important for understanding how and what animals perceive and learn. By using this form of conditioning coupled with appetitive conditioning in the framework of a differential conditioning procedure, it is possible to uncover discrimination capabilities that may remain otherwise unsuspected. We show that what an insect sees and learns may be significantly affected by experience rather than being only deductible from its visual machinery. Further

experiments studying visual discrimination capabilities of honeybees should consider using true negative reinforcements associated with the CS- in order to reveal what these insects can really perceive.

Materials and methods:

Experiment 1:

Individual free-flying honeybees *Apis mellifera*, Linnaeus, from a single colony located 100m from the test site were trained to collect 0.2M sucrose solution from an artificial feeder. The feeder was located 10 m from a Y-maze (Fig. 1a) to which individually marked honeybees selected from the feeder were trained to collect 1M sucrose solution [53]. Only one individual was present at a time in the Y-maze, which was covered by an ultraviolet-transparent Plexiglas ceiling. The maze was located on an outside table and illuminated by open daylight. The entrance of the maze led to a decision chamber, where the honeybee could choose between the two arms of the maze. Each arm was 40×20×20cm (L×H×W). The back walls of the maze (20×20 cm) were placed at a distance of 15cm from the decision chamber and were covered by a white reflecting UV background on which color targets were presented. Each color target consisted of a 7×7cm square cut from a HKS-N paper (K + E Stuttgart, Stuttgart-Feuerbach, Germany). Targets were placed in the middle of their corresponding back wall (Fig.1a). They therefore subtended a visual angle of 26° to the centre of the decision chamber and were thus large enough to recruit the chromatic pathways of the honeybee's visual system [53].

The reflectance spectra of the stimuli were measured with a spectrophotometer (Ocean Optics SD2000 with a DT1000 mini light source (200–1,100 nm) and R400-7 UV/VIS optical fibre). The perceptual distances between colors were calculated using the Color Opponent Coding (COC) space (Fig.2a; [54]) and the hexagon color space (Fig.2a; [55]). In both cases, for calculations we used the spectral sensitivities of the honeybee photoreceptors [56], a standard daylight function D65 [57] and the white background used in our experiments. Four colors were used in this experiment (HKS 8N, 26N, 44N and 54N, which appeared orange, pink, blue and green to humans respectively; see Fig. 2a). For each bee (N=18) three of these colors were chosen as CS+, CS- and NS, respectively (see below). Thus, a combination of

three colors was assigned to each bee among the 24 combinations possible (e.g. CS+: 8N, CS-: 26N and NS: 44N). All colors were easily distinguishable for bees as they were well separated from each other in both color spaces used to represent them (*color opponent coding space*: mean perceptual distance between stimuli \pm s.e.m. = 6.15 ± 1.10 COC units; *hexagon color space*: 0.31 ± 0.04 hexagon units).

During differential conditioning, the CS+ was rewarded with 1M sucrose solution while the CS- was associated either with tap water (water group) or 60mM quinine hydrochloride solution (quinine group) (N=9 for each group). Bees in the quinine group and in the water group (see below) were matched with respect to colors used. Solutions were delivered by means of a transparent micropipette 6mm in diameter located in the centre of each visual target.

Conditioning consisted of 15 training trials (i.e. 15 foraging bouts between the hive and the maze). The side of the rewarded stimulus was interchanged following a pseudorandom sequence to avoid positional (side) learning (i.e. the same stimulus was not presented more than twice on the same side). If the bee chose the rewarded stimulus CS+, it could drink sucrose solution *ad libitum*. If it chose the non-rewarded stimulus CS-, it was allowed to taste the water or quinine solution and then to fly to the alternative arm presenting the CS+ to find the sucrose. On each trial, only the first choice of the bee was recorded for statistical analysis.

Acquisition curves for both the quinine and the water group were obtained by computing the frequency of correct choices during 3 blocks of 5 trials each. After training, three transfer tests with fresh, non-rewarded stimuli were performed: a learning test presenting the CS+ vs. the CS-, an 'appetitive' test presenting the CS+ vs. a novel stimulus NS, and an 'aversive' test presenting the CS- vs. the NS. During the tests, contacts with the surface of the targets were counted for 45s. The choice proportion for each of the two test stimuli was then calculated. Each test was done twice, interchanging the sides of the targets to control for side preferences. Refreshing trials with the reinforced CS+ and CS- were intermingled between the tests to ensure motivation for the subsequent tests. The sequence of appetitive and aversive tests was randomized between bees.

Experiment 2:

The set-up and procedure used in this experiment were similar to those of experiment 1 (Fig.1a) except for the colors used for training, which, in this case, were perceptually similar. Colors used for training half of the bees (N=8) were HKS-3N vs. 68N, which appeared yellow to a human observer (perceptual distance separating them: 1.58 COC units; 0.08 hexagon units); the other half of the bees (N=8) was trained with HKS-43N vs. 47N, which appeared blue to a human observer (perceptual distance: 1.36 COC units; 0.08 hexagon units). CS+ and CS- were counterbalanced between bees within each group. After the 15-trial conditioning, a non-rewarded learning test with fresh CS+ and CS- stimuli was performed.

Experiment 3:

Experiment 3 consisted of two parts. In the first part, a Y-maze was used to train the bees in a discrimination task that was visually impossible as the identical color was presented as CS+ and CS- in the arms of the maze. For half of the bees (N=4), the training stimulus was HKS-8N (orange to humans) whereas for the other half (N=4) it was HKS-54N (green to humans) (Fig.2a).

The procedure used to train the bees was the same as in experiments 1 and 2, except that reinforcers were not delivered in micropipettes located in the middle of the color stimuli but on white plastic discs (26 mm diameter, 4 mm thick) lying on a horizontal landing surface standing on a 10 cm pole placed 1 cm in front of each stimulus (Fig. 1b). Thus, in each arm of the maze, a landing platform in front of the visual stimulus offered the reinforcer in a plastic disc. Each disc presented a small hole (4 mm diameter, 2.5 mm depth) to hold the fluid (Fig.1b). The landing platforms were used in order to record accurately feeding behavior with a video camera (see below).

Between trials the discs were cleaned with 10% ethanol solution to remove odor cues. During the first five trials one stimulus was rewarded with 35 μ L of 1M sucrose solution ('target') whilst the other stimulus contained no fluid ('distractor'). In the next five trials, the 'distractor' stimulus was associated with 35 μ L of 60mM quinine hydrochloride; finally, in the last five trials, it was associated with 35 μ L of tap water. For another group of bees, the sequence of the quinine – water trials was inverted.

During all trials a video camera (Canon MV920) was used to record the time spent by the imbibing the reinforcers (evaluated through proboscis extension time). To evaluate the volume (μL) fed using the drinking time (sec), we established a standard curve relating these two variables for 1M sucrose solution. The sequence of presentation of different volumes (2, 5, 10, 20, 30, 40, 50, 60, 70 μL) was randomized. The viscosities of water and of the 60mM quinine solution are lower than that of the 1M sucrose solution. Thus, the standard curve obtained for sucrose 1M was used to estimate the volume of water or quinine solution ingested.

In the second part of this experiment, we aimed at increasing the precision of our drinking measurements. We trained a new group of bees ($N=8$) to land on a $20\times 20\text{cm}$ UV transparent Plexiglas sheet housed within a $28.5\times 18.5\times 22.5\text{ cm}$ ($L\times H\times W$) box which had a UV transparent Plexiglas cover. To this end, individually-marked bees were allowed to collect 5-10 μL drops of a 1M sucrose solution on the Plexiglas sheet until satiation. Only one bee was present at a time in the box. After imbibing the sucrose solution, the bee selected for the experiments was allowed to return to the hive and recordings began when it came back to the set-up.

The bee had to visit the box six times (i.e. six foraging bouts or trials). Each trial lasted 180 seconds, and at the end of it the bee was fed until satiation with 10 μL drops of 0.5M sucrose placed on the Plexiglas sheet. After that, the bee was allowed to return to the hive. Within each trial the bee was allowed to collect small droplets (1 or 2 μL) of either sucrose (1M), tap water or 60mM quinine solution. In trials 1, 3 and 6 the bee was presented with 16 drops of either sucrose (4 \times 1 μL and 4 \times 2 μL) or water (4 \times 1 μL and 4 \times 2 μL), which were randomly arranged within a 4 \times 4 grid on the Plexiglas sheet. In trials 2, 4 and 5 the bee was presented with 16 drops of either sucrose (4 \times 1 μL and 4 \times 2 μL) or quinine (4 \times 1 μL and 4 \times 2 μL), which were randomly arranged within the same 4 \times 4 grid. Between trials the set-up was cleaned with 5% ethanol and afterwards with tap water. We recorded the number of drops of solution completely imbibed by a bee, and the proportion of solutions that were sampled.

Statistical analysis:

Data were checked for normality using the Shapiro-Wilk test and when necessary subjected to an arcsine transformation. Performance of balanced groups during acquisition was compared by means of a two-factorial ANOVA of repeated measurements in which the

stimuli used constituted one factor and the negative reinforcement (water or quinine) the other factor. The dependent variable was the percentage of correct first choices of each individual bee in each block of 5 trials.

Performance during the tests was analyzed in terms of the proportion of correct choices per test (i.e. a single value per bee). A one-sample t-test was used to test the null hypothesis that the proportion of correct choices in the test considered was not different from a theoretical value of 50%. Comparison between groups was made using an independent two-sample t-test. The alpha level for statistical significance was 0.05.

Acknowledgments:

We thank the beekeeper school of Pechbusque for access to their apiary and the Jock Marshall Reserve for the use of facilities. Thank you to Dr David Reser for careful reading and criticisms on an earlier draft of the manuscript. A.A.-W, M.G. de B.S and M.G. thank the National Research Agency (ANR) (Project INSAVEL), the French Research Council (CNRS) and the University Paul Sabatier for support. A.A.-W. was supported by a Travelling Fellowship from The Company of Biologists and by the University Paul Sabatier (ATUPS fellowship). A. G. D. acknowledges the Alexander von Humboldt Foundation, ARC DP0987989 and ARC DP0878968 for support.

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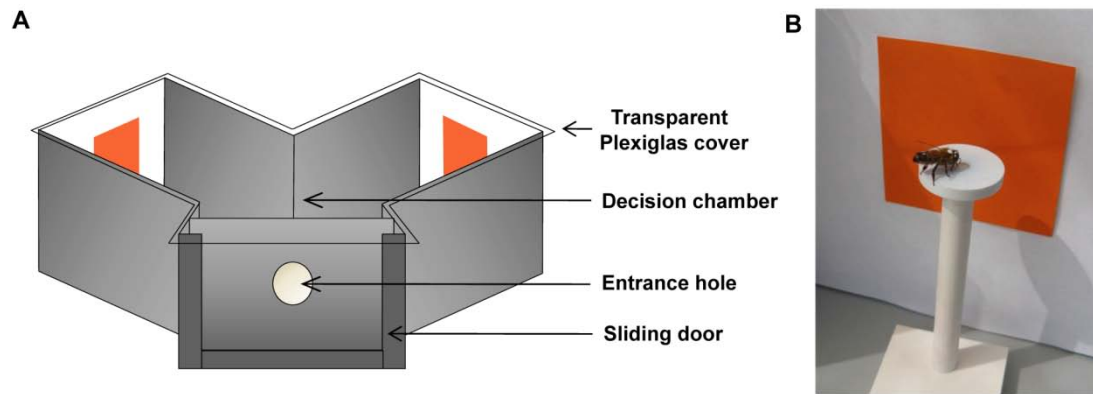


Figure 1: Set-up used in this study.

(A) Diagram of the Y-maze used in the experiments. (B) Photograph showing how stimuli were presented in association with a solution holder in each arm of the Y-maze during experiment 3.

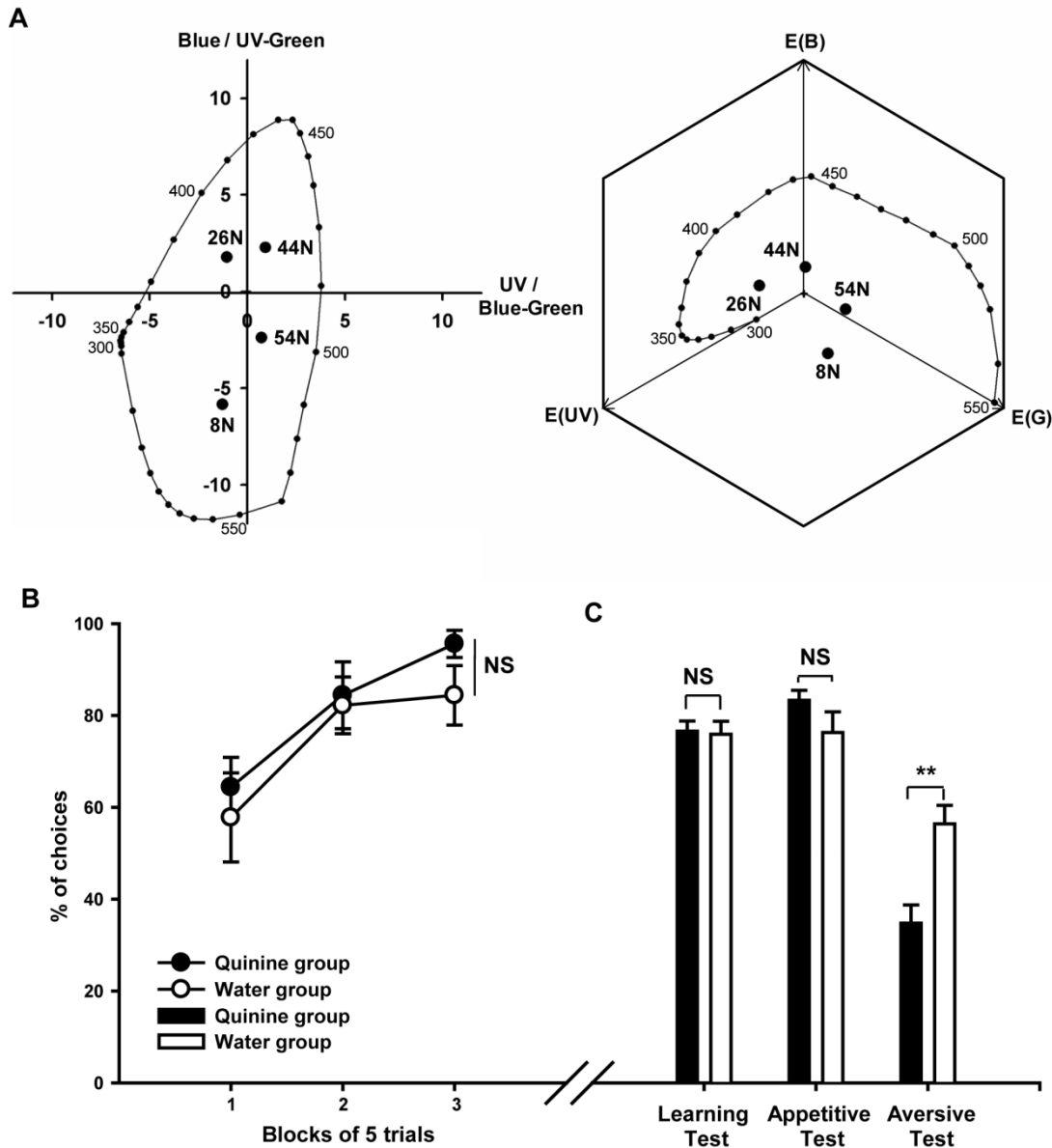


Figure 2: Results of Experiment 1: Does quinine improve visual discrimination of perceptually dissimilar colors when used as negative reinforcer?

(A) Plots of colored stimuli used in experiment 1 in a COC color space (left) and hexagon color space (right) for the trichromatic vision of honeybees. The colour distances between stimuli are above 2.5 COC units and above 0.2 hexagon units. Numbers refer to the HKS papers references.

(B) Learning acquisition (correct choices (%)) by blocks of 5 trials; means \pm s.e.m.; N=9 for each curve). The curve with black dots represents acquisition by the quinine group (CS-

reinforced with quinine); the curve with white dots represents acquisition by the water group (CS- reinforced with water).

(C) Performance (means + s.e.m. of percentages of CS+ choices ('learning' and 'appetitive' test) or CS- choices ('aversive' test); N=9 for each bar) in non-rewarded tests. Black bars represent the results of the quinine group; white bars represent the results of the water group.

The learning performance in this easy colour discrimination task was not significantly different between test groups. Bees from the quinine groups avoided the stimulus associated with quinine during training when proposed versus a neutral stimulus, however this avoidance was not found in the water group (**: $p < 0.005$).

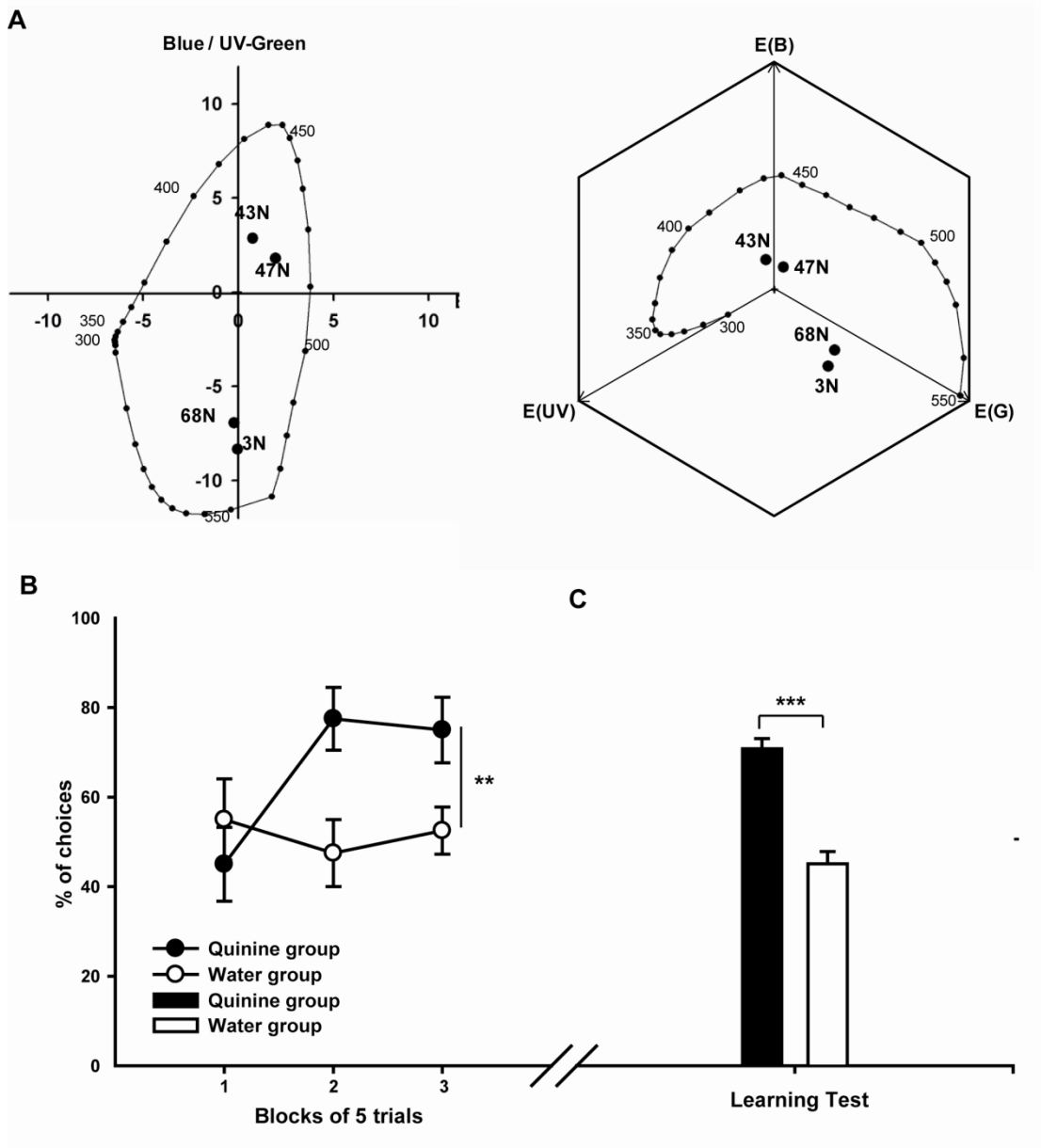


Figure 3: Results of Experiment 2: Does quinine improve visual discrimination of perceptually similar colors when used as negative reinforcer?

(A) Plots of colored stimuli used in experiment 2 in a COC color space (left) and in a hexagon color space (right) for the trichromatic vision of honeybees. Distances between the two stimuli used for each bee are 1.5 COC units and 0.08 hexagon units for the ‘yellow’ group (3N vs. 68N) and 1.3 COC units and 0.08 hexagon units for the ‘blue’ group (43N vs. 47N). Numbers refer to the HKS papers references.

(B) Learning acquisition (% of correct choices by blocks of 5 trials; means ± s.e.m.; N=8 for each curve). The curve with black dots represents acquisition by the quinine group (CS-

reinforced with quinine); the curve with white dots represents acquisition by the water group (CS- reinforced with water).

(C) Performance (means + s.e.m. of correct choices; N=8 for each bar) in non-rewarded learning test. The black bar represents the results of the quinine group; the white bar represents the results of the water group.

Only bees from the quinine group solved this difficult discrimination task (**: $p < 0.005$; ***: $p < 0.001$).

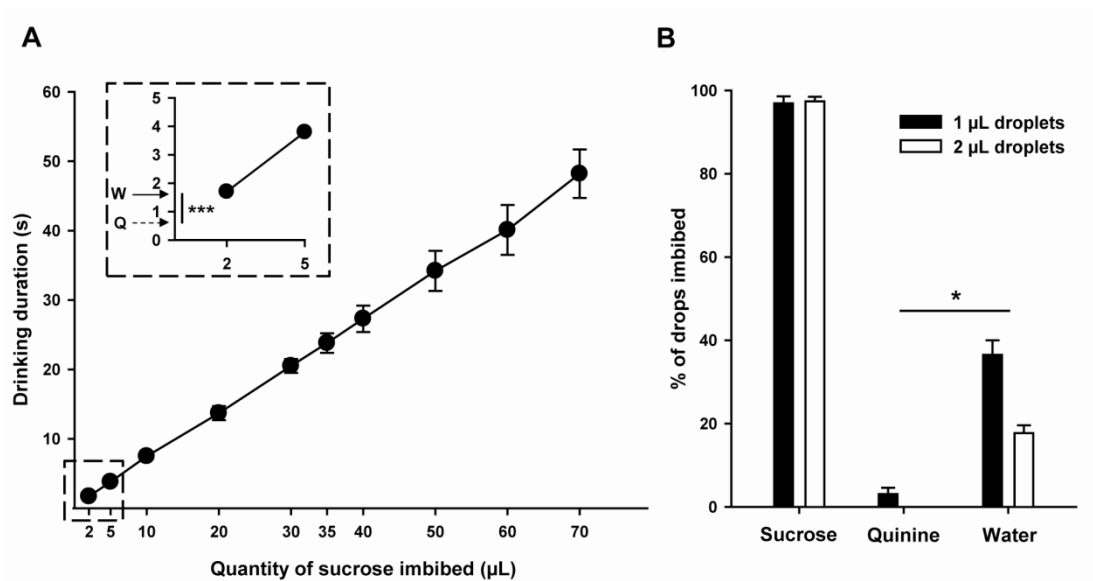


Figure 4: Results of Experiment 3: Possible mechanisms accounting for the aversive nature of quinine solution.

(A) Standard curve relating drinking duration (s) and volume of 1M sucrose solution imbibed (μL). These variables were linearly correlated ($p < 0.001$). The arrows in inset show the means of water (W) and quinine (Q) drinking duration (***: $p < 0.001$).

(B) Percentage of droplets (1 μL in black or 2 μL in white) of sucrose, quinine or water totally imbibed by bees when presented together on a plate. These data show that in a foraging context, free-flying honeybees don't imbibe quinine but do imbibe water (*: $p < 0.05$).

Article II

Configural processing enables discrimination and categorization of face-like stimuli in honeybees

Avarguès-Weber A, Portelli G, Benard J, Dyer AG, Giurfa M (2010)

The Journal of Experimental Biology 213, 593-601

Par cette étude, nous avons voulu tester la capacité des abeilles à catégoriser des stimuli visuels à partir de configurations ou topologies spécifiques. Les abeilles sont capables de classer des stimuli visuels en fonction de leur appartenance à une catégorie visuelle définie en fonction d'un trait particulier unique (symétrie, orientation, etc). Nous nous sommes demandé ici si, au-delà de cette capacité, les abeilles peuvent extraire et combiner les relations entre différents traits dans une configuration qui servirait de base à la catégorie visuelle.

Les abeilles ont été individuellement entraînées à discriminer des stimuli présentant une configuration de type « visage » (deux ronds à la place des yeux, situés au-dessus d'une barre verticale pour le nez, elle-même située au-dessus d'une barre horizontale pour la bouche (de type smiley)) versus des stimuli présentant une disposition aléatoire des mêmes éléments. Les abeilles testées ont alors réussi à transférer leur choix face à de nouveaux stimuli selon qu'ils présentaient ou non la bonne configuration. Nous avons vérifié, par ailleurs, la présence d'une discrimination intra-catégorie et l'absence d'utilisation d'indices de bas niveaux tels que le centre de gravité de l'image ou encore la distribution des fréquences spatiales. Cette performance est maintenue malgré l'ajout de bruit aux images par ajout d'un fond. Enfin, l'utilisation de photographies de visages humains a permis de montrer que les abeilles sont aussi sensibles à la configuration lors de l'apprentissage de stimuli complexes. En effet, la réorganisation spatiale des différents éléments des photographies induit une perte totale de reconnaissance.

Ces résultats démontrent la faculté des abeilles à combiner les inter-relations des éléments d'une image en une définition de catégorie et à utiliser la configuration pour reconnaître des stimuli complexes. Ce processus cognitif n'est donc pas la prérogative des seuls vertébrés.

Configural processing enables discrimination and categorization of face-like stimuli in honeybees

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Keywords: Vision – Visual Cognition – Configural Processing – Honeybee

Published on The Journal of Experimental Biology 213(4): 593-601

Summary

We studied whether honeybees can distinguish face-like configurations using standardized stimuli commonly employed in primate and human visual research. Furthermore, we studied whether irrespective of their capacity to distinguish between face-like stimuli bees learn to classify visual stimuli built up of the same elements in face-like vs. non-face like categories. We showed that bees succeeded in discriminating both face-like and non-face like stimuli, and categorized appropriately novel stimuli in these two classes. To this end, they used configural information and not just isolated features or low-level cues. Bees looked for a specific configuration in which each feature had to be located in an appropriate spatial relationship with respect to the others, thus showing sensitivity for first-order relationships between features. Although faces are biologically irrelevant stimuli for bees, the fact that they were able to integrate visual features into complex representations suggests that face-like stimulus categorization can occur even in the absence of brain regions specialized in face processing.

Introduction

Primates are very good at processing face-like stimuli (Rosenfeld and Van Hoesen, 1979; Parr et al., 2000). In particular, humans have remarkable capabilities for learning unfamiliar faces and recognizing familiar faces (Collishaw and Hole, 2000). This ability has been related to specialized brain areas both in primates (Tsao et al., 2006) and humans (Kanwisher, 2000). The capacity for recognizing familiar faces has largely been attributed to configural processing (Tanaka and Sengco, 1997; Collishaw and Hole, 2000; Maurer et al., 2002), which allows treating a complex visual stimulus by taking into account not only its individual components but also the relations among them (Palmeri and Gauthier, 2004; Peterson and Rhodes, 2003). It has often been assumed that this ability requires time to develop because children confronted with face recognition tasks move towards configural processing with increasing age and visual experience (Carey and Diamond, 1977; Carey and Diamond, 1994). However, experiments on how humans learn non-face objects using configural processing (Gauthier and Tarr, 1997; Gauthier et al., 2000; Busey and Vanderkolk, 2004) suggest that this ability might be learnt reasonably quickly if the appropriate visual experience is made available.

Putting these results into perspective is difficult given the various meanings that the term 'configural processing' may adopt. Indeed, although commonly used in visual cognition studies, the term '*configural processing*' remains ambiguous as it may refer to different levels of compound stimulus processing. Configural learning and processing *sensu* Pearce (1987, 1994), for instance, implies that an AB compound is treated as an entity different from the sum of its elements, i.e. the stimulus complex AB is not viewed as "A + B", but instead may be thought of as a distinct event that is related to A and B only through physical similarity. In visual cognition, the term 'configural processing' rarely refers to Pearce's theories and is used to refer to processing forms that involve perceiving relations among the features of a compound stimulus (Maurer et al., 2002). It is opposed to 'featural processing' (or 'analytical processing') in which only the features, but not the relationships among them, are taken into account. In the light of such ambiguity, Maurer et al (2002) proposed that studies on visual cognition, particularly face recognition studies, should distinguish three levels of configural processing: 1) *sensitivity to first-order relations*, in which basic relationships between features are taken into account (e.g. detecting a face because its features conform to a standard arrangement in which two eyes are located above and a nose is in turn located above a mouth,

etc.). 2) *holistic processing*, in which features are bound together into a gestalt, and 3) *sensitivity to second-order relationships* in which distances among features are perceived and used for discrimination (see Maurer et al., 2002 for review). In order to avoid the lack of consensus about terminology, and the fact that ‘configural processing’ is used indistinctly to characterize one or all three types of processing mentioned above, we will adopt here Maurer et al.’s three-level definition as the main framework for our study.

Besides humans and primates, insects constitute an interesting model to understand how brains learn to process complex images (Peng et al., 2007; Benard et al., 2006). Among insects, honeybees are particularly appealing because they learn and memorize a variety of complex visual cues to identify their food sources, the flowers. The study of their visual capacities is amenable to the laboratory as it is possible to train and test individual free-flying bees on specific visual targets, on which the experimenter offers a drop of sucrose solution as the equivalent of nectar reward (see review in Giurfa, 2007).

Using this protocol, it has recently been shown that bees are capable of previously unsuspected higher-order forms of visual learning that have been mainly studied in vertebrates with larger brains. Indeed, bees categorize both artificial patterns (see Benard et al., 2006 for review), and pictures of natural scenes (Zhang et al. 2004). They also learn abstract relationships (e.g. sameness) between visual objects in their environment (Giurfa et al., 2001) and exhibit top-down modulation of their visual perception (Zhang and Srinivasan 1994). Many of these experiments have shown that the way in which individual bees are conditioned is critical to uncover fine discrimination performances (Zhang and Srinivasan 1994; Giurfa et al., 1999; Stach and Giurfa, 2005). Bees trained in differential conditioning protocols, which imply learning to differentiate rewarded from non-rewarded targets, exhibit sophisticated discrimination abilities, some of which have been so far unsuspected in an invertebrate (Giurfa, 2007).

The possibility that small brains can learn to recognize human-face-like stimuli has considerable impact on several domains, from fundamental ones related to the neural architecture required to achieve this task, to applications based on how computer vision could benefit from using similar and potentially highly efficient mechanisms (Rind, 2004). Although the visual machinery of bees has definitely not evolved to detect and recognize human faces, but rather flowers (Chittka and Menzel, 1992) and other biologically relevant

objects, it might have the necessary capacities to extract and combine human face features in unique configurations proper to different persons. This ability may simply reflect the bees' use of similar strategies to recognize and discriminate food sources like flowers in their natural complex environment. In other words, testing whether bees learn to recognize and classify face-like stimuli should be contemplated as a test of configural processing in the visual domain, allowing an understanding of which of three levels of processing (see above) is used to this process a complex visual stimulus by a relatively simple visual machinery. We do not intend to raise the inappropriate question of whether human faces are biologically important for bees, which is certainly not the case. Nevertheless, if face-like stimuli classification and processing is achieved by a brain lacking specific areas devoted to the recognition of human faces such as those existing in humans and primates (Tsao et al., 2006; Kanwisher, 2000), one may conclude that basic mechanisms already available in more 'primitive' nervous systems allow reaching comparable goals in the absence of such brain specializations.

A recent study trained free-flying honeybees to discriminate pictures of human faces used in standard psychophysics tests (Dyer et al., 2005) and found that bees could indeed distinguish the pictures presented. This report was questioned (Pascalis, 2006; but see Dyer, 2006) as it could not control for the actual cues extracted from the pictures and used by the bees for recognition. Indeed, instead of responding to specific face configurations, bees could have used low-level cues to perform their choices. However, there is evidence that wasps recognize conspecific faces (Tibbetts, 2002) and honeybees learn multiple representations of human face stimuli and interpolate this visual information to recognize novel face viewpoints (Dyer and Vuong, 2008) leads to the question of what mechanisms might allow minibrains to perform apparently complex spatial recognition tasks like face recognition.

Here we asked whether honeybees can learn to classify visual stimuli that are constituted by the same visual features in face-like vs. non-face like categories. We used standardized stimuli commonly employed in primate and human visual research and we analyzed the processing mechanisms, *sensu* Maurer et al. (2002), used by the bees to solve the visual discriminations proposed in our experiments.

Material and methods

Experimental setup and procedure

Y-maze

In Experiments 1 to 3, free-flying honeybees, *Apis mellifera*, were individually trained to collect sucrose solution on visual targets presented on the back walls of a Y-maze (Giurfa et al., 1996). Only one honeybee marked individually with a color spot on the thorax was present at a time.

The maze was covered by an ultraviolet-transparent Plexiglas ceiling to ensure the presence of natural daylight. The entrance of the maze led to a decision chamber, where the honeybee could choose between the two arms of the maze. Each arm was 40x20x20 cm (length x height x width). Visual targets (20x20 cm) were black-and-white parameterized line drawings presented vertically on the back walls of both arms and were placed at a distance of 15 cm from the decision chamber. They subtended thus a visual angle maximum of 67° to the center of decision chamber. One of the two stimuli was rewarded with 50% (weight/weight) sucrose solution while the other was non-rewarded. Sucrose solution was delivered by means of a transparent micropipette 6 mm in diameter located in the center of the stimulus. The micropipette was undetectable to the bees from the decision chamber and did not provide a sucrose-predicting cue as the non-rewarded stimulus presented a similar but empty micropipette in its center.

During training the side of the rewarded stimulus (left or right) was interchanged following a pseudorandom sequence in order to avoid positional (side) learning. If the bee chose the rewarded stimulus, it could drink sucrose solution *ad libitum*. When it chose the non-rewarded stimulus, it was gently tossed away from the maze such that it had to re-enter it to get the sucrose solution. In such cases, only the first incorrect choice was recorded. After training, transfer tests with different non-rewarded stimuli were performed. Such stimuli were novel to the bees as they were never used during the training. Contacts with the surface of the patterns were counted for 1 minute. The choice proportion for each of the two stimuli was calculated. Each test was done twice, interchanging the sides of the patterns to control for side preferences. Refreshing trials, in which the training patterns were represented and the animal

got reward on the appropriate ones, were intermingled among the tests to ensure motivation for the subsequent test.

Rotating screen

In Experiment 4, free-flying bees were trained and tested with visual targets presented on a rotating grey screen which was 50 cm in diameter (Dyer et al., 2005). The screen was located outdoors and was therefore illuminated by natural daylight. Four visual targets were presented at different, interchangeable positions on the screen. Visual targets were 6x8 cm achromatic photographs presented vertically. At the base of each target a small platform allowed the bee to land. Two correct landing positions were rewarded with a drop of sucrose solution 30% (weight/weight) placed on the platform while the two alternative positions presented a drop of quinine solution 0.012%. Thus, the presence of a liquid drop could not be used by the bees to discriminate correct from incorrect targets. A choice was recorded whenever the bee touched a landing platform. When the bee landed on a correct target it could drink the sucrose solution (see Dyer et al., 2005 for details). When, on the contrary, it landed on the incorrect target, it experienced the quinine solution. Between foraging bouts, landing platforms and stimuli were cleaned with 30% ethanol. After training, the bee experienced a non-rewarded test in which fresh stimuli were presented. Landings on the non rewarded stimuli were counted until the bee flew more than one meter away from the screen. A minimum of 20 landings were counted for each test and the test ended when the bee made 30 choices or after 5 minutes.

Refreshing trials, in which the training patterns were presented again and the animal got reward on the appropriate ones, were intermingled between the tests to ensure motivation for the subsequent test.

Experiment 1

In a first experiment, we trained bees with face-like stimuli (F1 to F6) and/or non-face like stimuli (NF1 to NF6) (Fig. 1) presented in a Y-maze. Face-like stimuli consisted of parameterized line drawings presenting the main features constitutive of a face (eyes, nose and mouth). Such features could be varied systematically in order to create different face-like alternatives. Non-face like stimuli (NF1 to NF6) presented the same features in a scrambled way so that they exhibited no common configuration. Stimuli were printed on white paper with a high-resolution laser printer. Similar stimuli are commonly used in primate and Human visual research (e.g. Sigala and Logothetis, 2002) as they allow independent variation of

dimensions like mouth or nose length, interocular distance, etc. Each element (bar or disc) subtended a minimum visual angle of 8° while the global stimuli subtended a visual angle between 25° and 48° depending of the stimulus. The stimuli were therefore perfectly resolvable to the bees' eyes. We first verified that bees were able to distinguish stimuli belonging to the same category, face or non-face (i.e. *within-class discrimination*) after 48-trial training (e.g. F4 vs. F6 in the face-like class and NF3 vs. NF5 in the non-face class). Each discrimination experiment was balanced as it involved two groups of bees: in one of them, one stimulus was rewarded and the other stimulus was non-rewarded while in the other group the stimulus contingencies were reversed. After training, tests with non rewarded stimuli were performed.

We then studied whether bees learn to classify face-like vs. non-face like stimuli (i.e. *between-class discrimination*). We trained bees with 5 pairs of F vs. NF stimuli (Fig. 1), which were presented in a random succession during 48 trials. Experiments were balanced as half of the bees were rewarded with sucrose on the F stimuli while the other half was rewarded on the NF stimuli. The continuous alteration of the stimuli precluded that bees memorized a single stimulus pair. We determined whether bees extract the common configuration underlying the rewarded patterns (e.g. F or NF) and transfer appropriately their choice to a test pair of F vs. NF stimuli that were never used during the training (6th pair) and that did not present sucrose reward. Performance in such transfer tests should thus reveal if bees possess the capacity to build generic face vs. non-face categories.

Four kinds of transfer tests were performed: 1) in a *first transfer test*, bees were confronted with a novel pair of F vs. NF stimuli; bees trained to faces should transfer their choice to the novel F stimulus while bees trained to non-faces should choose the novel NF stimulus; 2) in a *second transfer test*, bees were confronted with an ambiguous situation as they had to choose between a novel F stimulus and a novel NF stimulus in which scrambled features presented the spatial configuration of a face; this test should reveal if bees focus on the configuration irrespective of its content or if they expect specific features at the appropriate position; 3) in a *third transfer test*, bees had to choose between a novel face-like stimulus and the same image rotated by 180° (i.e. upside-down); bees trained to faces should choose the novel face configuration while bees trained to non-faces should choose the inverted face as an example of non-face stimulus; this test allows ruling out bilateral symmetry as the cue predicting pattern reward given that both test stimuli are perfectly

symmetric; 4) finally, in a *fourth transfer test*, bees were presented with the inverted face vs. a novel, scrambled non-face like stimulus. If bees classify novel stimuli in the face vs. the non-face categories, random choice should be expected in both bees trained with F (no test stimuli would have a face configuration) and bees trained with NF stimuli (both test stimuli would belong to the non-face category).

To control for potential effects of the set up used, the same experiment were conducted using the rotation screen.

Experiment 2

In a further experiment performed in the Y-maze, we tested whether bees used the face configuration or low-level features to classify stimuli in the appropriate category. Features like the centre of gravity of the figures (COG; Ernst and Heisenberg, 1999), the main visual angle subtended by a visual pattern to the bee's decision point in the maze (Horridge et al., 1992; in our case the decision point was the centre of the triangular imaginary space between both arms of the maze) and the position of the eyes (two dots at the top) may be used as predictive cues allowing category discrimination without the necessity of configuration learning (COG: F stimuli: 8.9 ± 0.7 cm, NF stimuli: 10.2 ± 0.5 cm; Mann Whitney test: $Z_5 = 1.92$, $p = 0.055$; visual angle: F stimuli: $32.4 \pm 2.6^\circ$; NF stimuli: $43.6 \pm 1.6^\circ$; $Z_5 = 2.56$, $p < 0.01$).

To control for this possibility, we trained bees to categorize face vs. non-face stimuli following the procedure of the previous experiment. Given that the previous experiment did not show differences in performance between bees trained to choose faces and those trained to choose non-faces, we analyzed the performance of bees trained to face-like stimuli only. After training, we performed two tests with novel stimuli (Fig. 4). In one these test, bees were confronted with F6 (not used during the training) vs. a variant of F6 in which mouth and nose were swapped (F6'). If bees use only the position of the eyes (two dots on the top) to classify stimuli, random choice should be expected in this test. In the other test, the same bees were presented with a rough drawn stimulus (RD) versus F6' used in the previous test (mouth and nose swapped). The RD stimulus was designed in such a way that it had a COG value similar to those of the non-face stimuli (10.8 cm) while F6' had a COG close to those of the face stimuli (9.4 cm) despite not presenting a face configuration. Thus, if bees used the COG, they should prefer F6' to RD, even if RD corresponds better to the face category than F6'. Moreover, F6' and RD subtended the same visual angle to the decision point of the maze (39.4°) so that this feature could not be used as predictive cue. In this case, a random choice

should be expected if bees base their choice on this cue. Finally, a fast-Fourier analysis (Zhang et al., 2004; Dyer et al. 2008) showed that the spatial frequency energy distribution of RD differed widely from that of all the stimuli used during training. Thus, bees should always prefer F6' if they base their choice on this cue.

Experiment 3

In this experiment, we studied the effect of enriching or impoverishing the face-like configuration learned. In one case, bees were trained in the Y-maze to distinguish two simple F stimuli that consisted of the parameterized line drawings (F1 vs. F4 in Fig. 1; see also Fig. 5a, 'learning test') and were afterwards tested with the same configurations superimposed onto real face layouts derived from achromatic photographs of human faces (See Fig. 5a, 'transfer test'). Such photographs were obtained from standardized psychophysics tests of human visual recognition (Warrington, 1996) and they subtended a visual angle of 67° from the center of the decision chamber. In the other case, the reverse protocol was conducted, i.e. bees were trained to discriminate between F1 vs. F4 configurations superimposed onto real face layouts and then tested with the line drawing stimuli (Fig 5b). In each experiment, one half of the bees were rewarded on F1 (superimposed or not onto a real face layout) while the other half was rewarded on F4 so that experiments were balanced.

Experiment 4

We performed two further transfer experiments using photographs of real human faces to determine whether findings on parameterized line stimuli also apply to the recognition of more complex pictures. Pictures of human faces were obtained from standardized psychophysics tests of human visual recognition (Warrington, 1996). They were presented on a circular screen apparatus, which could be rotated to change the position of the figures (Dyer et al., 2005).

Bees were first trained to distinguish two photographs of real human faces (Fig. 6, 'learning test') and then tested with altered versions of these photographs. For one group of bees, the outer features (hair and ears) were removed (Fig. 6, 'transfer test 1'). For another group, the inner features (eyes, nose and mouth) were removed (Fig. 6, 'transfer test 2'). For the last group, the photographs were scrambled along the vertical axis (see Fig. 6, 'transfer test 3'). The scrambling method we used exactly matches the method used by Collishaw and Hole (2000) and reorders the spatial arrangement of the major human facial features (hair,

eyes, nose, mouth and chin) without causing a disruption to any of the particular features that bees could use to solve the recognition task in the transfer test.

Within each group, half of the bees were rewarded on one face (F1: left face on Fig. 6) while the other half was rewarded on the other face (F2: right face on Fig. 6) so that experiments were balanced.

Statistics

In all cases we checked for normality using the Lilliefors test. When necessary and depending on the test to be used, data were subjected to an arcsine transformation in order to normalize them. The performance of balanced groups within each experiment (e.g. group trained to discriminate face-like stimuli rewarded from non-face like stimuli non-rewarded vs. group trained with the reversed contingency) was compared by means of a 2-factorial ANOVA of repeated measures in which the groups constituted one factor and the test stimuli the other factor. For each individual bee, we calculated the proportion of correct choices per test (i.e. a single value per bee). Performance in a given test was therefore assessed through a sample of such values. This situation allowed a one-sample approach in which our null hypothesis was that the proportion of correct choices in the test considered was not different from a theoretical value of 50%. Such a hypothesis was evaluated by means of a one-sample t-test. In all cases the alpha level was 0.05.

Results

Experiment 1

We first studied within-class discrimination to ensure that transfer performances, if any, are not due to a lack of discrimination. Bees differentiated between F stimuli on one hand and between NF stimuli on the other hand, thus showing that within-class discrimination was possible. As an example, Fig. 2 shows discrimination for the F pair (F4 vs. F6) and the NF pair (NF3 vs. NF5) in which stimuli were more similar and thus in principle difficult to distinguish (see Fig. 1).

In the task F4 vs. F6 (face-like stimuli), discrimination was the same irrespective of which stimulus was rewarded (two sample t-test, $t_6=1.97$, $p=0.10$) so that results were pooled and presented as a single black bar (Fig. 2). Bees chose the correct F stimulus in the absence

of sucrose reward in $68.7 \pm 3.1\%$ of the cases (mean \pm S.E.; $n=8$ bees; one sample t-test against a 50% random choice, $t_7=5.72$, $p<0.001$), thus showing a capacity to distinguish between closest face-like figures. A similar conclusion applies to non-face like stimuli. In the task NF3 vs. NF5 (non-face like stimuli), discrimination did not depend on which stimulus was rewarded ($t_6=0.08$, $p=0.94$) so that results were pooled and presented as a single white bar (Fig. 2). In this case, bees preferred the correct NF stimulus in $67.7 \pm 2.0\%$ of the cases ($n = 8$ bees; $t_7=8.22$, $p<0.001$), thus showing a capacity to discriminate between highly similar non-face like stimuli.

Bees were then trained to classify face-like vs. non-face like stimuli in a Y-maze with 5 pairs of F vs. NF stimuli (Fig. 1), which were presented in a random succession Figure 3 shows the performance during the four transfer tests performed after training (*black bars*: bees trained on F stimuli; *white bars*: bees trained on NF stimuli).

In the **first transfer test**, bees of both groups (F-trained and NF-trained) transferred appropriately their choice to the corresponding stimulus of the novel pair. Thus, bees trained to faces chose the novel face-like configuration ($78.4 \pm 7.3\%$ correct choices; $n=6$; black bar in Fig. 3) while bees trained to non-faces chose the novel non-face like configuration ($64.3 \pm 9.8\%$ correct choices; $n=6$; white bar in Fig. 3). There were no significant differences in transfer performances between these two groups ($t_{10}=1.41$, $p=0.19$) so that data were pooled. Pooled performance was significantly different from a random choice ($71.3 \pm 6.2\%$ correct choices; $t_{11}=3.14$, $p<0.01$), thus showing that bees extracted the correct configuration irrespectively of the configuration trained.

In the **second transfer test**, bees rewarded on F stimuli transferred their choice appropriately to the novel F stimulus ($79.5 \pm 2.8\%$ correct choices; $n=6$; black bar in Fig. 3) while bees rewarded on NF stimuli preferred the novel NF stimulus in which the wrong features occupied the correct places of the face array ($71.4 \pm 5.0\%$ correct choices; $n=6$; white bar in Fig. 3). There were no significant differences in transfer performances between the two groups of bees ($t_{10}=1.25$, $p=0.24$) so that their data were pooled. Pooled performance was significantly different from a random choice ($75.4 \pm 3.0\%$; $t_{11}=7.59$, $p<0.001$). These results show that neither did bees trained to faces confuse the novel face-like stimulus with the ambiguous alternative nor did bees trained to non-faces interpret the ambiguous stimulus as a face. In other words, in extracting a face configuration, bees assigned features to a specific

position so that if the spatial array was preserved but the position assigned to each feature was inappropriate, the stimulus was not recognized as belonging to the category learned.

In the **third transfer test**, bees rewarded on F stimuli chose the novel F configuration ($67.3 \pm 6.1\%$ correct choices; $n=6$; black bar in Fig. 3) while bees rewarded on NF stimuli preferred the inverted face ($74.2 \pm 6.6\%$ correct choices; $n=6$; white bar in Fig. 3). There were no significant differences in transfer performances between these two groups ($t_{10}=0.91$, $p=0.38$). Pooled performance was significantly different from a random choice ($70.8 \pm 4.4\%$ correct choices; $t_{11}=3.86$, $p<0.005$). These results indicate that bees lack rotational invariance as they do not treat an image and its 180° -rotated version as equivalent. A rotated face-like configuration is therefore a non-face configuration, a result that excludes bilateral symmetry, distinctive of F stimuli, as the cue used to classify stimuli.

In the **fourth transfer test**, both groups of bees chose randomly between an inverted face-like stimulus and a novel non-face like stimulus with scrambled features. Bees rewarded on F stimuli exhibited a random level of choices for the inverted face ($50.6 \pm 2.8\%$ choices; $n=5$; black bar in Fig. 3) while bees rewarded on NF stimuli exhibited a similar performance for the novel non face-like stimulus ($51.1 \pm 2.9\%$ choices; $n=5$; white bar in Fig. 3). There were no significant differences in transfer performances between these two groups ($t_8=0.40$, $p=0.70$) so that their data were pooled. Pooled performance did not differ from a random choice (mean choice of the inverted face: $49.8 \pm 1.9\%$, $t_9=0.12$, $p=0.91$). These results show, therefore, that bees trained to classify faces did not interpret a rotated face configuration as a face, thus reaffirming the lack of rotational invariance, and that bees trained to classify non-face like stimuli treated a rotated face and a scrambled version of a face as equivalent. These performances reveal the use of specific configurations (i.e. face-like) in which the use of symmetry can be excluded.

We repeated this experiment by using the rotating screen to control for potential effects of the set up used. The results were not significantly different from those obtained in the same experiment performed in the Y-maze (paired sample t-test. First transfer test: $t_9=0.28$, $p=0.79$; second transfer test: $t_9=2.03$, $p=0.07$; third transfer test: $t_9=0.08$, $p=0.94$; the fourth transfer test was not performed). We conclude, therefore, that configural processing is a strategy employed by honeybees to recognize visual targets, which is independent of the experimental set-up used.

Experiment 2

This experiment was conceived to determine whether bees solved the previous task using low-level cues like the center of gravity of the stimuli (COG; Ernst and Heisenberg, 1999), the visual angle subtended by their main axis (Horridge et al., 1992), their spatial frequency (Horridge, 1997) or the position of the two dots typical of face-like stimuli.

Bees trained to face-like stimuli were confronted with F6 (not used during the training) vs. a variant of F6 (F6') in which mouth and nose were swapped (Fig. 4, left). Bees significantly preferred F6 to F6' ($n = 8$; black bar in Fig. 4: 72.9 ± 8.7 correct choices; $t_7=6.87$, $p<0.001$), thus showing that they did not only use the position of the eyes (two dots on the top) to classify stimuli.

In a further test, the same bees were presented with a rough drawn stimulus (RD) versus F6' (Fig. 4, right). Bees significantly preferred RD to F6' ($n = 8$; white bar in Fig. 4: 73.7 ± 8.3 correct choices; $t_7=7.44$, $p<0.001$), thus showing that neither COG (predicting preference for F6'), nor the visual angle subtended by the stimuli to the decision point of the maze (39.4° in both cases), nor spatial frequency energy distribution (which predicted preference for F6') accounted for stimulus choice. Stimulus configuration was therefore the main information used by the bees to achieve discriminations.

Experiment 3

To what extent basic face-like configurations like the ones used in the previous experiments can be recognized as such if additional visual cues pertaining to real human faces are added to them? And vice versa, can bees trained on a simple face-like configuration enriched by real human-face features recognize the correct configuration after depriving it of such features? To answer these questions, we performed two series of experiments, testing the effect of enriching or impoverishing the face-like configuration learned.

In the first series, bees trained with the parameterized line drawings alone discriminate very well between the two stimuli during the learning test. Bees rewarded on F1 ($n=9$; left black bar in Fig. 5a) reached $63.9 \pm 4.3\%$ correct choices while bees rewarded on F4 ($n=9$; left white bar in Fig. 5a) reached $64.9 \pm 2.2\%$ correct choices. Both performances did not differ significantly ($t_{16}=0.02$, $p=0.98$) so that they could be pooled. The resulting performance ($64.4 \pm 2.3\%$ correct choices) was significantly different from a random choice ($t_{17}=5.46$,

$p < 0.001$), thus showing that bees learned to recognize their respectively trained face-like configuration. In the transfer test, both groups of bees chose the correct face-like configuration despite being enriched by a human face background (Fig. 5a); bees originally rewarded on F1 chose preferentially the enriched version of F1 (66.3 ± 3.4 ; right black bar in Fig. 5a) while bees trained on F4 preferred the enriched version of F4 ($65.6 \pm 2.0\%$; right white bar in Fig. 5a). There were no significant differences between groups ($t_{16}=0.23$, $p=0.82$). The pooled performance was significantly different from a random choice (66.0 ± 1.9 % of correct choices; $t_{17}=7.93$, $p < 0.001$), thus showing that adding a visual background did not alter the recognition of the configuration learnt.

In the second series of experiments, bees were first trained with the parameterized line drawings (F1 or F4) superimposed onto the real face layouts and then tested with impoverished stimuli presenting only the parameterized line drawings. Training was successful in both groups of bees. Bees rewarded on the enriched F1 reached a level of $66.1 \pm 2.8\%$ correct choices (left black bar in Fig. 5b) while bees rewarded on the enriched F4 performed at $68.2 \pm 3.1\%$ correct choices (left white bar in Fig. 5b). There were no significant differences between these groups ($t_{16}=0.53$, $p=0.60$; Fig 5b). The pooled performance (67.2 ± 2.0 % of correct choices) differed significantly from a random choice ($t_{17}=7.94$, $p < 0.001$), and was similar to that obtained in the learning tests of Fig. 5a (two samples t-test, $t_{34}=0.76$, $p=0.45$). In the transfer tests, both bees trained on F1 ($72.0 \pm 3.5\%$; right black bar in Fig. 5b) and on F4 ($68.9 \pm 3.7\%$; right white bar in Fig. 5b) transferred correctly their choice to the impoverished F1 and F4 configurations (Fig. 5b) with comparable performances ($t_{16}=0.23$, $p=0.82$). The pooled choice level (70.4 ± 2.5 %) was significantly different from a random choice ($t_{17}=7.64$, $p < 0.001$) and did not differ from the transfer performance found in Fig. 5a ($t_{34}=1.47$, $p=0.15$). Transfer in both directions was, therefore, equally possible, thus showing that enriching or impoverishing a simplified face-like configuration by adding or suppressing visual cues from real human faces did not affect visual recognition in bees.

Experiment 4

Further experiments using real human photographs were performed to determine whether findings on parameterized line stimuli apply to the recognition of complex pictures such as those of human faces. Bees were trained on the rotating screen to distinguish two photographs of real human faces (Fig. 6, 'learning test') and then tested with altered versions of these photographs. Half of the bees were rewarded on one face (F1: left face on Fig. 6) while the

other half was rewarded on the other face (F2: right face on Fig. 6) so that experiments were balanced.

Bees learned to discriminate the two training stimuli. In the learning test, bees rewarded on F1 reached $74.0 \pm 1.0\%$ correct choices ($n=21$; black bar in Fig. 6, 'learning test') while bees rewarded on F2 reached $78.0 \pm 1.1\%$ correct choices ($n=21$; white bar in Fig. 6, 'learning test'). Both performances did not differ significantly ($t_{40}=0.41$, $p=0.068$) so that they could be pooled. The resulting performance ($76.0 \pm 1.1\%$ correct choices) was significantly different from a random choice ($t_{41}=17.02$, $p<0.001$), thus showing that bees learned to recognize the human-face photograph on which they were rewarded.

In the transfer test in which the outer features (hair and ears) were removed (Fig. 6, 'transfer test 1'), bees originally rewarded on F1 chose preferentially the inner part of F1 (60.0 ± 2.4 ; black bar in Fig. 6, 'transfer test 1') while bees trained on F2 preferred the inner part of F2 ($60.7 \pm 3.0\%$; white bar in Fig. 6, 'transfer test 1'). There were no significant differences between the performances of these two groups ($t_{12}=0.20$, $p=0.85$) so that results could be pooled. The resulting performance was significantly different from a random choice ($60.4 \pm 1.9\%$ of correct choices; $t_{13}=5.47$, $p<0.001$), thus showing that the inner parts of the faces were used by the bees to discriminate between the two human- face photographs. However, discrimination was significantly poorer than that obtained in the learning test with the complete photographs (paired samples t-test, $t_{13}=7.86$, $p<0.001$).

In the transfer test in which the inner features (eyes, nose and mouth) were removed (Fig. 6, 'transfer test 2'), bees trained on F1 significantly preferred the photograph presenting the outer parts of F1 ($67.9 \pm 1.8\%$; black bar in Fig. 6, 'transfer test 2') while bees trained on F2 significantly preferred the photograph presenting the outer parts of F2 ($70.7 \pm 1.7\%$; white bar in Fig. 6, 'transfer test 2'). Performance was similar in both cases ($t_{12}=1.14$, $p=0.28$). The pooled choice level ($69.3 \pm 1.3\%$) was significantly different from a random choice ($t_{13}=10.26$, $p<0.001$). However, discrimination was again significantly poorer than that obtained in the learning tests with the complete photographs ($t_{13}=2.97$, $p=0.01$). In addition, recognition based on the outer features of the faces was significantly better than that based on the inner features ($t_{26}=3.95$, $p<0.001$; Fig 6, 'transfer tests 1 and 2'). This experiment shows therefore that bees use both internal and external features of human-face photographs to

discriminate between them, and that both kinds of features are bound together in a configural representation.

Finally in the transfer test in which the photographs were scrambled along the vertical axis (see Fig. 6, 'transfer test 3'), both groups of bees failed to choose the correct scrambled face (Fig. 6, 'transfer test 3'). Bees originally rewarded on F1 chose the scrambled image of F1 in 51.6 ± 2.5 of the cases (black bar in Fig. 6, 'transfer test 3') while bees trained on F2 chose the scrambled image of F2 in $50.0 \pm 3.1\%$ of the cases (white bar in Fig. 6, 'transfer test 3'). There were no significant differences between groups ($t_{12}=0.39$, $p=0.70$) so that their results could be pooled. The resulting performance (50.8 ± 1.9 % of correct choices) was not different from a random choice ($t_{13}=0.42$, $p=0.68$). These results show that scrambling the photographs completely disrupts face recognition and suggests that bees employ holistic processing (as defined in Maurer et al., 2002) to discriminate the photographs. Indeed this manipulation alters the configuration of the face but not the features (Collishaw and Hole, 2000). The use of the average picture brightness as a discriminative low-level cue can be discarded given that it was the same in the scrambled photographs.

Discussion

The present work shows that configural visual processing is present in an insect and underlies its learning and classification of complex images such as face-like stimuli. Bees succeeded in categorizing face-like vs. non-face like stimuli using configural information and not only isolated features, low-level cues such as the symmetry, center of gravity, visual angle, spatial frequency or background cues present in face-like stimuli. Whether bees can use configural information to recognize complex visual stimuli remained an important question to be answered as it has been argued that bees can only use simple, unconnected features for object recognition (Horridge, 2009). Our findings exclude this possibility since stimulus recognition was possible even when low level cues were removed or confusing (Figs. 4 and 5), and because recognition was not possible in the case of face-like stimuli in which the first-order relationship between features was slightly modified (Fig. 3). Moreover, pictures of real faces that contained all cues presented in a scrambled arrangement were not recognized as the training stimulus given that the original configuration was disrupted (Fig. 6).

Following differential conditioning bees thus looked not for isolated features but for a specific configuration in which each feature had to be located in an appropriate relationship with respect to the others. In that sense, their performance is consistent with Maurer et al.'s (2002) first level of configural processing termed '*sensitivity to first-order relations*', in which basic relationships between features are taken into account. The second level proposed by Maurer et al. (2002), '*holistic processing*', constitutes an appealing framework to interpret the bees' performance, but so far the evidence obtained is contradictory and does not allow concluding that such a processing form is available in bees. Holistic processing implies that features are bound together into a gestalt, which is more than the simple sum of its components. From this perspective, it corresponds to Pearce's configural theories (1987; 1994) so that it can be predicted that partial suppression of one or more components should severely affect gestalt recognition. This is not what we observed in Experiment 3 (Fig. 5b) in which bees were trained with the parameterized line drawings superimposed onto the real face layouts and then tested with impoverished stimuli presenting only the parameterized line drawings. In this case, suppression of the real face background did not affect recognition. On the other hand, experiments in which pictures of real human faces were used (see Fig. 6) yielded evidence consistent with holistic processing as suppressing external or internal features of the faces induced a significant decrease in recognition. These contradictory results may be explained by differences in salience and/or similarity between components, which may affect the capacity of configuring elements into a compound (Deisig et al., 2002). The fact that more salient cues may be easier to extract to build a configured representation may explain why bees did not exhibit a decay in performance when the real human face background was suppressed, leaving the parameterized line configuration alone (Fig. 5b). In this case the high contrast provided by the black features could promote focusing on the simplified configuration. On the contrary, when real human faces were deprived of part of their features (Fig. 6), a decay in performance was observed probably due to the absence of highly salient cues in this case. More experiments are, therefore, necessary to determine whether or not holistic processing occurs in the framework of complex visual stimuli recognition by honeybees. Finally, no evidence allows discussing Maurer et al.'s third level of processing, '*sensitivity to second-order relationships*', in which distances among features are perceived and used for discrimination. These results support, therefore, the notion that configural processing in bees reaches at least the '*sensitivity to first-order relations*' level,

based on extracting the relevant, predictive features common to a given category, and combining them in a general representation.

Such a capacity allows constructing a high number of different representations on the basis of a limited number of features, thus providing the basis for complex categorization abilities. Visual categorization in bees has been shown in several independent experiments (see Benard et al., 2006 for review). Such experiments focused on single-feature categorization and showed that bees transferred their choice to novel stimuli presenting the predictive feature of a category. Recent work shows that bees can construct complex image representations following extended differential conditioning (Stach et al., 2004; Stach and Giurfa, 2005; but see Horridge, 2009). Here we move a step further by showing that such a task can involve various, different features as long as these preserve the spatial relationship defining the category. This ability may underlie categorization of natural objects in classes such as radial flowers, plant stems or landscapes as shown in free-flying honeybees (Zhang et al., 2004) and may thus be very useful in the bee life to forage efficiently in a complex visual environment.

A critical feature in visual discrimination experiments in bees is the visual angle at which targets to be discriminated are presented. Indeed, local or global processing may be promoted depending on how stimuli are perceived by the bees at the decision point in a Y maze (Zhang et al., 1992). In our parameterized-line drawing experiments the visual targets subtended a mean visual angle of 38° to the bee's eye when it had to decide between visual alternatives. This angle was chosen to ensure perception of a figure as a whole. Given the low spatial resolution of the insect compound eye, focusing on global configurations may be an appropriate strategy before closing-up to a visual target. Indeed, while spatial details are still unclear at farther distances, basic configurations are preserved and may be perceived in low-frequency visual patterns. For example, honeybees are able to learn images of natural shapes like trees, which could at a distance assist in navigational tasks in complex visual environments like forests (Dyer et al., 2008). It is interesting that bees did not choose to use low level cues like symmetry (Fig. 3), COG, spatial frequency distribution (Fig. 4), or brightness (Fig. 5) to recognize the stimuli in the current study. This may be due to configural cues offering more robust information on which to make decisions in complex environments where some low-level cues like brightness are often highly variable. Note also that individual features were resolvable in our stimuli so that it was not the lack of discrimination (due for

instance, to a lack of visual resolution) which may have lead to prioritizing configural information.

Our results show that a non-specialized brain can learn to do this complex recognition task using a mechanism of configural processing, despite the absence of brain specialized areas such as the fusiform face area (Sergent et al., 1992; Kanwisher et al., 1997), or its homologous region in the macaque brain (Tsao et al., 2006), which have been proposed as dedicated modules for the recognition of faces. This result has significant implications for understanding how larger brains may learn face processing tasks if specialised neural circuitry is not available (Pierce et al., 2001; Koshino et al., 2008). We maintain nevertheless that face-like stimulus recognition is, in our experiments, an artificial situation far from the biological background of visual recognition tasks to which bees are naturally confronted. The various simplified and complex stimuli used in our experiments were simply uncommon flowers on which they were rewarded with sucrose solution as the equivalent of nectar and which they could recognize using configural processing. Although they could use a similar processing to distinguish between human faces, nothing prepares them to do so in evolutionary terms. The performance exhibited in our work underlines, nevertheless, that higher-order forms of visual processing and categorization of complex stimuli are not a prerogative of vertebrates. They are already present in more ‘simple’ brains, thus showing that simplicity refers to the number of neurons but not necessarily to the sophistication of performances that can be achieved with such a reduced number of neurons.

Acknowledgements

We thank two anonymous referees for comments and corrections. M. Giurfa and A. Avargues-Weber thank the French Research Council (CNRS) and the University Paul Sabatier for support. A. Avargues-Weber was supported by a Travelling Fellowship of The Journal of Experimental Biology and by the University Paul Sabatier (ATUPS fellowship). A. Dyer acknowledges the USAF AOARD, the Alexander von Humboldt Foundation and ARC DP0878968 for funding support.

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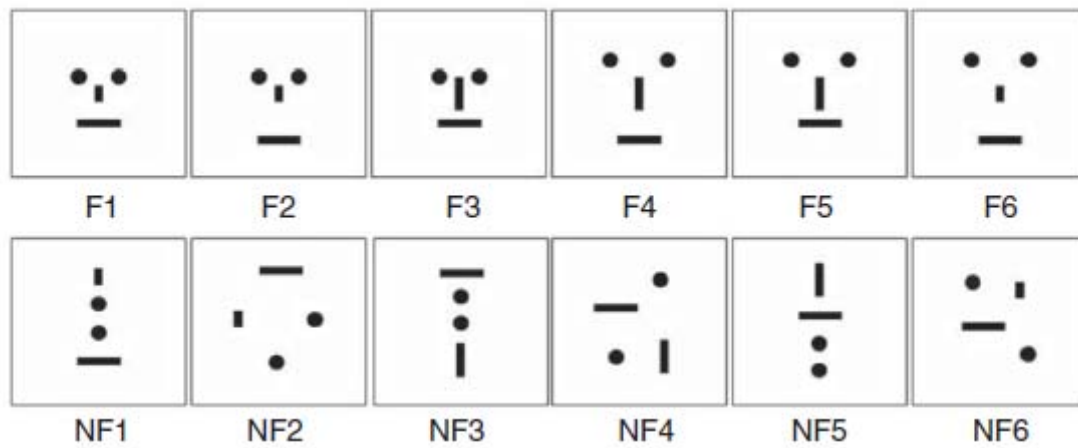


Figure 1: The six face-like (F1-F6) and six non-face like (NF1-NF6) stimuli used in Experiments 1 and 2. Both stimulus classes were made of the same elements arranged differently.

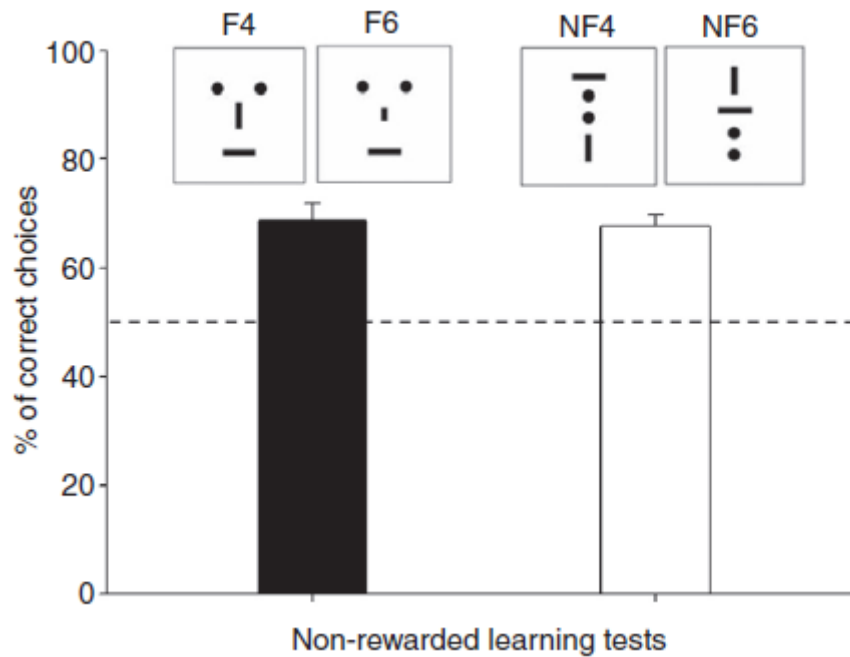


Figure 2: Examples of within-class discrimination performances (mean + SE; $n = 8$ for each bar) in non-rewarded tests. The black bar shows discrimination between highly similar F4 and F6 face-like stimuli; the white bar shows discrimination between NF3 and NF5 non-face like stimuli. Bars show pooled performances of two groups of bees trained with either stimulus. Both for face-like and non-face like stimuli, bees recognized the stimulus they were trained to.

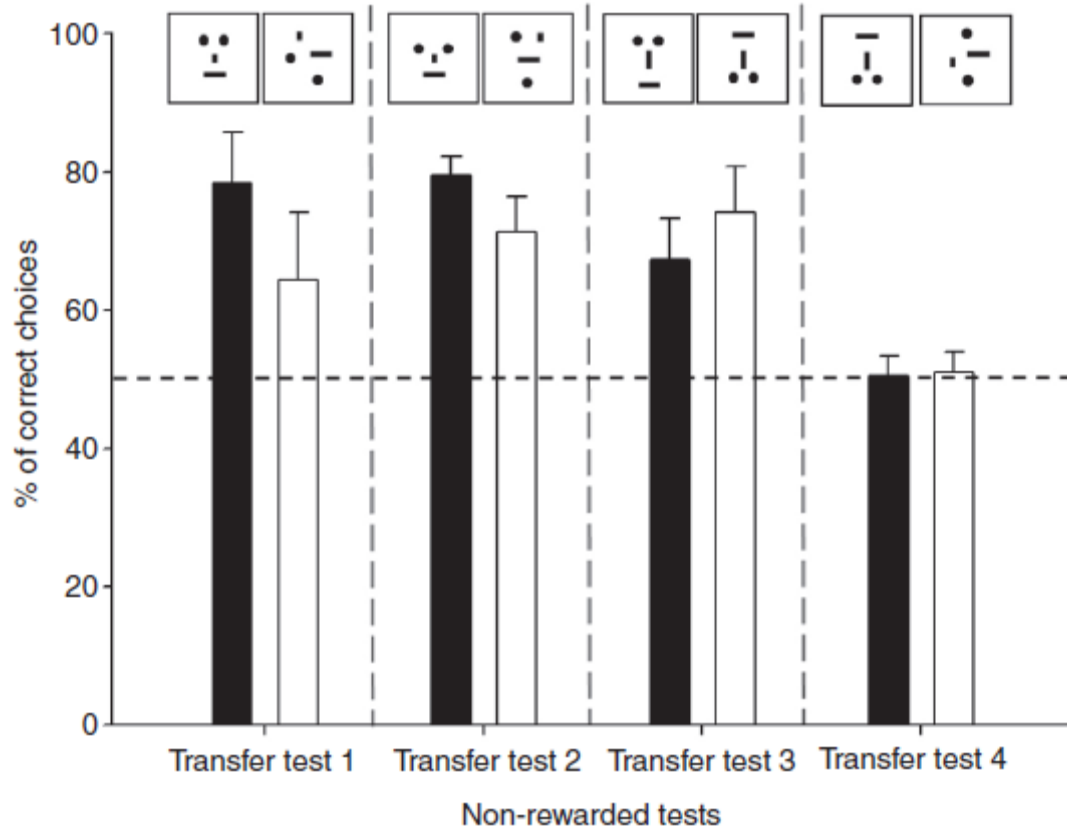


Figure 3: Performance (mean + SE; $n = 6$ for each bar) in non-rewarded transfer tests. Black bars represent the performance of bees rewarded on face-like stimuli while white bars represent the performance of bees rewarded on non-face like stimuli. In the *first transfer test*, bees transferred appropriately their choice to the novel stimulus (F or NF) belonging to the category they were trained to. In the *second transfer test*, bees treated the novel, ambiguous NF stimulus (presenting scrambled features in the spatial configuration of a face) as a non-face stimulus. In the *third transfer test*, bees did not treat a face-like stimulus and its 180°-rotated version as equivalent, thus showing a lack of rotational invariance. A rotated face-like configuration was treated as a non-face. In the *fourth transfer test*, bees trained to classify faces did not interpret a rotated face configuration as a face, thus reaffirming the lack of rotational invariance, while bees trained to classify non-face like stimuli treated a rotated face and a scrambled version of a face as equivalent.

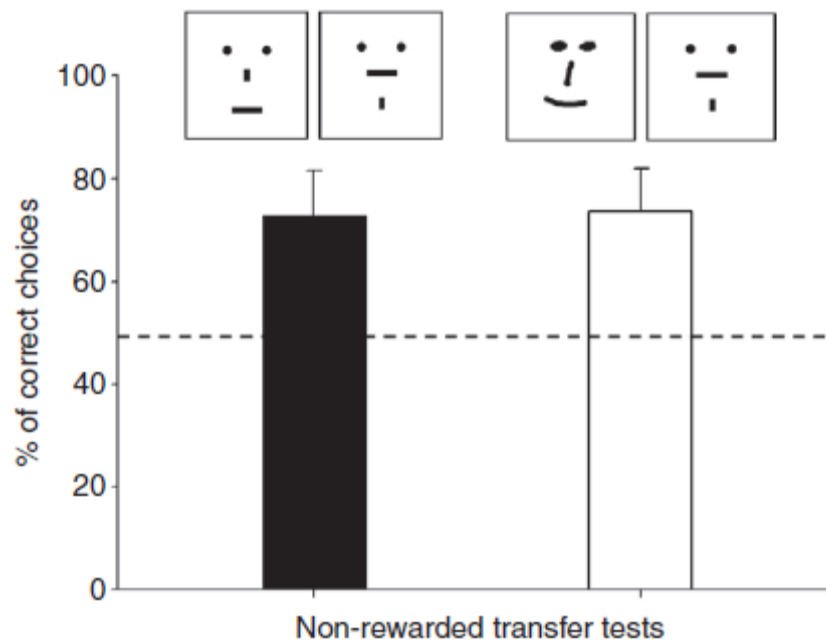


Figure 4: Performance (mean + SE; $n = 8$ for each bar) in non-rewarded transfer tests designed to control the implication of low level cues such as the position of the dots (black bar: percentage of choice for the face-like stimulus), the center of gravity (COG), the main visual angle or the spatial frequency distribution. In these experiments bees were trained to choose human-like faces. In both transfer tests, bees showed a preference for the novel stimulus that was closer to the face-like category, irrespective of the low-level cue considered (black bar: % of choices for the face-like stimulus F6; both the top position of the eyes and bilateral symmetry were excluded as predictive cues of reward; white bar: % of choices for the rough drawing face-like stimulus, RD; COG, visual angle and spatial frequency distribution did not mediate stimulus preference) (see material and methods for more details).

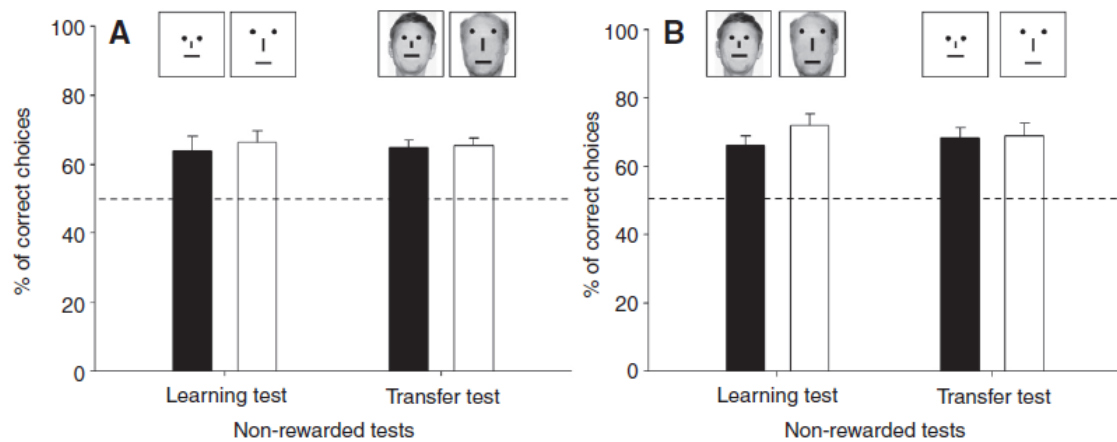


Figure 5: Performance (mean + SE; $n = 9$ for each bar) in non rewarded transfer tests. **a)** Bees were trained to distinguish F1 and F4 stimuli. Black bars represent the results of bees rewarded on F1 while white bars represent the results of bees rewarded on F4. Bees discriminated between the two trained configurations (learning test) and their recognition was not altered by enriching the original training stimuli with real human-face features (transfer test). **b)** Bees were trained to distinguish enriched F1 from enriched F4 stimuli. Black bars represent the performance of bees rewarded on enriched F1 while white bars represent the performance of bees rewarded on F4. Bees discriminated between the two enriched face-like stimuli (learning test) and their recognition ability was not affected when the real face background was removed.

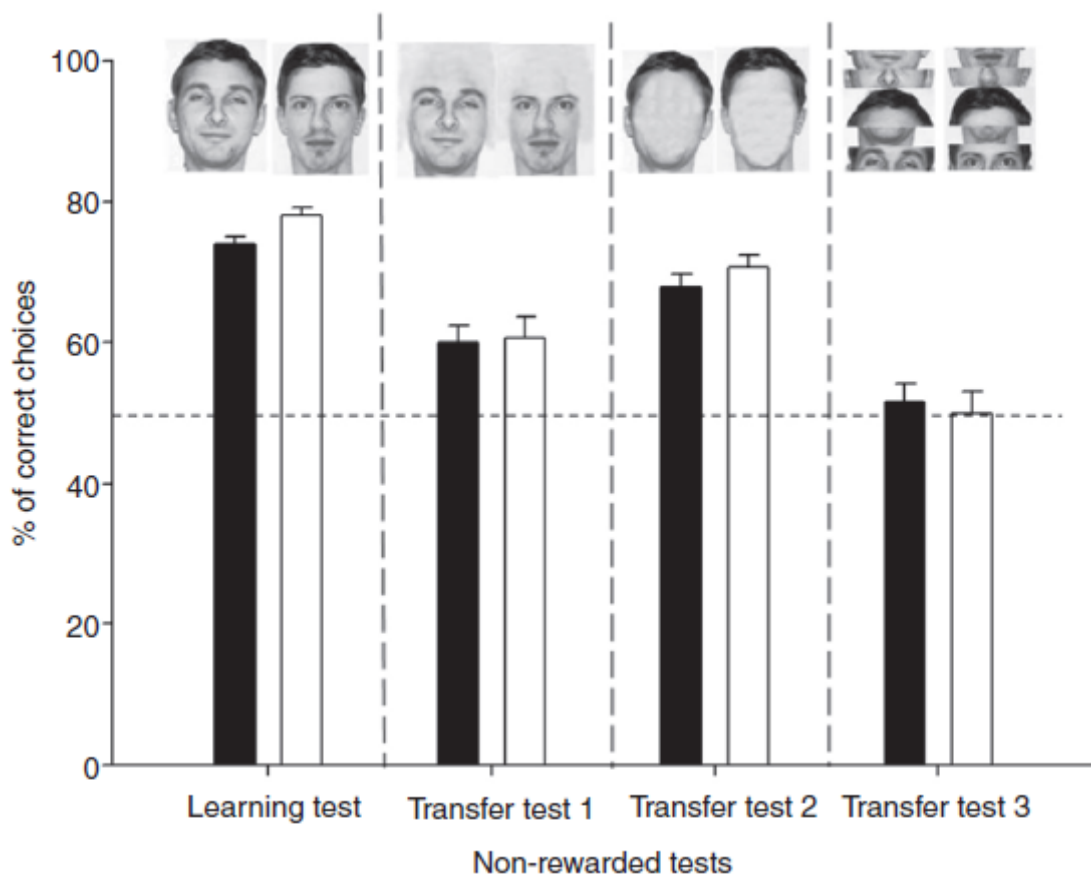


Figure 6: Performance (mean + SE; $n = 21$ for each learning test bar and $n = 7$ for each transfer test bar) in non rewarded tests. Black bars represent the results of bees rewarded on the F1 face photograph while white bars represent the results of bees rewarded on the F2 face photograph. Bees discriminated between the two photographs of human faces (learning test) and also recognized the appropriate face when only the inner features (transfer test 1) or the outer features were available (transfer test 2). In these two latter cases, performance was significantly lower than that obtained with the complete faces (see text for statistics). Bees were also unable to recognize these faces when their features were scrambled along the vertical axis (transfer test 3).

Article III

Preference for global properties of hierarchical compound stimuli in the honeybee *Apis mellifera*

Avarguès-Weber A, Dyer AG, Giurfa M (en préparation)

Ce travail a été motivé par nos études précédentes démontrant la capacité des abeilles à traiter les relations entre les éléments d'une image et à les assembler en une configuration. Nous avons souhaité mieux comprendre la perception des configurations chez l'abeille en testant l'existence d'une prédominance potentielle d'un niveau de traitement (global ou local) des configurations spatiales par le système visuel de l'abeille.

Les abeilles ont été entraînées avec des stimuli composés, présentant plusieurs répétitions d'un symbole agencées de façon à décrire une forme géométrique globale (ex : des petits carrés disposés de façon à dessiner la forme d'un triangle). Ainsi, de tels stimuli peuvent être discriminés sur la base des symboles utilisés (information locale ; carré dans notre exemple) ou sur la base de la forme géométrique générée par la disposition des symboles (information globale ; triangle ici). Les abeilles ont été entraînées à associer une solution sucrée à un stimulus conditionné (CS+), présenté simultanément avec un autre stimulus non récompensé (CS-). Nous avons ensuite soumis les abeilles à une situation de choix conflictuelle : nous leur avons présenté un stimulus composé des symboles locaux du CS- mais disposés de façon à former la forme globale du CS+ face à un stimulus composé cette-fois-ci des éléments locaux du CS+ mais dont la disposition génère la forme globale du CS-. Les abeilles ont alors préféré utiliser la forme globale du CS+ plutôt que l'information locale pour faire leur choix. Ce résultat est maintenu si l'on diminue le nombre d'éléments composant la forme globale, ce qui rend sa perception plus difficile. Des tests de contrôle ont permis de vérifier que les deux niveaux d'information ont bien été perçus et mémorisés lors de l'entraînement, bien que la forme globale soit plus saillante pour les abeilles au vu du résultat du test précédent. Enfin, une dernière expérience a mis en évidence la capacité des abeilles à lier mentalement les symboles entre eux de façon à extrapoler la forme géométrique composée.

Ce résultat permet donc de penser que l'abeille analyse et utilise naturellement un niveau de traitement global de perception dans sa recherche de nourriture. Ceci remet en cause la spécificité humaine concernant une prédominance de la perception des configurations globales par rapport à la perception des détails avec de tels stimuli.

Global preference in the processing of hierarchical compound stimuli in honeybees

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Keywords:

Global/local processing – Hierarchical stimuli - Visual perception – Honeybee – *Apis mellifera*

Manuscript in preparation

Abstract

Honeybees (*Apis mellifera*) preference to use either global shape or local features of hierarchical visual stimuli when making choice was assessed. The results showed a robust saliency of global features despite a local elements density manipulation. Bees were able to resolve both level of perception thus showing abilities to discriminate geometric plain shapes (local feature) and geometric configurations (global feature). In addition, bees succeeded in interpolating the global shape generated by the binding of discrete local elements. These findings, which challenge a Human specificity, are consistent with theories on adaptive value of global perception when processing visual objects.

Introduction

The relation between whole object processing and perception of its parts is a key issue to understand object recognition by a visual system (Kirkpatrick, 2001; Peterson and Rhodes, 2003). In particular, do we integrate the entire object from its components or do we perceive the whole object prior to the analysis of its parts or details (Maurer et al., 2002; Peterson and Rhodes, 2003; Tanaka and Farah, 1993)? Using hierarchical compound stimuli is a classic approach to compare processing of both levels (Navon, 1977; Navon, 1981). Hierarchical stimuli present small identical *local* features arranged in a spatial configuration drawing a large *global* feature (see examples with geometric symbols in Figure 1A). Such artificial compound stimuli assure equivalence of meaning for both local and global information thus allowing processing comparison. In humans, several studies suggest a priority for perception of the global configurations (Fagot and Deruelle, 1997; Fagot and Tomonaga, 1999; Fagot and Tomonaga, 2001; Navon, 1977; Navon, 1981). People indeed show a global advantage as discrimination response time for global features is faster than for local ones. In addition, it is difficult to ignore the global information to focus on the local level (global-to-local interference), whereas the local information doesn't interfere with global feature recognition. Such a particularity of our visual system can be argued as adaptive considering that fast object classification relies on shape configuration perception rather than on details analysis (Biederman, 1987; Cave and Kosslyn, 1993; Maurer et al., 2002; Navon, 1977; Peterson and Rhodes, 2003).

Comparative studies, however, tend to show that this global precedence is specific to human vision (Cavoto and Cook, 2001; Fagot and Deruelle, 1997; Fagot and Tomonaga, 1999; Fagot and Tomonaga, 2001; Spinozzi et al., 2003). Indeed, non-human primates, show difficulty in linking elements together to perceive the global shape (Fagot and Tomonaga, 2001). As non-human primates share, in many aspects, a similar visual system than Humans, the global precedence human specificity may be mediated by cognitive processes. It has thus been proposed that demands on human vision of complex artificial forms, like symbols or text, have influenced the evolution of highly efficient global and local processing (Fagot and Tomonaga, 1999).

In this comparative context, investigating potential honeybee global or local precedence is of great value due to the evolutionary distance and the visual systems differences between bees and primates (Wehner, 1981). Among invertebrate species, the bee is particularly appealing because of its known visual cognitive abilities to process visual configurations (Avarguès-Weber et al., 2010; Stach et al., 2004). There are moreover some evidences that the honeybee can process stimuli both at a global and local level and tend to show global preference (Zhang et al., 1992).

Here we tested bees with hierarchical stimuli presenting a global geometric shape composed of local geometric symbols. We first assessed the bee ability to perceive both local and global features and then tested a potential saliency of one level on the other when making their choice. Two different densities of stimuli (number of local element constituting the global feature) were used to test the robustness of the preference observed. Finally, to precise bee perception of the global feature, we asked if they were able to interpolate (i.e. create link between local features) the global shape from the particular spatial configuration of local features.

Methods

Stimuli

Experiment 1

Hierarchical compound stimuli were composed of black geometric shapes (local feature). The local features spatial arrangement constituted also a geometric shape (global feature) (Examples Fig. 1A). A 1 cm equilateral triangle, a 1cm disc, a 1cm square diamond

and a 1 cm square were used as local features. Global features were composed of the spatial arrangement of twelve repetitions of one identical local form (disposed every 2 cm), thus drawing an equilateral triangle, a circle, a square diamond or a square. All features were above discrimination threshold considering bees' visual acuity (Srinivasan and Lehrer, 1988) and performances in discrimination tasks involving geometric shapes (Campan and Lehrer, 2002; Hempel de Ibarra and Giurfa, 2003; Lehrer and Campan, 2005). The global shape could not be identical as the local shape. Twelve different hierarchical compound stimuli were thus obtained. For each particular bee, the rewarded and non-rewarded stimuli used for training were randomly chosen among the pool of twelve stimuli available. The only restriction was that all four geometric shapes were used in each stimuli pairs either at the global or local level.

Experiment 2

In this experiment, global features were composed by eight repetitions instead of twelve, thus reducing the visual density of stimuli (Examples Fig. 2A).

Experiment 3

All bees were trained with the same pair of stimuli composing of a global square made of twelve repetitions of local 1 cm equilateral triangles and a global diamond made of twelve repetitions of local 1 cm inverted triangles (Fig. 3A). In transfer conditions, black empty square and diamond (line width: 1 cm) were proposed to bees in order to evaluate their ability to interpolate a shape from spatial arrangement of discrete elements (Fig. 3A).

Procedure

Individually free-flying marked honeybees were trained to collect 1M sucrose solution in a Y-maze (Giurfa et al., 1999). The maze was covered by an ultraviolet-transparent Plexiglas ceiling and illuminated by natural daylight. During both training and tests only one honeybee was present in the apparatus. The entrance of the maze led to a decision chamber, where the bees could choose between two arms. The back walls of the maze (20×20 cm) on which hierarchic compound stimuli were presented were placed at a distance of 20 cm from the decision chamber. Following a differential conditioning procedure (Giurfa et al., 1999),

one stimulus was rewarded with 1M sucrose solution while the alternative stimulus was not. Sucrose solution was delivered in the centre of the stimulus. At each trial, if the bee chose the rewarded stimulus, it was allowed to drink sucrose solution ad libitum and resumed afterward its foraging trip, thus returning to the hive. If the bee chose the non-rewarded stimulus, it was gently tossed away from the maze and had to re-enter until the bee flew to the rewarded stimulus. Only the first choice was registered. During training the side of the rewarded stimulus (left or right) was interchanged in a pseudorandom sequence. After training, transfer tests with fresh, non-rewarded stimuli were performed. The numbers of contacts with the surface of the patterns for 45 seconds were recorded. The choice proportion for each of the two stimuli was calculated from the cumulative contacts. Each test was done twice, interchanging the sides of the stimuli. Three to six refreshing trials were intermingled among the tests. The positions of the different tests were interchanged from one bee to another.

Experiment 1 & 2

Bees (N=10 in Experiment 1 and N=6 in Experiment 2) were trained for 36 trials with a particular pair of stimuli described above (CS+: G+/L+ vs. CS-: G-/L-; Figs. 1A and 2A, 'Training'). Afterward, bees learning abilities were tested by analysing their choices in front of the stimuli used during training (CS+ vs. CS-; Figs. 1A and 2A, 'Learning Test'). Then, three different transfer tests were performed. First, bees were presented with a conflict situation to assess a potential preference for one level processing, in which one test stimulus was composed of CS+ local features arranged in the CS- global configuration (G-/L+) whereas the alternative was composed of CS- local features arranged in the CS+ global configuration (G+/L-; Figs. 1A and 2A, 'Conflict Test'). The two other tests were control tests in which bees' discrimination performance between both local features (Local Test) and global features (Global Test). Bees were presented with the rewarded trained stimulus (CS+) versus the stimulus composed of CS- local features in the CS+ global configuration (G+/L-) (Figs. 1A and 2A, 'Local Test') or the stimulus composed of the CS+ local features in the CS- global configuration (G-/L+) (Figs. 1A and 2A, 'Global Test').

Experiment 3

Experiment 3 was designed to test the bees' ability to interpolate the global features as a geometric shape instead of learning specific local features locations. The bees (N=8) were trained for 36 trials with a particular pair of stimuli (see above and Fig. 3A, 'Training'). One

group of bees were rewarded on the global square composed of local triangles whereas another group was rewarded on the global diamond composed of local inverted triangles. After training, bees were subjected to a learning test presenting the two training stimuli (CS+: G+/L+ vs. CS-: G-/L-; Fig. 3A, 'Learning Test'). To control the discrimination performance at the global level a control test was performed in which bees faced the CS+ (G+/L+) versus the stimulus composed of the CS+ local features in the CS- global arrangement (G-/L+) (Fig. 3A, 'Global Test'). The bees' interpolation ability was tested by presenting an empty square vs. an empty diamond at the global level size (G+ vs. G-; Fig. 3A, 'Transfer Test').

Statistics

Performance was analyzed in terms of the proportion of correct choices per test, producing a single value per bee to exclude pseudo-replication. Mann-Whitney U tests were used to test the null hypothesis that the proportion of correct choices cumulated during 45 sec of a test was not different from a theoretical value of 50%. Comparisons between tests were performed thanks to Wilcoxon tests. The alpha level for all analyses was 0.05.

Results

Experiment 1

Bees learned to discriminate the hierarchical stimuli as they chose the correct conditioned stimulus in the non-rewarded learning test, (N=10; $73.7 \pm 2.9\%$ (mean \pm s.e.m.) of correct choices; Mann-Whitney U-test: $Z_{adj} = -4.04$; $p < 0.001$; Fig. 1B, 'Learning Test').

In the conflict situation, bees preferred the stimulus presenting the conditioned global feature from the stimulus presenting the conditioned local features ($65.1 \pm 3.7\%$ of G+/L- choices; $Z_{adj} = -3.23$; $p = 0.001$; Fig. 1B, 'Conflict test'), thus suggesting that global configurations are more salient or hierarchically represented for the honeybee than the local constitutive elements.

Bees succeeded in discriminating the conditioned stimulus from the alternative differing only by its local features ($65 \pm 3.5\%$ of correct choices; $Z_{adj} = -3.23$; $p = 0.001$; Fig. 1B, 'Local Test') or by its global feature ($65.1 \pm 4.5\%$ of correct choices; $Z_{adj} = -3.23$; $p = 0.001$; Fig. 1B, 'Global Test'). These results show that bees were able to extract and memorize from the

hierarchical compound stimuli both the details (local features) and the spatial configuration (global features). Consequently, the bees' preference toward global feature in the conflict test is not due to a lack of perception or discrimination of the local features. In addition, local and global feature perception reached similar level as there was no significant difference in performance between the respective local and global tests (Wilcoxon Test: $T=27$, $Z=0.05$, $p=0.96$). The results observed in the conflict situation are not the consequence of an easier perception of global features.

It can thus be concluded from Experiment 1 that there is a global precedence tendency in the honeybee's compound hierarchical stimuli processing. To test the robustness of these results, we decided to use less dense stimuli (eight local features composing the global configuration instead of twelve) from which the global feature is more difficult to perceive.

Experiment 2

In the Experiment 2, a new set of bees ($N=6$) learned to discriminate the less-dense hierarchical stimuli as they reach significant level of learning performance in the non-rewarded learning test, ($72.1\pm 5.6\%$ of correct choices; $Z_{adj}=-3.08$; $p<0.01$; Fig. 2B, 'Learning Test').

In the conflict situation, bees chose the stimulus presenting the conditioned global feature rather than the stimulus presenting the conditioned local features ($60.8\pm 7.3\%$ of G+/L-choices; $Z_{adj}=-2.05$; $p<0.05$; Fig. 2B, 'Conflict test'), thus confirming that global configurations are preferentially used by the honeybee to make choice than the local constitutive elements.

Results of the local test ($68.4\pm 3.1\%$ of correct choices; $Z_{adj}=-3.08$; $p<0.01$; Fig. 2B, 'Local Test') and the global test ($73.3\pm 1.8\%$ of correct choices; $Z_{adj}=-3.08$; $p<0.01$; Fig. 1B, 'Global Test'), showed that removing one third of local information doesn't alter bees' capacity to discriminate stimuli both at the local and global level (no significant difference between performance of the local and global tests: $T=6$, $Z=0.94$, $p=0.35$).

This experiment 2 reveals that the global precedence observed in the experiment 1 is robust as decreasing the saliency of the global geometrical form didn't change the bee choice in the conflict situation. However, it can be argued that the bees may not have perceived the

geometric shape drawing by the local elements but may rather have learned the combination of specific elements locations. In that case, decreasing the number of constitutive elements is not supposed to increase difficulty in processing global information. To approach this question, we designed a new experiment in which transfer performance toward the global geometric shapes were tested.

Experiment 3

The new bees trained in Experiment 3 learned to discriminate the two hierarchical stimuli as they chose the correct conditioned stimulus in the non-rewarded learning test, (N=8; 68.4±3.9% of correct choices; $Z_{adj}=3.59$; $p<0.001$; Fig. 3B, 'Learning Test'), thus confirming that bees are able to discriminate hierarchical stimuli based on geometric shapes even when the features differ only by rotation (Global level: the diamond corresponds to a 45° rotated square; Local level: the inverted triangle is a 180° rotated triangle; Fig. 3A).

Consistent with results from Experiment 1, the bees tested in Experiment 3 did extract and memorize the global feature. Specifically, bees preferred the conditioned stimulus from the alternative differing only by its global shape (70.2±3.1% of correct choices; $Z_{adj}=3.59$; $p<0.001$; Fig. 3B, 'Global Test').

Finally, bees were able to transfer their choice to the geometric shape corresponding of the global feature learned (63.1±4.5% of correct choices; $Z_{adj}=2.90$; $p<0.01$; Fig. 3B, 'Transfer Test'). This result show that bees can create link between local features to interpolate the global shape rather that memorize simply all local elements specific locations.

Discussion

The present work shows a global preference of the honeybee when processing hierarchical stimuli. Bees rely on global configurations to make choices between complex visual pictures although they were able to resolve both vision levels. Even when not specifically trained for, bees not only extract the spatial arrangement of the picture details but also possess ability to bound element together in order to create a shape by interpolation.

Taken together these results tend to show a natural advantage to process objects at a whole rather than analysing its constituent parts.

Although these results need to be confirmed by using different type of stimuli, the bee's visual system appears to have evolved toward a more global perception of surrounding objects. This assumption seems reasonable knowing that the honeybee foraging behaviour rely on complex navigation skills (Menzel et al., 2005) and flower constancy (Giurfa, 2007; von Frisch, 1967) which require important landmarks (Collett, 1996; Collett and Zeil, 1998) and flowers (Chittka and Raine, 2006) visual recognition abilities. Indeed, object recognition and classification are facilitated by configurational processing (Maurer et al., 2002; Peterson and Rhodes, 2003; Tanaka and Farah, 1993). In addition, due to the relatively poor spatial acuity of the bees (Srinivasan and Lehrer, 1988), global shape must be easier to perceive at distance for a flying bee.

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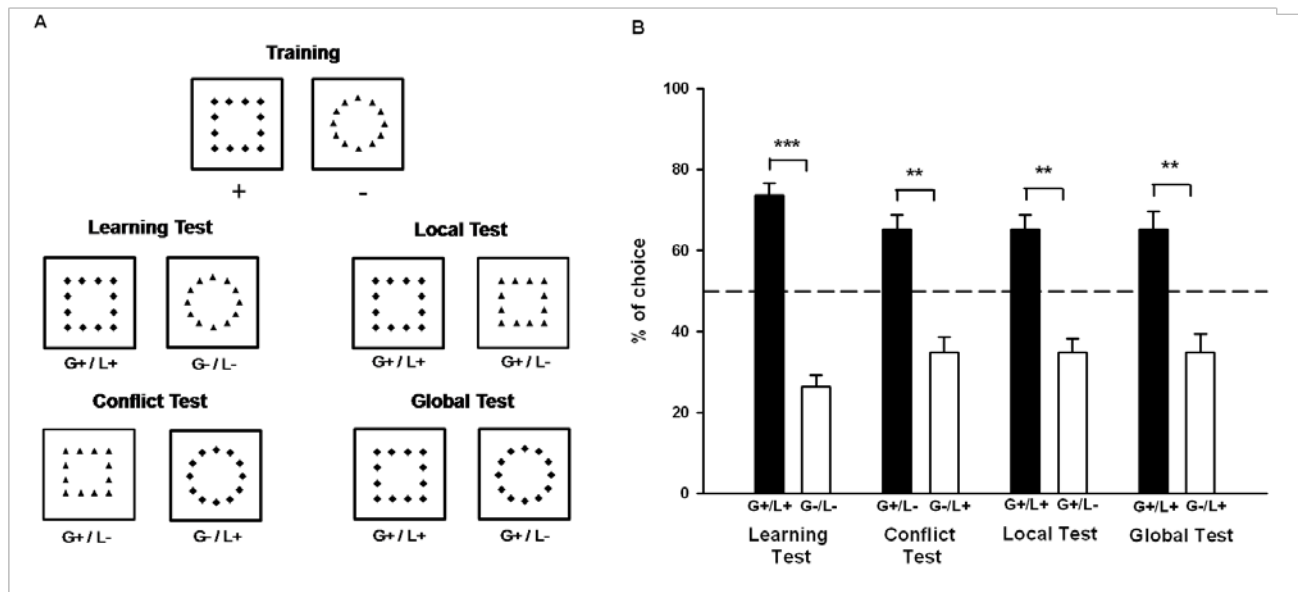


Figure 1: Experiment 1. (A) Example of conditioning and testing procedure. Each particular bee was trained with different stimuli. Training lasted 36 trials. Tests were not rewarded. (B) Performance in tests (% of choices). Bees learned the trained stimulus both at local and global level and showed a preference for global information in a conflict situation. Data shown are means + s.e.m (N=10; **: $p < 0.01$; ***: $p < 0.001$).

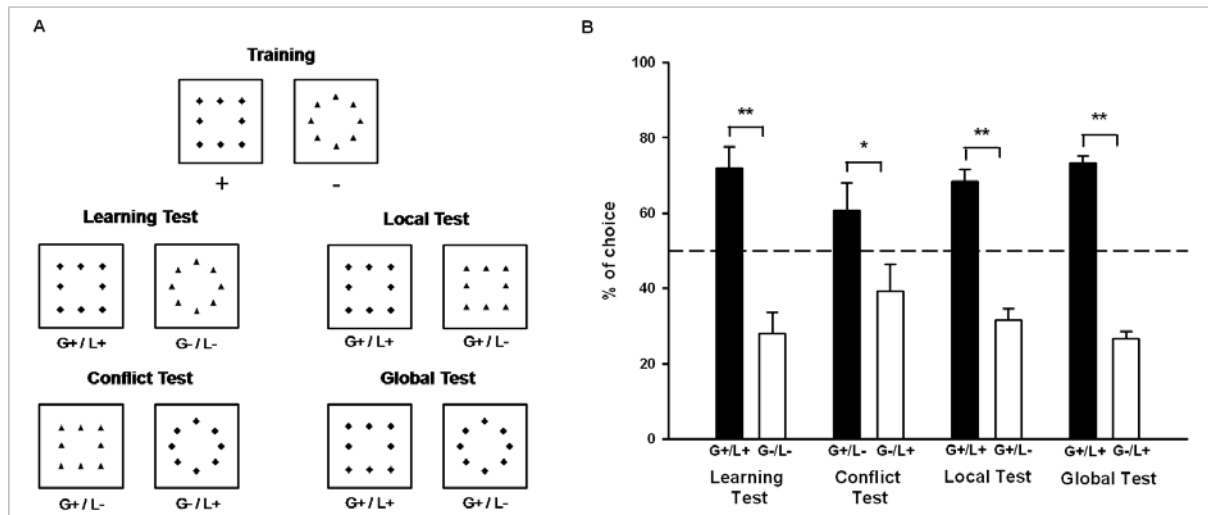


Figure 2: Experiment 2. (A) Example of conditioning and testing procedure. Each particular bee was trained with different stimuli. Training lasted 36 trials. Tests were not rewarded. (B) Performance in tests (% of choices). Bees learned the trained stimulus both at local and global level and showed a preference for global information in a conflict situation. Thus, making the global form harder to perceive by decreasing the stimuli density doesn't affect bees global preference. Data shown are means + s.e.m (N=6; *: $p < 0.05$; **: $p < 0.01$).

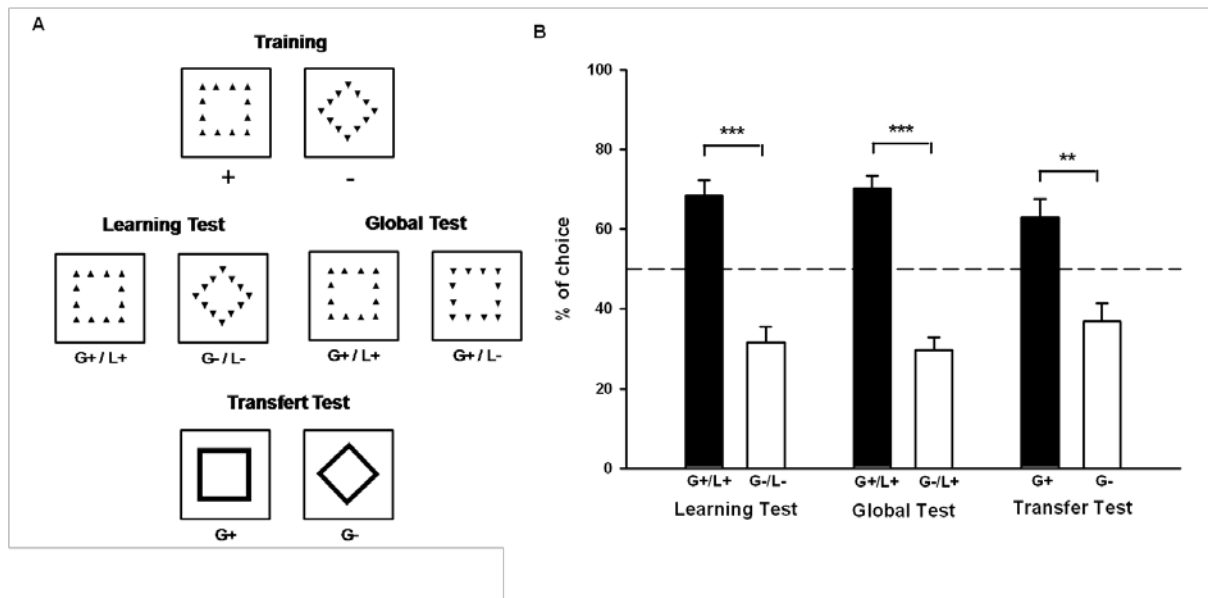


Figure 3: Experiment 3. (A) Conditioning and testing procedure. Half of the bees are rewarded on the square as shown whilst the other half was rewarded on the diamond. Training lasted 36 trials. Tests were not rewarded. (B) Performance in tests (% of choices). Bees were able to interpolate a geometric form from the spatial arrangement of local forms. Data shown are means + s.e.m (N=8; **: $p < 0.01$; ***: $p < 0.001$).

Article IV

Conceptualization of above and below relationships by an insect

Avarguès-Weber A, Dyer AG, Giurfa M (sous presse)

Proceedings of the Royal Society B

Dans ce travail, nous avons voulu étudier la capacité des abeilles à maîtriser des concepts abstraits au-delà de l'utilisation de la similarité perceptive, employée dans les tâches de catégorisation précédentes. Contrairement aux catégorisations visuelles, les concepts font appel à l'implémentation de règles qui sont indépendantes de la nature physique et sensorielle des stimuli. Sachant, aux vues des articles précédents que les abeilles peuvent combiner les relations entre éléments d'une image en une configuration, nous avons choisi de tester la capacité des abeilles à discriminer des stimuli visuels en fonction de concepts spatiaux, à savoir 'au-dessus de' vs. 'en-dessous de'. Ces relations sont définies par la position relative d'un stimulus de référence ('le référent').

Nous avons tout d'abord utilisé une barre horizontale comme référent. Les abeilles devaient donc discriminer des stimuli en fonction de la position spatiale d'un symbole (croix, losange, etc.) par rapport au référent (au-dessus ou en-dessous de la barre). D'un essai à l'autre, les symboles, leur position par rapport à la barre (distance verticale et latérale) et la position de l'ensemble dans le champ visuel de l'abeille variaient constamment pour favoriser l'abstraction de la relation récompensée au-delà de stimuli particuliers. Les abeilles n'ont montré aucune difficulté à maîtriser cette tâche et ont réussi à transférer le concept appris à de nouveaux stimuli présentant un nouveau symbole situé au-dessus ou en-dessous de la barre.

Dans une seconde expérience, nous avons augmenté la similarité perceptive entre les deux relations spatiales en utilisant deux symboles (au lieu d'un symbole et une barre de référence, un des symboles agissant alors comme référent tout au long de l'expérience. Dans ce cas-là, un nouveau groupe d'abeilles devait donc reconnaître le symbole référent préalablement appris et choisir en fonction de sa position relative (au-dessus ou en-dessous) d'un autre symbole variable. La distance entre les deux symboles ainsi que leur position variaient elles-aussi. Là encore, les abeilles ont appris rapidement à maîtriser le concept spatial et ont su transférer leur choix à de nouveaux stimuli qui préservaient la relation apprise. Nous avons pu enfin vérifier qu'elles n'ont pas utilisé d'informations plus simples comme la position du symbole référent dans le champ visuel (majoritairement dans la moitié supérieure du champ visuel quand il était au-dessus par exemple).

Ces travaux démontrent donc pour la première fois que le cerveau de l'abeille possède effectivement l'architecture et la capacité de traitement nécessaire à l'extraction et le transfert de concepts relationnels de type spatial.

Conceptualization of above and below relationships by an insect

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Keywords: Above/below Relationship – Concept Learning – Rule Learning – Visual Cognition - Honeybee – *Apis mellifera*

Published on Proceedings of the Royal Society B (*In Press*)

ABSTRACT

Relational rules such as 'same' or 'different' are mastered by humans and non-human primates and are considered as abstract conceptual thinking as they require relational learning beyond perceptual generalization. Here we investigated whether an insect, the honeybee (*Apis mellifera*), can form a conceptual representation of an above/below spatial relationship. In Experiment 1 bees were trained with differential conditioning to choose a variable target located above or below a black bar that acted as constant referent throughout the experiment. In Experiment 2, two visual stimuli were aligned vertically, one being the referent, which was kept constant throughout the experiment, and the other the target, which was variable. In both experiments, the distance between target and referent, and their location within the visual field was systematically varied. In both cases, bees succeeded in transferring the learned concept to novel stimuli preserving the trained spatial relation thus showing an ability to manipulate this relational concept independently of the physical nature of the stimuli. Absolute location of the referent into the visual field was not a low-level cue used by the bees to solve the task. The honeybee is thus capable of conceptual learning despite having a miniature brain, showing that such elaborated learning form is not a prerogative of vertebrates.

1. INTRODUCTION

In cognitive sciences, concepts or categories constitute representations of typical entities or situations encountered in an individual's world [1-3]. They promote cognitive economy by sparing the learning of every particular instance of such entities or situations. Instead, individuals generate broad classifications of items so that they can transfer appropriate responses to novel instances that fulfil the basic criteria defining the concept or category.

Categorical classification is thought to be governed to some extent by perceptual similarities. In other words, it is possible to classify different items within a class based on specific features that define the category. For instance, a robin, a sparrow and even a dodo are positive instances of the category 'bird' as they share specific features such as a beak, feathers, wings, etc, that allow grouping them together in the same class. A higher level of classification corresponds to stimulus grouping that is not necessarily based on perceptual similarity but on relational rules linking different instances together. Relational rules such as 'same' or 'different' 'more than' or 'less than' are mastered by humans and non-human primates (e.g. [4-6]) and are considered as a form of abstract conceptual cognition as they involve learning beyond perceptual generalization.

For many animals that must operate in complex natural environments, spatial concepts such as right, left, above and below are of crucial importance to generate appropriate relational displacements and orientation in their environment. Currently however, relatively few studies have investigated if and how animals master conceptual spatial relations such as above/below or left/right. Because the few studies performed so far have focused on pigeons [7] and non-human primates such as chimpanzees [8], baboons [9] or capuchins [10], it is relevant from the perspective of comparative animal cognition, to study if the mastering of a spatial concept such as above/below is a prerogative of vertebrates traditionally characterized as efficient learners such as the species cited above or can also be found in a miniature brain. We have therefore focused on an insect that despite its phylogenetic distance to vertebrates is capable of efficient learning and retention. Such an insect is the honeybee *Apis mellifera* [11].

Honeybees are appealing to study whether abstract rule learning occurs in an insect because they learn and memorize a variety of complex visual cues to identify food sources

including flowers. While foraging in the field, bees learn to associate visual, olfactory, mechanosensory and spatial cues of flowers, with flower rewards of nectar and pollen [11-12]. The study of their visual capacities is possible because free-flying bees can be trained to choose specific visual targets, on which the experimenter offers a drop of sucrose solution as the equivalent of nectar reward (see review in [11]). After the completion of training, bees can be tested with different stimuli in order to determine the visual strategies employed for stimulus recognition. Using this protocol, it has been shown that bees are capable of higher-order forms of visual learning that have been mainly studied in vertebrates [11, 13-14]: they categorize both artificial patterns [15-16; see 17 for a review], and pictures of natural scenes [18], they exhibit top-down modulation of their visual perception [19] and they learn to master both delayed matching-to-sample and delayed non-matching-to-sample discriminations by following a rule of sameness and of difference, respectively [20, 21] or a rule of numerosity [22]. These performances indicate that bees can effectively deal with relational concepts.

Here we studied whether honeybees learn an above/below relationship between visual stimuli and transfer it to novel stimuli that are perceptually different from those used during the training. We therefore analyzed whether conceptual learning based on spatial relationships is possible in a brain that due to its reduced amount of neurons (less than one million compared to 100 billions in humans) could be considered as limited in its computational capacities.

2. MATERIAL AND METHODS

(a) General procedure

Free-flying honeybees *Apis mellifera*, Linnaeus, from a single colony located at 30m from the test site were allowed to collect 0.2M sucrose solution from a gravity feeder. Individual bees marked with a colour spot on the thorax were trained to fly into a Y maze located at 10m from the feeder to collect 1M sucrose solution delivered on the back walls of the apparatus [23]. The maze covered by an ultraviolet-transparent Plexiglas ceiling was located on an outside

table and illuminated by open daylight. Only one bee was present at a time in the Y-maze to insure independence of decision making.

The entrance of the Y-maze led to a decision chamber, where the honeybee could choose between the two arms of the maze. Each arm was 40×20×20cm (L×H×W). The back walls of the maze (20×20 cm) were placed at a distance of 15cm from the decision chamber and were covered by a white reflecting UV background on which stimuli patterns were presented.

Bees were trained using a differential conditioning protocol, in which one stimulus, presented on one of the back walls of the Y-maze, was rewarded with 1M sucrose solution while a different stimulus was penalized with 60mM quinine solution [24-25]. Solutions were delivered by means of a transparent micropipette (5mm diameter) located in the centre of each visual target. The pipette subtended a visual angle of 1.9° to the decision chamber and was below the angular threshold for visual detection established for honeybees [26]. Moreover, as both maze arms presented the same pipette, it could not be used as discrimination cue.

Each training trial consisted in a visit to the maze of the experimental bee, which ended with its uptake of sucrose solution (i.e. a foraging bout). If the bee chose the rewarded stimulus, it could drink sucrose solution *ad libitum*. If, however, it chose the non-rewarded stimulus, it was allowed to taste the quinine solution and then to fly to the alternative arm presenting the rewarded stimulus to imbibe the sucrose solution. The bee then flew back to the hive and 3 to 5 min later returned to the maze for the next training trial. During its absence, the side of rewarded and punished stimuli was interchanged following a pseudorandom sequence (i.e. the same side was not rewarded more than twice) in order to avoid positional (side) learning. On each training trial, only the first choice of the bee was recorded for statistical analysis. Acquisition curves were obtained by calculating the frequency of correct choices per block of 5 trials.

Following the last acquisition trial, non-rewarded tests were performed with fresh, non-rewarded stimuli. During the tests, both the first choice and the cumulative contacts with the surface of the targets were counted for 45s. The choice proportion for each of the two test stimuli was then calculated. Each test was done twice, interchanging the sides of the stimuli to

control for side preferences. Three refreshing trials with the reinforced training stimuli were intermingled between the tests to ensure that foraging motivation did not decay due to non-rewarded test experiences.

(b) Stimuli

Stimuli were presented on a white UV-reflecting paper of constant quality covering the back walls of the maze. Pairs of vertically aligned stimuli were presented on each back wall. Per definition, the '*referent*' was the stimulus that was below or above and acted as a reference for the '*target*', which was located above or below it, respectively. In Experiment 1, the referent was always a horizontal black bar, 10cm long, above or below which different targets were presented. In Experiment 2, the referent was either a black disk 3cm in diameter, or a 4×4cm black cross, depending of the group of bees considered. In both experiments, the targets were variable, so that the bee had to learn the relationship rule rather than a specific pattern. Within a trial or a test, stimulus pairs in the left and right arms of the maze were identical except for their spatial relationship, thus excluding the use of low level cues for discrimination such as differences in the centre of gravity, black area, or total pattern frequency [27].

In Experiment 1 (Fig. 1a), six targets were presented above the black bar used as referent. Three of the targets - a 5×5cm vertical grating, a 4×4cm radial three-sectored pattern and a 5×6cm concentric diamond pattern - were achromatic 'black' and were printed with a high resolution laser printer. The other three targets - a 4×4cm cross, a disk 3cm in diameter and a 1×3cm small bar - were chromatic and were respectively cut from HKS-N coloured papers 3N, 71N and 26N (K + E Stuttgart, Stuttgart-Feuerbach, Germany), which appeared yellow, ochre and purple to the human eye. All colours were well above discrimination threshold and distinguished by bees according to both the Colour Opponent Coding space [28] and the Hexagon colour space [29] models of bee visual processing.

The black bar used as referent subtended a visual angle of 37° to the centre of the maze's decision chamber. In their largest extension, the targets subtended visual angles that varied from 12° to 22°. The grating stripes, the cross bars and the black and white diamonds that composed the concentric diamond pattern were all 1cm-width, which corresponded to a visual angle of 4° from the decision chamber. Sectors in the radial pattern covered 2cm in their largest extension thus subtending a visual angle of 8° to the bees' eye when deciding

between stimuli. An angular threshold of 5° has been reported for stimulus detectability using chromatic or achromatic discs of varying size [26]. In a different experiment [30] where horizontal and vertical black and white gratings of varying spatial frequency had to be discriminated, single stripes could be resolved if they subtended a threshold angle of 2.3° . These angular values ensure that our disc and striped stimuli were detectable and resolvable for honeybees [26, 30].

In Experiment 2 (Fig. 1b), only achromatic, black stimuli were used. The referent was either a cross or a disk as described for Experiment 1. The six stimuli used as target were as described for Experiment 1, except that for Experiment 2 all stimuli were achromatic.

(c) Experiment 1

Compound stimuli consisted of a target (Fig. 1a) placed above or below a referent black bar (Fig. 1a; Fig. 2a). One group of bees ($N=4$) was trained to choose the rewarded ‘target above bar’ spatial relation and to avoid the penalized ‘target below bar’ spatial relation; another group of bees ($N=4$) was trained with inversed contingencies (i.e. ‘target above bar’ penalized and ‘target below bar’ rewarded). Each bee was trained for 30 trials using 5 of the 6 available target stimuli. During training each of the 5 targets was presented 6 times, in a randomized sequence. From one trial to the next, the side of reward, the target, its distance to the referent bar and the bar’s location were pseudo randomized (Fig. 2a), only keeping constant the fact that the ‘target above bar’ relation was rewarded and the ‘target below bar’ relationship was penalized for bees of the ‘above group’ while the opposite was true for the ‘below group’. Following the training procedure, each bee was presented with a non-rewarded transfer test with a novel target located above or below the referent. The transfer-test target had not been used during the training and was thus novel to the individual bee (Fig. 1a). The stimulus selected as transfer-test target varied randomly between bees.

(d) Experiment 2

In a pre-training phase (*Phase 1*), bees were subjected to an absolute conditioning protocol [23] during 15 trials in which the stimulus subsequently used as a constant referent throughout the experiment was rewarded with sucrose solution when presented in one arm of the maze. The other arm presented a white background alone associated with quinine solution. This

absolute conditioning was performed to encourage the bees to focus on the referent during the subsequent training phase, in which the above/below relationship was inculcated relative to it. For one group of bees, the stimulus used as referent was a cross (N=10) while it was a disk for another group of bees (N=10). The target used within each group was varied in a pseudo random fashion between trials (Fig. 3a). After pre-training, a discrimination test opposing the referent and a pattern chosen randomly among the set of available targets ('test pattern') was performed to verify that bees did indeed discriminate the referent (see Fig. 3a). This pattern was not used during the following training phase.

After pre-training, bees were subjected to a training phase (*Phase 2*) during 50 trials to select either the 'target above referent' ('above group') or the 'target below referent' relationship ('below group') in a differential conditioning task. Bees in the 'above group' were rewarded whenever a variable target was above the referent (disk or cross depending on the group of bees); for these bees, the same stimuli presented in a reversed relationship (i.e. with the target always below the referent) were penalized with quinine solution. Bees in the 'below group' were trained with the reversed contingencies. From one trial to the next, the positions and distance between target and referent were constantly varied, keeping only constant the above/below relationship as predictor of reward and punishment (Fig. 3a). Excluding the pattern used for the test following pre-training (see above) and the pattern used as referent, all 4 remaining patterns (Fig. 1b) were used as targets during the training. Each of the 4 patterns was therefore presented 12 or 13 times in a random sequence during the 50 trials.

After training was completed, bees were subjected to a transfer test in which the test pattern was introduced as target in an above/below relationship (Fig. 3a). Additionally, 8 of 20 bees studied were subjected to two further controls aimed at verifying that bees used the relative position of both target and referent and not just the fact that in most cases the referent appeared in the lower or in the upper visual field in the 'above' or the 'below group', respectively. In control 1, the referent was positioned in the middle of the background for both the rewarded and the punished stimulus (Fig. 3a) so that its absolute position could not help discrimination. As a target was nevertheless also present in this test, the above/below relationships were preserved. In control 2, the referent was presented singly in the lower vs. the upper part of the background (Fig. 3a) to determine whether its absolute position was an

orientation cue used by the bees, instead of its position relative to the target, which was now absent.

(e) Statistical analysis

Performance of balanced groups during acquisition was compared using ANOVA for repeated measurements in which groups and trial blocks constituted factors of analysis. The dependent variable was the percentage of correct first choices of each individual bee in each block of 5 trials. Performance during the tests was analyzed in terms of the proportion of correct choices per test, producing a single value per bee to exclude pseudo-replication. A binomial test was used to analyze performance in terms of the bees' first choice while a one-sample t-test was used to test the null hypothesis that the proportion of correct choices cumulated during 45sec of a test was not different from a theoretical value of 50%. Comparisons between groups and between tests were made using independent two-sample t-tests and paired samples t-tests, respectively.

3. RESULTS

(a) Experiment 1

To test if honeybees can extract an abstract above/below relationship and to classify visual instances based on this relative spatial relationship we used target stimuli presented above or below a horizontal bar (Fig. 1a). Honeybees were individually trained in a differential conditioning procedure in which one spatial relation (e.g. 'target above bar') was associated with sucrose solution whilst the other relation (e.g. 'target below bar') was associated with quinine solution (Fig. 2a). One group of bees was rewarded on the 'target above bar' relation while another group was rewarded on the 'target below bar' relation. After completing the training, bees were subjected to a non-rewarded transfer test in which a novel target (not used during the training) was presented above or below the bar (Fig. 2a). This experiment allows the testing of two alternative hypotheses. First, if bees responded on a strictly perceptual basis, deterioration of performance should be observed in the transfer test because of the introduction of the unknown target stimulus. By contrast, if performance in the transfer test

remained high, then an above–below abstract concept was formed by the bee in order to solve the novel visual problem.

Both groups of bees ('above group' and 'below group') learned the task as their acquisition curves significantly increased during the six blocks of 5 trials (ANOVA for repeated measurements; $N=8$ bees; block effect: $F_{5,30}=3.5$, $p<0.05$). There was no group effect ($F_{1,6}=0.1$, $p=0.81$), thus showing that acquisition was not influenced by the particular spatial relationship (above or below) rewarded. Data of both groups were therefore pooled and presented as a single curve in Fig. 2b.

In the transfer test, performance was also independent of the particular spatial relationship (above or below) rewarded (two sample t-test, $t_6=0.55$, $p=0.60$) so that results of both groups were pooled and presented as a single bar (Fig. 2b). Here and henceforth, test performances shown correspond to the cumulative choices of the bees during 45 sec test; similar performance levels were obtained when considering the first choice in the tests (not shown). Bees chose the correct spatial relationship despite the introduction of an unknown stimulus as target in $75.4 \pm 1.8\%$ of the cases (mean \pm S.E.; $N=8$ bees). This performance was significantly different from chance both if the first choice (binomial test: $p<0.005$) or the cumulative choices during 45sec (one sample t-test against a 50% choice, $t_7=12.57$, $p<0.001$) were considered, thus showing that bees were able to classify visual instances on the basis of a conceptual representation of the above/below relationship.

(b) Experiment 2

In this experiment, we extended our testing of how bees can learn the concept of above/below, but instead of using a salient horizontal bar as referent, we used two stimuli positioned one above the other so that one acted as the target and the other as the referent. For one group of bees, the stimulus used as referent was a cross ($N=10$) while for another group of bees, it was a disk ($N=10$) (Fig. 1b). The target used within each group varied from trial to trial in order to promote the extraction of an abstract above/below relationship.

In Phase 1 (pre-training phase), bees were rewarded to choose the referent stimulus alone (cross or disk, depending on the groups of bees; see Fig. 3a). During the three blocks of five pre-training trials, bees learned quickly to choose the arm presenting the target

independently of the stimulus used (cross or disk) ($N=20$; block effect: $F_{2,36}=5.6$, $p<0.01$; group effect: $F_{1,18}=2.7$, $p=0.12$); data for both groups were therefore pooled and presented as a single curve in Fig. 3b. Results from the discrimination test showed that honeybees discriminated significantly different to chance expectation between the referent and the alternative stimulus both considering the first choice (binomial test: $p<0.001$) and the cumulative choices ($N=20$; $t_{19}=11.2$, $p<0.001$; Fig.3b), irrespective of the referent trained ($t_{18}=0.3$, $p=0.78$).

After pre-training, bees were trained in Phase 2 to select either the ‘target above referent’ (‘above group’) or the ‘target below referent’ relationship (‘below group’) in a differential conditioning task in which the referent was constant and the targets were variable. During ten blocks of five trials, bees significantly improved their correct choices ($N=20$, trial effect: $F_{9,144}=3.1$, $p<0.005$) irrespective of the group considered (above/below effect: $F_{1,16}=0.9$, $p=0.36$; cross/disk effect: $F_{1,16}=3.4$, $p=0.08$; Fig. 3c). Performance of groups was therefore pooled and presented as a single curve in Fig. 3c. The fact that the first block of trials yielded a performance that was already significantly above chance ($t_{19}=2.8$, $p=0.011$) was due to the fact that some bees rapidly learned to make correct choices after the first choice. The inset in Fig. 3c shows the choice performance for the first 5 acquisition trials that integrate the first block. The data indicate that bees started choosing randomly and then rapidly improved their performance to a 60 -70% correct choices.

After training was completed, bees were subjected to a transfer test in which a novel stimulus was introduced as target in an above/below relationship (Fig. 3a). In the transfer test bees significantly preferred the spatial relationship for which they were trained ($N=20$; first choice: $p<0.001$; cumulative choices: $t_{19}=13.0$, $p<0.001$; Fig.3c) irrespective of the group considered (above/below: $t_{18}=0.4$, $p=0.70$; cross/disk: $t_{18}=0.6$, $p=0.55$). The bees were consequently able to learn the concept above/below even when the task was rendered more difficult.

In addition, 8 of 20 bees studied were subjected to two further controls aimed at verifying that bees used the relative position of both target and referent and not just the fact that in most cases the referent appeared in the upper or in the lower visual field in the ‘below’ or the ‘above group’, respectively. In control 1, the referent was located in the middle of the

background both for the rewarded and the non-rewarded stimulus pairs so that it could not help the bees choosing between them. In this control, bees chose appropriately the stimulus pair presenting the spatial relationship for which they were trained (Fig. 3c; N=8; first choice: $p < 0.05$; cumulative choices: $t_7 = 2.9$, $p < 0.05$) irrespective of the group considered (above/below: $t_6 = 1.1$, $p = 0.33$; cross/disk: $t_6 = 1.9$, $p = 0.10$). Performance of bees in this test did not differ from that observed in the transfer test ($t_7 = 1.6$, $p = 0.16$). In control 2, only the referent was presented in the upper or the lower part of the background to determine whether its absolute position was an orientation cue used by the bees, instead of its position relative to the target, which was now absent. Bees showed no preference between the two referent locations (Fig. 3c; N=8; first choice: $p = 0.40$; cumulative choices: $t_7 = 0.7$, $p = 0.50$), irrespective of the group considered (above/below: $t_6 = 0.08$, $p = 0.94$; cross/disk: $t_6 = 0.4$, $p = 0.74$; Fig. 2c). Performance in this test was, therefore, significantly different from that in the transfer test ($t_7 = 4.6$, $p < 0.005$) and in control 1 ($t_7 = 2.6$, $p < 0.05$). These results show that the honeybee possesses the faculty to extract a conceptual above/below relationship from a set of training stimuli and to transfer this concept to newly encountered stimuli.

4. DISCUSSION

The present work shows that honeybees learn a conceptual spatial relationship based on an above/below relationship between stimuli, irrespectively of the physical nature of the stimuli. In both experiments, bees exhibited the faculty to transfer an appropriate response to novel instances of the trained concept in spite of variations in the distance separating the referent and the target, the spatial location within the visual field, the fact that targets were variable and randomized during training and that novel stimuli were introduced in the tests. None of these manipulations affected the performance of the bees, which learned to choose stimuli based on an above/below relationship.

Several examples of perceptual categorical learning have been provided for honeybees (see [17] for review). Honeybees can indeed categorize visual stimuli based on single features such as bilateral symmetry [15], global orientation [31], concentric and radial organization [31], and on configurations of features [16, 33]. This capacity also applies to natural pictures which bees can categorize in classes such as closed and radial flower types, plant stems and

landscapes [18]. In all these examples, perceptual similarity plays a critical role as bees classify stimuli based on their physical similarity with a prototype or because they present the basic perceptual features that define the category [34]. Examples of conceptual relational learning, in which the animal's response is not driven by the physical similarity of stimuli but rather by an abstract relationship or rule binding items irrespective of their physical nature, are however rare in the case of the honeybee. So far, bees have been reported to learn both a concept of sameness and of difference [20, 21] so that they learned to solve a delayed matching-to-sample (DMTS) and a delayed non-matching-to-sample (DNMTS) problem, respectively. In these experiments, the bees' response was not driven by stimulus similarity as bees matched (DMTS) or non-matched (DNMTS) colours with achromatic gratings (and vice versa) and even colours with odours [20]. A delayed-matching-to-sample procedure was also used to demonstrate the existence of numerosity in honeybees [22]. In this case bees had to match the novel stimulus containing the same number of items as the sample. The authors controlled for low-level cues such as cumulated area and edge length, configuration identity, and illusionary shape similarity formed by the elements. Their results showed that honeybees have the capacity to match visual stimuli as long as the number of items does not exceed four. In a different setup, inspired by field experiments on honeybee navigation [35], bees were trained to fly into a tunnel to find a food reward after a given number of landmarks [36]. The shape, size, and positions of the landmarks were also changed in the different testing conditions in order to avoid confounding factors. As in the delayed matching-to-sample experiment, bees showed a stronger preference to land after the correct number of landmark in non-rewarded tests and showed the same limit of four in their counting capacity.

Our results add another example of conceptual learning to the relatively few set of examples documenting this capacity in honeybees. Our new finding demonstrates that bees can indeed process 'above' and 'below' spatial relations between visual stimuli and provide the first example of above/below conceptual learning in an invertebrate. This ability seems thus to be present in a variety of animals. Until now, this capacity has only been studied in pigeons [7], in chimpanzees [8], in baboons [9] and in capuchins [10]. Our results thus support the hypothesis that the learning the conceptual spatial relations is independent of language abilities [37] and challenges the idea that a relatively large vertebrate brain is necessary to perform such high cognitive tasks.

Acknowledgements

We thank two anonymous reviewers and L. Chittka for helpful comments and corrections. M. Giurfa and A. Avarguès-Weber thank the French Research Council (CNRS) and the University Paul Sabatier (Project APIGENE) for generous support. A. Avarguès-Weber was supported by a Travelling Fellowship of The Journal of Experimental Biology, and by the University Paul Sabatier. A.G. Dyer acknowledges the ARC DP0878968 and DP0987989 for funding support.

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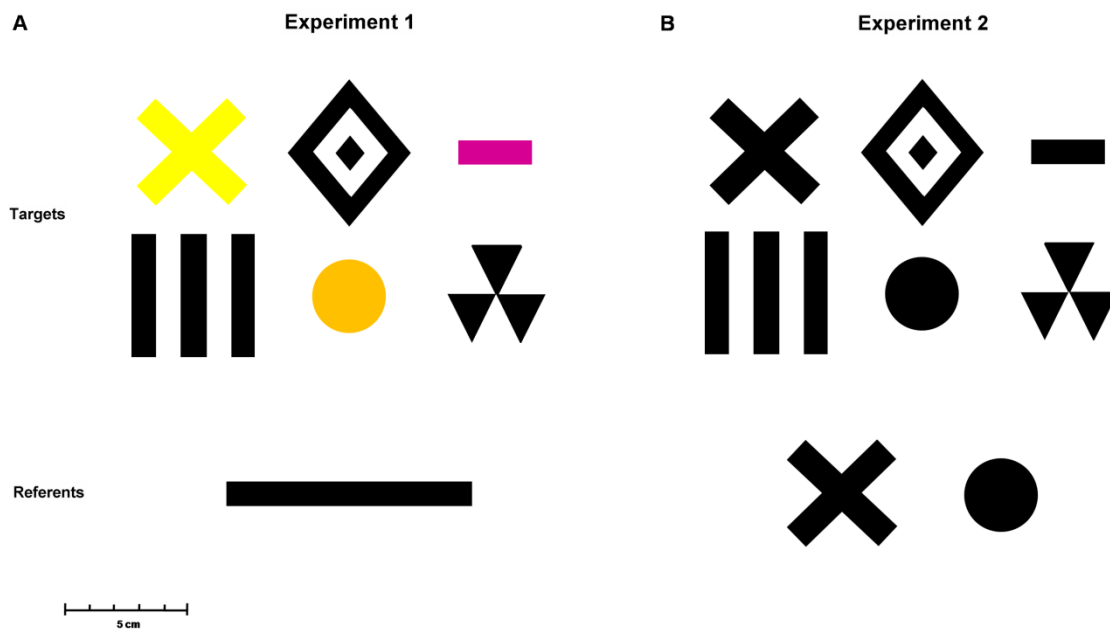


Figure 1: Stimuli used in the experiments. (A) Experiment 1: During training, the targets were presented above and below the referent bar. (B) Experiment 2: Only achromatic patterns were used. During training, the targets were presented above and below the referent pattern which was a cross or a disk depending on the group of bees trained.

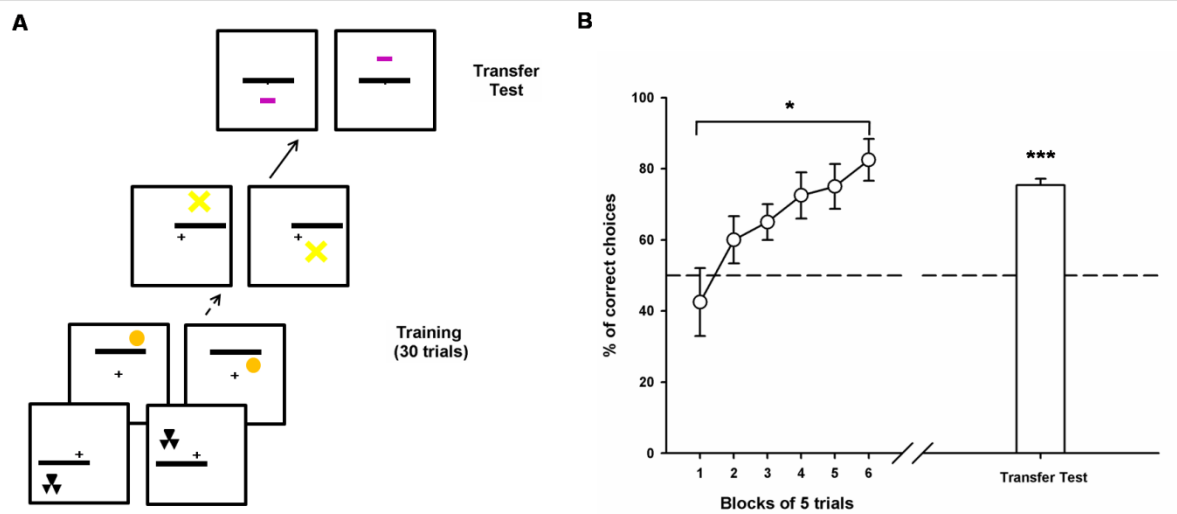


Figure 2: Experiment 1. (A) Example of the conditioning and testing procedure. Half of the bees were rewarded on the ‘target above bar’ relation whereas the other half was rewarded on the ‘target below bar’ relation. The transfer test was not rewarded. (B) Acquisition curve during training (percentage of correct choices as a function of blocks of 5 trials) and performance (cumulative choices during 45sec test) in the non-rewarded transfer test (white bar). Data shown are means and s.e.m. (N=8). Bees succeeded in learning the rule based on the ‘above’ vs. ‘below’ relationship and in transferring the concept to novel stimuli (*: $p < 0.05$; ***: $p < 0.001$).

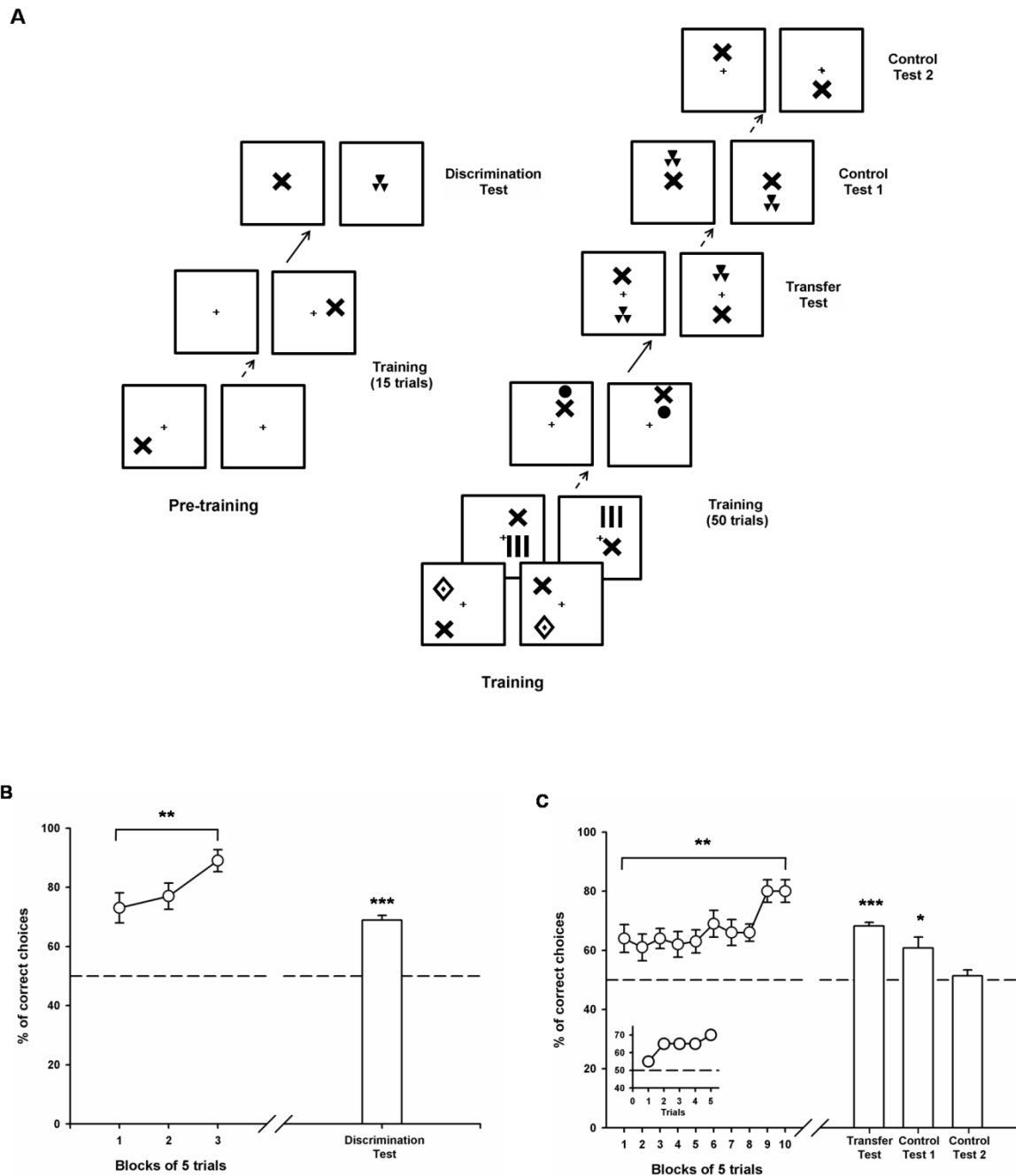


Figure 3: Experiment 2. (A) Example of the conditioning and testing procedure. Half of the bees were rewarded on the ‘target above referent’ relation whereas the other half was rewarded on the ‘target below referent’ relation. The referent pattern was either the disk or the cross depending on the group of bees trained. The transfer test was not rewarded. (B) Phase 1 (pre-training): Acquisition curve during pre-training

(percentage of correct choices as a function of blocks of 5 trials) and performance (cumulative choices during 45sec test) in the non-rewarded discrimination test (white bar). Data shown are means and s.e.m. (N=20). Bees learned to choose the referent pattern and to discriminate it from other patterns used as targets in the subsequent training phase (**: $p < 0.01$; ***: $p < 0.001$). (C) Phase 2 (training): Acquisition curve during training (% of correct choices as a function of blocks of 5 trials) and performance (cumulative choices during 45sec test) in the non-rewarded tests (white bars). Data shown are means and s.e.m. (N=20 for acquisition curve and transfer test, and N=8 for controls 1 and 2). The inset shows acquisition performance during the first 5 trials that integrate the first training block. Bees learned the concept of 'above/below' and transferred it to novel stimuli. Controls 1 and 2 show that the spatial location of the referent on the background was not using as a discrimination cue to resolve the task (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

Article V

Learning abstract concepts with a miniature brain

Avarguès-Weber A, Dyer AG, Giurfa M (en procédure de soumission)

Suite aux résultats de l'article IV, nous avons souhaité aller pour loin dans l'analyse des facultés d'abstraction de l'abeille en testant leur capacité à maîtriser les relations 'au-dessus / en dessous' vs. 'droite / gauche' pour lesquelles il n'y a pas conservation d'un 'référent' d'un stimulus à l'autre.

Dans ce but, nous avons entraîné les abeilles à choisir parmi deux relations spatiales (au-dessus/en-dessous vs. gauche/droite) présentées simultanément dans un labyrinthe Y. A chaque essai de conditionnement, une branche du Y présentait deux motifs achromatiques *différents* (carrés composés de rayures, quadrillage, ronds concentriques, etc.) situés l'un au-dessus de l'autre tandis que l'autre branche présentait les mêmes motifs situés l'un à gauche de l'autre. Les motifs utilisés variaient d'un essai à l'autre ainsi que leur position afin d'initier un apprentissage de la relation entre les éléments plutôt que celui d'une image particulière. Chaque abeille testée était récompensée par une solution sucrée sur une des deux relations spatiales tandis que l'autre était associée à de la quinine. A la suite de l'entraînement, les abeilles ont été capables de reconnaître la bonne relation spatiale bien que celle-ci soit alors composée de disques colorés. Les abeilles ont donc su utiliser le concept relationnel comme une règle, indépendamment des stimuli utilisés. Un autre groupe d'abeilles, entraîné avec les disques colorés et testé avec des motifs achromatiques, a montré des résultats similaires. Nous avons aussi pu vérifier que les abeilles n'ont pas simplement utilisé l'orientation globale des stimuli pour résoudre la tâche.

De plus, les abeilles ont extrait un autre concept relationnel de l'entraînement : deux motifs ou couleurs différents étaient utilisés pour construire chaque relation. Bien que cette propriété des stimuli soit partagée par les deux relations spatiales lors de l'entraînement et n'est donc pas prédicatrice de la récompense, les abeilles préfèrent les images composées de deux stimuli différents dans des tests de transfert présentant la même relation spatiale composée de stimuli différents vs. identiques. Enfin, dans une situation conflictuelle présentant la relation spatiale récompensée composée de deux stimuli identiques et la relation non récompensée composée de deux stimuli différents, l'abeille ne montre aucune préférence. Ainsi, les abeilles ont extrait de l'entraînement à la fois la relation spatiale et la différence entre les stimuli et ont combiné ces concepts afin de définir la catégorie récompensée.

Ces travaux montrent donc que l'abeille possède les capacités suffisantes pour extraire, combiner et manipuler des concepts relationnels. L'abeille possède donc un cerveau capable d'un niveau d'abstraction élevé malgré une complexité très en-deçà des cerveaux de Vertébrés.

Learning abstract concepts with a miniature brain

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Keywords: Concept learning – Relational learning – Visual cognition – Insect – Honeybee – *Apis mellifera*

Submitted to Nature

Sorting objects and events into classes is a fundamental cognitive capacity that spares us the cost of learning every particular situation encountered in our daily life^{1,2}. A sophisticated level of classification is based on grouping objects on the basis of abstract relationships, irrespective of stimulus similarity^{2,3}. For instance, humans and non-human primates learn conceptual relationships such as ‘same’, ‘different’, ‘larger than’, ‘better than’, ‘above’ and ‘below’ among others^{1,2,4-7}. Spatial concepts are particularly useful as they facilitate orientation⁸ and stimulus recognition⁹⁻¹¹. Whether spatial concepts can be learned by an insect brain remains unknown. Here we show that the miniature brain of a honeybee can rapidly form a conceptual representation of spatial relationships between visual stimuli and transfers the learned concept to novel stimuli. Individual honeybees learned to classify visual targets using above/below and right/left relationships, independently of the physical nature of the stimuli. In addition, they learned to pay attention to an additional concept derived from the training, namely that the two related stimuli had to be different. They combined these two forms of generic knowledge to choose efficiently when facing novel stimuli. This finding has wide implications for understanding how brains can form abstract conceptual relations, even in the absence of language. It raises questions about what is unique to humans and non-human primates and how cognitive processing can be achieved by minimal neural architectures such as that of a bee brain.

Honeybees are commonly used for studies on visual cognition as they can be easily trained to associate visual stimuli with sucrose reward¹². In this experimental context, they learn to navigate in complex mazes¹³, to categorize objects based on visual features^{14,15}, and to master discriminations based on identity^{16,17} and numerosity¹⁸⁻²⁰. Here we investigated whether honeybees can form abstract concepts based on spatial relationships such as above/below and right/left and use these concepts to choose appropriately between perceptually novel stimuli.

A Y-shaped maze was used to train individually marked bees on a discrimination task that involved simultaneous presentation of two spatial relationships: above/below and left/right. To this end, one arm of the maze presented either two achromatic patterns or two chromatic discs arranged vertically (one above the other), while the other arm presented the same stimuli arranged horizontally (one on the right of the other) (Figs.1a, 2a, 3a; ‘Training’). Bees were trained to enter into the Y-maze to collect a drop of sucrose solution delivered

whenever they chose the appropriate spatial relationship. During 30 learning trials (i.e. 30 foraging bouts between the hive and the Y-maze), half of the bees ($N = 14$) was rewarded on the above/below relation while the other half ($N = 14$) was rewarded on the right/left relationship. Within each group, half of the bees were trained with achromatic patterns while the other half was trained with colour discs (Figs.1a, 2a, 3a). Only one bee was trained at a time within the maze. The stimuli and their positions were systematically varied between trials, just preserving the above/below or left/right relationship as predictor of reward. In this way, we prevented bees from memorizing specific stimuli and absolute spatial locations to solve the discrimination task. To determine if bees were capable of extracting the spatial relationships *per se* irrespective of the physical appearance of the stimuli, and to uncover the nature of the concepts inculcated by the training, we performed non-rewarded transfer tests presenting novel stimuli (Figs.1a, 2a, 3a; ‘Transfer Tests’).

Bees learned to choose the rewarded spatial relationship between stimuli, irrespective of the relationship trained (above/below or left/right) and the visual modality used during the training (achromatic patterns or chromatic discs) ($P < 0.01$ in all cases; see acquisition curves in Fig. S1, Suppl. Mat.). Learning was extremely robust despite a systematic trial-to-trial variation of both stimuli and their absolute position in the bees’ visual field.

In a first experiment, we studied whether bees were able to transfer the learned spatial relationship to novel stimuli differing from those used during the training (Fig.1a, ‘Transfer Test’). In this way we determined whether stimulus similarity or a true relational concept was used by the bees while choosing in the Y-maze. Bees trained with achromatic patterns (Group 1; $N = 5$) were tested with colours while bees trained with colours were tested with achromatic patterns (Group 2; $N = 5$). In both cases, bees chose the appropriate spatial relationship in the transfer test (Fig. 1b; $P < 0.001$ for both Groups 1 and 2). No differences were found between transfer performances of Groups 1 and 2. Bees, therefore, used an abstract relational concept between stimuli (above/below or left/right) instead of being guided by their physical nature and perceptual similarity.

In a second experiment, we analyzed whether bees did indeed use a spatial concept or rather responded to broad orientation cues extracted by orientation detectors in the visual areas of their brains²¹. In other words, we aimed at distinguishing between learning that two stimuli were connected by a specific relationship (say, one above the other, or one on the right

of the other) and learning that a global cue (say, vertical, or horizontal, respectively) was associated with sucrose reward. Two groups of bees ($N = 5$ each) were trained either with achromatic patterns (Group 1) or colours (Group 2) to choose a specific spatial relationship (above/below or left/right). In two transfer tests (Fig. 2a; 'Transfer Tests'), we confronted bees with stimuli that were never used during the training. In Transfer Test 1, they had to choose between a large bar - vertical for above/below-trained animals and horizontal for left/right-trained animals - and two stimuli that were placed in the appropriate spatial relationship but which were slightly shifted with respect to each other (Fig. 2a; 'Transfer Test 1'). In Transfer Test 2, the same large bar was presented against two stimuli exhibiting the non-rewarded spatial relationship (left/right for above/below-trained animals and vice versa).

In Transfer Test 1, bees of both groups significantly preferred the two stimuli over the large bar (Fig. 2b: $P < 0.001$ for both Group 1 and Group 2; 'Transfer Test 1'), thus showing that they were guided by the presence of two stimuli in a specific spatial relationship (even if stimuli were slightly shifted with respect to each other) instead of using a strong orientation cue. Differences were neither found between transfer performances of Groups 1 and 2, nor between bees trained for above/below and bees trained for left/right (NS in all cases). In Transfer Test 2, bees of both groups showed no preference for either stimulus (Fig. 2b: NS for both Group 1 and Group 2; 'Transfer Test 2'), thus showing that the bar providing the salient orientation cue was as ineffective as the two stimuli in the inappropriate spatial relationship. These results confirm, therefore, that bees learned to choose two stimuli arranged in a specific spatial relationship, irrespective of the stimuli used to this end.

Finally, in a third experiment, we investigated if, besides learning that two stimuli had to be present in the appropriate spatial relationship, bees also paid attention to an additional concept derived from the training, namely that the two stimuli should be different. Bees were trained with either achromatic patterns (Group 1; $N = 4$) or colour discs (Group 2; $N = 4$) and then subjected to three transfer tests (Fig. 3a) in which they had to choose between two different stimuli and twice the same stimulus. In Transfer Test 1, the correct spatial relationship linked both kinds of stimuli; in Transfer Test 2, the incorrect spatial relationship linked them; finally, in Transfer Test 3, conflictive information was presented as the correct spatial relationship linked two identical stimuli while the incorrect spatial relationship linked two different stimuli.

In Transfer Test 1, bees of both groups always preferred two different stimuli arranged in the appropriate spatial relationship over two identical stimuli arranged in the same relationship (Fig. 3b: $P < 0.01$ for both Group 1 and Group 2; ‘Transfer Test 1’). No differences were found between transfer performances of Groups 1 and 2, or between bees trained for above/below and bees trained for left/right (NS in all cases). A similar result was found in Transfer Test 2; in this case, bees facing twice the incorrect spatial relationship always preferred two different stimuli over the same stimulus presented twice (Fig. 3b: $P < 0.01$ for both Group 1 and Group 2; ‘Transfer Test 2’). Again, no differences were found between transfer performances of Groups 1 and 2, or between bees trained for above/below and bees trained for left/right (NS in all cases). These results confirm that in addition of a specific spatial relationship, bees extracted from the training that they had to paid attention to the presence of two different stimuli. Finally, in Transfer Test 3, bees set in the conflict of choosing between the correct relationship linking two identical stimuli and the incorrect relationship linking two different stimuli showed no preference for either situation (Fig. 3b: NS for both Group 1 and Group 2; ‘Transfer Test 3’). No differences were found between transfer performances of Groups 1 and 2, or between bees trained for above/below and bees trained for left/right (NS in all cases). Thus, the rule guiding the bees consisted of two combined concepts: one that defined the spatial relationship between visual stimuli, and another that determined that stimuli had to be different.

These results demonstrate that the miniature brain of honeybees is capable of extracting relational concepts from experience and that such concepts can be combined to determine sophisticate performances that are independent of the physical nature of stimuli. Irrespective of whether stimuli were chromatic or achromatic, bees learned that they had to choose stimuli that were arranged in a specific spatial relationship and that were different. Thus, conceptual rules rather than perceptual similarity guided the choices of honeybees in these experiments.

This performance may appear surprising from an anthropocentric perspective that puts the accent on human and primate cognitive capabilities. Abstract concepts are not necessarily expected in an insect brain with its reduced size and amount of neurons (950 000 neurons in a honeybee brain vs. 100 billions in a human brain). However, honeybees learn to master sameness and difference relationships in delayed matching-to-sample and non-matching-to-sample protocols, respectively^{16,17,20}, thus exhibiting cognitive capacities that were

unsuspected in an invertebrate^{12,22}. Here we showed that such capacities even allow them to combine two abstract rules together (spatial and difference) to solve a complex discrimination task.

Relational thinking is considered to be a cornerstone of human perception and cognition³. From this perspective, psychological and modeling studies have attempted to establish the conditions underlying the learning of a conceptual relation. Three basic problems should be solved by a cognitive architecture capable of relational learning³: 1) to detect invariant features across instances of the relation; for instance to learn an above/below relation, the cognitive architecture must detect the invariant perceptual property of having one object being above another, regardless of the objects involved. In the case of honeybees, these invariants may be extracted through the retinotopic organization of the visual pathways in the brain, which would inform that there is stimulation on the upper and lower visual field upon above/below conditioning; 2) to isolate these invariants from other properties of the objects engaged in the relation to be learned; for instance, the cognitive architecture should become independent of object properties such as shape, size, colour, etc., thus focusing on the relevant relation; in other words, bees should extract the regularities of the visual scene from experience, a capacity that has been repeatedly shown¹⁵ and which could occur by synchronization of neurons firing through repeated exposure to different figures sharing regularities²³; 3) to code the binding *per se* in such a way that it can be applied to novel objects or situations; thus, in learning an above/below relationship, the cognitive architecture should possess neural circuits responding to the co-activation of units that signal that there are two simultaneous stimulations, one in the upper and the other in the lower visual field; such circuits are so far unknown in the case of the honeybee.

What do bees gain from extracting spatial concepts such as the ones trained in our work? Identifying spatial relationships between objects is useful for learning about generic configurations that underlie specific stimulus categories; if for instance, bilateral flowers share a common layout with upper and lower petals and a left/right symmetry, spatial relationships may help constructing the general configuration corresponding to what a bilateral flower should be^{9-11,24}. Furthermore, honeybees are central-place foragers navigating between the central place of the hive and their food sources, the flowers. Navigational strategies implemented by these insects are extremely sophisticated and may even rely on map-like representations of space²⁵. In this navigational context, dealing with abstract spatial

relationships such as ‘on the right of’ or ‘above of’ may be extremely helpful in terms of where landmarks are expected en route to the goal.

Methods

Free-flying honeybees were marked with a colour spot on the thorax and individually trained to collect 1M sucrose solution within a Y-maze²⁶. The maze was covered with an ultraviolet-transparent Plexiglas ceiling and illuminated by natural daylight. During both training and tests only one bee was present in the apparatus. The entrance of the maze led to a decision chamber defined by the intersection of the maze arms. Once in that chamber the bee could choose between the stimuli presented on the back walls of both arms. The back walls (20×20 cm) were placed at a distance of 15cm from the decision chamber.

In order to improve discrimination, a differential conditioning procedure was used²⁶ so that one stimulus was rewarded with 1M sucrose solution while another stimulus was penalized with 60mM quinine hydrochloride dihydrate solution²⁷. Solutions were offered in a micropipette inserted in the centre of the back walls. Each bee was trained along 30 trials (i.e. 30 foraging bouts between the hive and the laboratory) during which the sides of rewarded and non-rewarded stimuli (left or right) were interchanged in a pseudorandom sequence. In each trial, the first choice of a bee was quantified after it entered the maze. Tests lasted 45 sec during which the number of contacts with the surface of the stimuli was recorded. Neither sucrose nor quinine was offered during tests. Each test was done twice, interchanging the sides of the stimuli. Three to six refreshing training trials were intermingled between the tests. The sequence of tests was randomized from bee to bee.

Stimuli

Stimuli were presented on a grey background which covered the back walls of the maze. The background was cut from HKS-92N paper (K + E Stuttgart, Stuttgart-Feuerbach, Germany). Pairs of vertically or horizontally aligned achromatic black patterns (7×7cm) or colour discs (7cm in diameter) were used to establish above/below or left/right relationships. Six different colours discs were used. They were cut from HKS papers 1N, 3N, 29N, 32N, 68N and 71N

and could be well detected against the grey background. They could also be well discriminated from each other according to both the colour opponent coding space²⁸ and the colour hexagon²⁹, two perceptual spaces proposed for honeybee colour vision. The achromatic patterns were printed with a high-resolution laser printer on UV-reflecting white paper of constant quality. Six different patterns were used: a vertical grating, a horizontal grating, a checkerboard, a radial three-sectored pattern, and two concentric patterns (rings and squares). Various studies have shown that these patterns are well discriminated from each other by honeybees³⁰. Additionally, in transfer tests of the second experiment, achromatic or colour large bars (7×18 cm) were used in order to provide strong orientation cues, vertical or horizontal, depending on the orientation of the bar.

During the training, two groups of bees were trained in parallel in each experiment, one with achromatic patterns and another with colour discs. From the six stimuli available within each category, four were randomly selected as training stimuli, thus leaving two other stimuli for the transfer tests. The four training stimuli were arranged in pairs in order to define above/below and left/right relationships. All six possible combinations between the four training stimuli (six stimulus pairs) were used during the training, i.e. each pair was presented five times (30 learning trials in total). Additionally, vertically (above/below) and horizontally (left/right) aligned stimuli were presented in three different positions, either on the central axis or on both distal axes of the back wall. Stimulus pairs as well their position were randomly varied from trial to trial.

Statistics

Data were checked for normality using the Shapiro Wilk test. Acquisition was measured in terms of the percentage of correct choices during three blocks of training trials. Performance of balanced groups during training was compared using ANOVA for repeated measurements with groups and trial blocks as factors of analysis. Performance during the tests was analyzed in terms of the proportion of correct choices per test, producing a single value per bee to exclude pseudo-replication. A one-sample t-test was used to test the null hypothesis that the proportion of correct choices cumulated during the 45 sec of a test was not different from a random value of 50%. Data of all bees (above/below- and left/right-trained bees, as well as colour- and achromatic-pattern trained bees) were pooled upon analysis of test performances

as no significant difference were found between these different conditions. Between-group and between-test comparisons were made using t-tests for independent and paired samples, respectively. The alpha level for all analyses was 0.05.

Acknowledgements

A. Avarguès-Weber and M. Giurfa thank the French Research Council (CNRS), the University Paul Sabatier (Project APIGENE) and the National Research Agency (ANR: Project Apicolor) for generous support. A. Avarguès-Weber was supported by a Travelling Fellowship of The Journal of Experimental Biology, and by the University Paul Sabatier (ATUPS fellowship). A.G. Dyer acknowledges the ARC DP0878968 and DP0987989 for funding support.

Author contribution

A.A.W., A.G.D. and M.G. conceived and designed the experiments. A.A.W. performed the experiments. A.A.W. analyzed the data. A.A.W. and M.G. wrote the manuscript.

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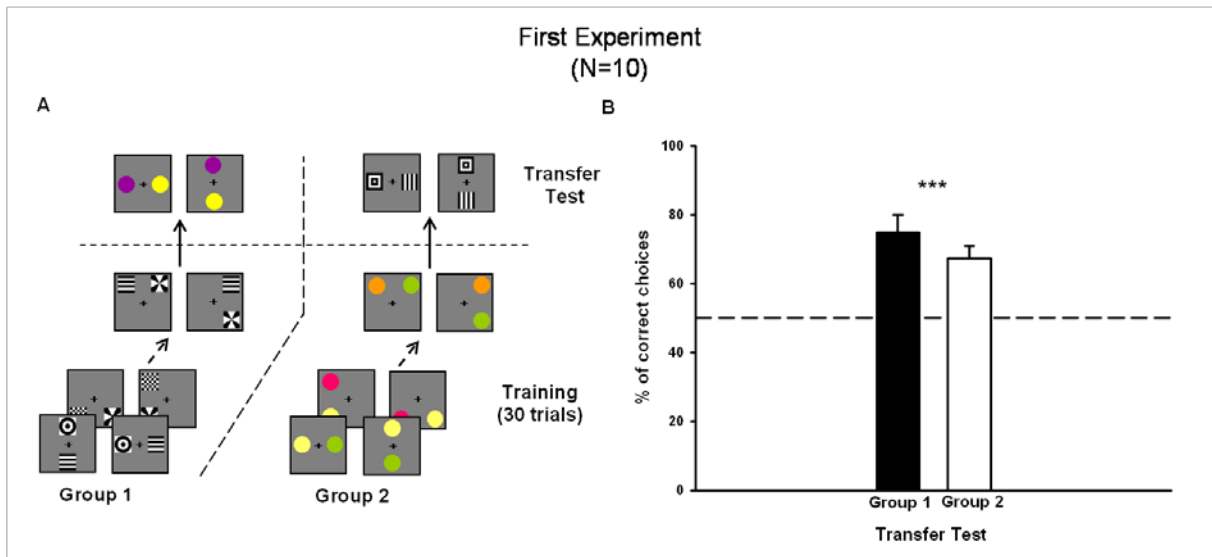


Figure 1: First experiment.

(A) Example of conditioning and testing procedure for two groups of bees: Group 1 trained with achromatic patterns and Group 2 trained with colour discs. Within each group, half of the bees were rewarded on the above/below relation whereas the other half was rewarded on the right/left relation. Novel stimuli were presented in the transfer test. (B) Performance in the transfer test (% of correct choices) of Groups 1 and 2. Bees preferred the novel stimuli arranged in the trained spatial relationship. Data shown are means + s.e.m. (N=5 for each bar; ***: $p < 0.001$).

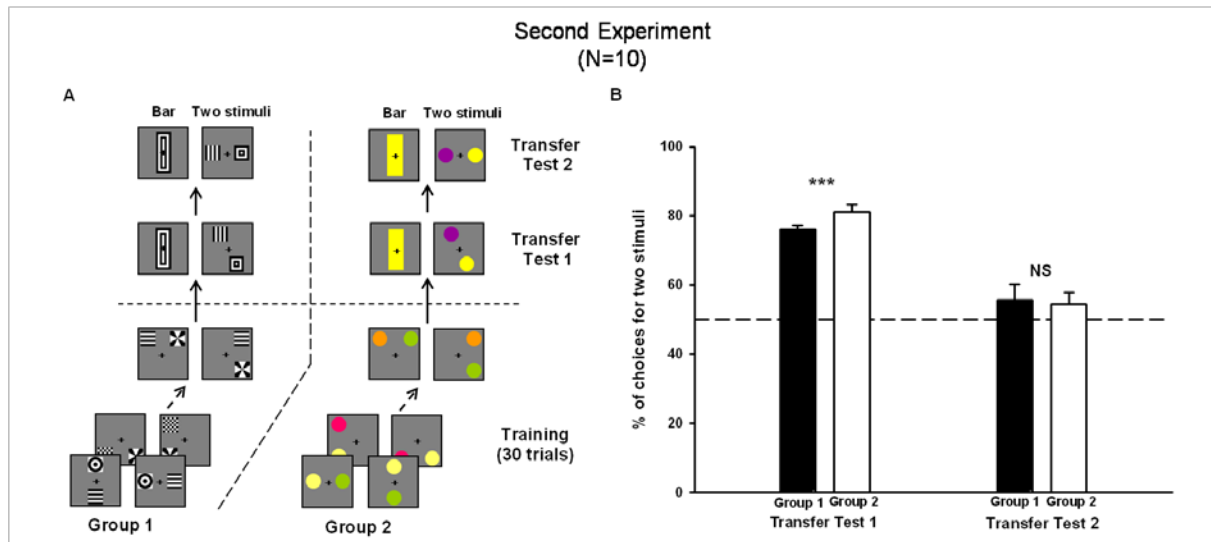


Figure 2: Second experiment.

(A) Example of conditioning and testing procedure for Group 1 trained with achromatic patterns and Group 2 trained with colour discs. Within each group, half of the bees were rewarded on the above/below relation whereas the other half was rewarded on the right/left relation. Transfer tests shown correspond to bees rewarded on the above/below relation. (B) Performance in the transfer tests (% of choices for the option displaying two stimuli). Bees preferred the two stimuli to the bar if they were linked by the appropriate relationship (Transfer Test 1) but not if they displayed the inappropriate relationship (Transfer Test 2). Data shown are means + s.e.m. (N=5 for each bar; ***: $p < 0.001$; NS: $p > 0.05$).

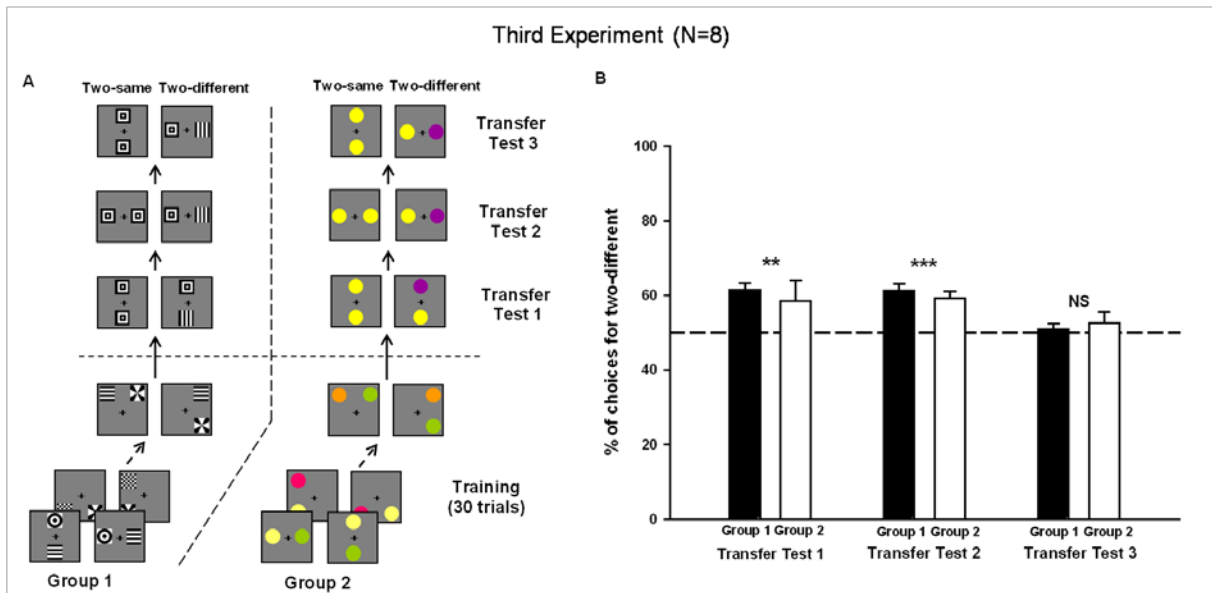


Figure 3: Third experiment.

(A) Example of conditioning and testing procedure for Group 1 trained with achromatic patterns and Group 2 trained with colour discs. Within each group, half of the bees were rewarded on the above/below relation whereas the other half was rewarded on the right/left relation. Transfer tests shown correspond to bees rewarded on the above/below relation. (B) Performance in the transfer tests (% of choices for the option displaying two different stimuli). Bees preferred two different to two identical stimuli both in the appropriate (Transfer Test 1) and the inappropriate relationship (Transfer Test 2) and chose randomly in a conflict situation (Transfer Test 3). Data shown are means and s.e.m. (N=4 for each bar; **: $p < 0.01$; ***: $p < 0.001$; NS: $p > 0.05$).

Supplementary information

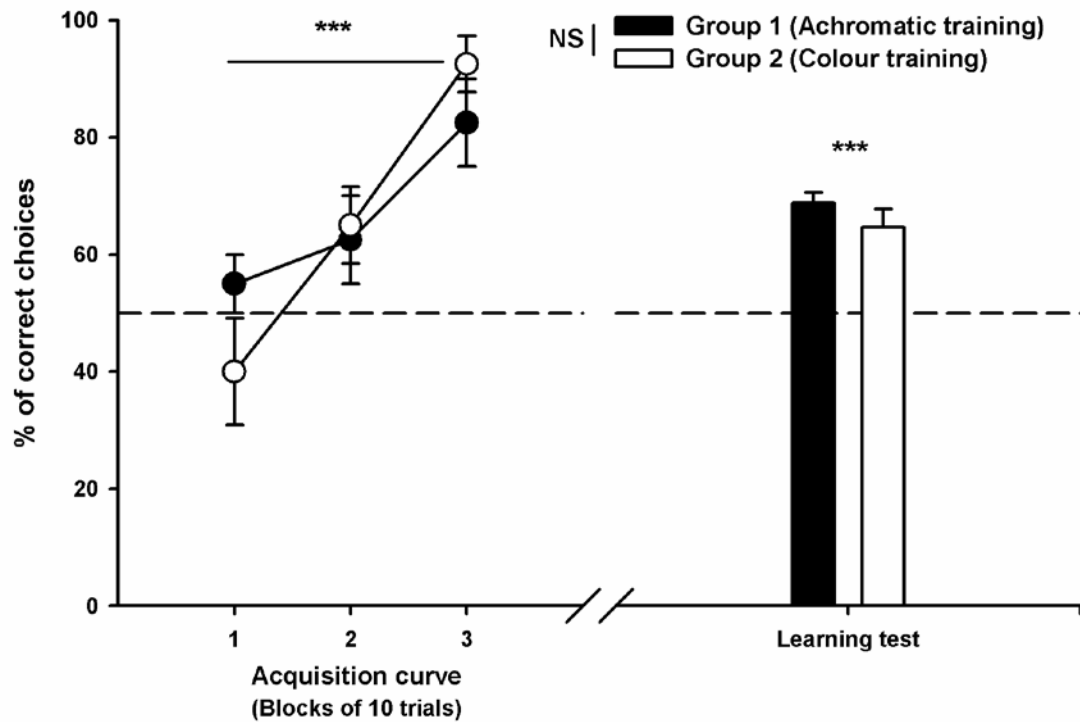


Figure S1: Acquisition curves during training (percentage of correct choices as a function of blocks of 10 trials) and performance (cumulative choices during 45sec test) in the non-rewarded learning test. After training, all bees were subjected to a learning test in which they faced novel stimuli from the trained visual modality (achromatic patterns or colours). Data shown are means and s.e.m. on all bees tested ($N=14$ for each group). The curve with black dots and the black bar represent data from the group 1 trained with achromatic patterns while the curve with white dots and the white bar represent data from the group 2 trained with coloured patterns. Differences were neither found between performance of Groups 1 and 2, nor between bees trained for above/below and bees trained for left/right (NS: $p>0.05$ in all cases). Pooled data for both groups show a significant improvement in the acquisition curve and are significantly different from a 50% random choice in the Learning test (***: $p<0.001$).

Discussion

Dans ce travail de thèse, nous nous sommes intéressés, dans une perspective comparative, à la sophistication cognitive de l'abeille dans l'analyse de son environnement visuel. Plus précisément, nous avons souhaité étudier la capacité d'un système visuel d'insecte à classer des stimuli visuels et des situations nouvelles à partir de processus de catégorisation basée sur des configurations spatiales et formation de concepts relationnels. Dans les deux cas, un traitement cognitif élaboré est nécessaire soit pour extraire et combiner les inter-relations entre les éléments d'une image en une configuration spatiale, soit pour manipuler des relations abstraites, indépendamment des éléments impliqués dans ces relations.

Le premier article de cette thèse nous a permis d'améliorer les protocoles traditionnels de conditionnement visuel chez les abeilles en libre vol par l'utilisation d'une solution amère de quinine comme renforçateur négatif associée aux stimuli non récompensés. Cette méthode permet d'améliorer les performances d'apprentissage et de discrimination visuelle des abeilles, notamment quand il s'agit de différencier des stimuli proches d'un point de vue perceptif. Ce résultat met donc en évidence que la perception des abeilles est un phénomène modulable par l'expérience et le degré de motivation des individus. Ce travail nous a permis ainsi d'élaborer un protocole de conditionnement plus efficace chez l'abeille, indispensable pour des études poussées de cognition visuelle nécessitant de longs apprentissages.

Dans le deuxième article présenté dans cette thèse, nous avons montré que les abeilles peuvent utiliser la configuration des éléments constituant une image pour établir des catégories. Ainsi, les abeilles ont été capables de répondre correctement face à de nouveaux stimuli présentant une configuration de type 'visage' après avoir été entraînées à associer cette configuration particulière à une récompense. La capacité à catégoriser des configurations visuelles en tant que visage ou non-visage n'a pas de contexte écologique évolutif en soi pour une abeille. En effet, les visages humains sont des stimuli sans signification biologique pour elles. Ce type de stimuli constitue cependant, grâce aux différents leviers de variations possibles n'affectant pas la configuration particulière (exemple : longueur du nez, écartement des yeux, etc...), un vecteur approprié pour l'étude des stratégies de catégorisation des abeilles. Il nous a ainsi permis de montrer que le système visuel des abeilles est capable d'analyser les stimuli visuels en extrayant et en combinant la disposition spatiale relative des éléments les composant. Cette faculté peut leur être particulièrement utile pour reconnaître rapidement les objets de leur environnement malgré des variations de texture, couleur, luminosité, taille, point de vue ou encore une obstruction partielle.

Nous avons aussi montré dans un troisième article que la perception et l'utilisation des configurations spatiales mises en évidence dans notre second article n'est pas un artefact dû à un entraînement spécifique mais semble au contraire être un comportement naturel pour l'abeille. En effet, grâce à l'utilisation de stimuli ayant la propriété de solliciter un niveau

d'analyse global et local, nous avons montré que, non seulement les abeilles ont pu extrapoler la forme géométrique globale en reliant les symboles géométriques locaux la composant, mais aussi qu'elles préfèrent suivre la configuration globale apprise au détriment des éléments locaux de l'image dans leur prise de décision.

Dans les quatrième et cinquième articles, nous avons mis en évidence que les relations spatiales entre les éléments d'une image pouvaient être appréhendées en tant que concepts, indépendamment des éléments impliqués. Les abeilles se sont en effet montrées capables d'utiliser la relation spatiale 'au dessus de', 'au dessous de' ou encore 'droite/gauche' entre deux éléments comme critère de récompense et ce, malgré l'introduction de nouveaux éléments très différents d'un point de vue perceptif. De plus, elles ont su combiner la relation spatiale apprise avec une seconde caractéristique commune aux stimuli proposés en une règle de choix face à de nouveaux stimuli. En effet, l'abeille a extrait de l'entraînement la notion que les deux éléments impliqués dans les relations spatiales doivent être différents. De telles facultés apparaissent comme nécessitant un traitement cognitif particulièrement sophistiqué afin d'élaborer des concepts abstraits à partir d'un jeu de stimuli et applicables de façon indépendante des propriétés physiques particulières des stimuli.

L'ensemble de ces résultats nous amène donc à discuter ici, dans un premier temps, l'influence de processus de type attentionnels pour expliquer les niveaux variables de perception des abeilles en fonction des méthodes d'entraînement. Dans un deuxième temps, nous discuterons de l'existence, chez l'abeille, d'une organisation cérébrale et neuronale du système visuel permettant la catégorisation de stimuli suivant leur configuration spatiale ou la détection et la conceptualisation d'une relation spatiale particulière. Nous verrons ensuite comment nos résultats peuvent s'inscrire dans les modèles de reconnaissance des objets existants avant de conclure sur l'intérêt de la présence et de l'étude de tels phénomènes cognitifs chez l'abeille.

Influence de processus de type attentionnel et de l'entraînement dans la perception de l'abeille

Nous avons montré dans le premier chapitre de cette thèse que l'utilisation d'une punition sous forme d'une solution aversive induit une amélioration sensible des performances d'apprentissage et de discrimination de couleurs, en particulier si celles-ci sont difficiles à discriminer. Ainsi, des abeilles entraînées à discriminer deux couleurs similaires, l'une étant associée à une récompense sucrée et l'autre à de la quinine, ont pu percevoir la différence entre les couleurs, tandis qu'à l'inverse, les abeilles pour lesquelles la quinine était remplacée par de l'eau dans les stimuli non récompensés, n'ont pas fait preuve de discrimination. Ce résultat met en évidence le fait que l'acuité visuelle de l'abeille ainsi que sa capacité d'apprentissage n'ont pas de limites absolues, qui seraient directement et purement

sous-tendues par ses caractéristiques physiologiques, mais sont au contraire dépendantes de l'expérience et du degré d'investissement de l'animal dans la tâche.

En effet, l'utilisation d'une substance aversive induit une augmentation du coût de l'erreur, ce qui peut être à l'origine d'une plus grande attention dans la prise de décision. Il a ainsi été montré chez le bourdon que l'utilisation de la quinine comme punition induit un changement de comportement vers une prise de décision plus lente et par conséquent plus précise (Chittka et al., 2003). Les bourdons sont en effet capables d'adapter leur rapidité de choix selon un compromis vitesse/réussite. Par conséquent, lorsque la difficulté de la tâche augmente au point d'induire de trop nombreuses erreurs en cas de réponse rapide, les bourdons prennent plus de temps pour choisir, ce qui permet une diminution de leur taux d'erreurs (Dyer et Chittka, 2004a). Il apparaît donc que le coût de l'erreur et la difficulté de la tâche influent sur le degré d'attention de l'animal.

Le niveau de précision de l'information apprise est lui aussi influencé par le mode de conditionnement. Lorsque l'on entraîne l'abeille avec une couleur seule associée à une récompense (conditionnement absolu) ses capacités de discrimination de la couleur apprise parmi d'autres couleurs proches sont limitées. Par contre, si l'abeille est entraînée à choisir entre deux couleurs présentées simultanément (conditionnement différentiel), ses facultés de discrimination sont fortement augmentées (Giurfa, 2004). Un résultat comparable est observé chez le bourdon (Dyer et Chittka, 2004b) ou des fourmis (Camlitepe et Aksoy, 2010). Par ailleurs, si les deux couleurs alternatives sont présentées l'une après l'autre pendant le conditionnement, les performances observées sont plus faibles que si on les présente simultanément (Dyer et Neumeyer, 2005). Cet effet montre que, lorsque l'insecte doit choisir entre plusieurs alternatives, la précision de l'objet mémorisé est plus importante, lui permettant ainsi de mieux le reconnaître dans les tests. Ceci peut être interprété comme la conséquence d'une sélectivité de l'attention. Dans le premier cas (conditionnement absolu), l'abeille se focalise seulement sur la présence d'une couleur tandis que dans le cas d'un conditionnement différentiel, elle doit se focaliser sur la différence entre les stimuli présentés. Apprendre la différence requiert la mise en place d'une analyse visuelle comparative entre les deux stimuli et solliciterait donc plus de ressources attentionnelles. Cette interprétation est en accord avec un résultat obtenu en entraînant des abeilles avec des motifs achromatiques complexes. Dans le cas d'un conditionnement absolu, l'abeille n'utilise qu'une partie du stimulus dans des tests de discrimination (la partie correspondant au champ visuel inférieur de l'abeille) tandis que des abeilles entraînées en situation de conditionnement différentiel élargissent leur analyse du stimulus visuel pour le traiter dans son intégralité (Giurfa et al., 1999). Ainsi, le fait d'avoir à différencier entre deux stimuli induit un apprentissage plus complet et précis de ses caractéristiques grâce à la nécessité d'une attention sur ce qui les différencie.

Par ailleurs, nos résultats des chapitres II à V ainsi que les travaux montrant une capacité de catégorisation visuelle basée sur un trait unique (symétrie bilatérale, symétrie radiale, etc... ; Benard et al., 2006) montrent qu'avec l'entraînement, les abeilles extraient les

caractéristiques communes (configuration spatiale ou concept relationnel) du jeu de stimuli présenté, afin de définir la catégorie récompensée. Ceci peut être interprété comme impliquant une focalisation de l'attention de l'abeille sur les éléments essentiels afin de résoudre la tâche, c'est à dire les traits communs définissant la catégorie. Ceci permet une simplification de l'information apprise et facilite le transfert de réponse à de nouveaux stimuli sur la base de la détection facilitée de ces éléments-clés (Benard et al., 2006). De même, il a été observé qu'avec l'augmentation du nombre d'essais de conditionnement, les abeilles ont plus de facilité à généraliser leur réponse face à de nouveaux stimuli partageant la même configuration que les stimuli de conditionnement (Stach et Giurfa, 2005). Là encore, ce résultat peut être interprété en termes de restriction des informations mémorisées aux seuls éléments utiles pour résoudre la tâche de discrimination.

Une implication de processus attentionnels se retrouve aussi dans les résultats obtenus par Zhang et Srinivasan (1994), montrant une perception d'objets camouflés rendue possible seulement après avoir pré-entraîné les abeilles avec les mêmes objets non camouflés. Ainsi, la focalisation sur les caractéristiques des objets appris a permis aux abeilles de les percevoir ensuite malgré leur camouflage (Figure 1).

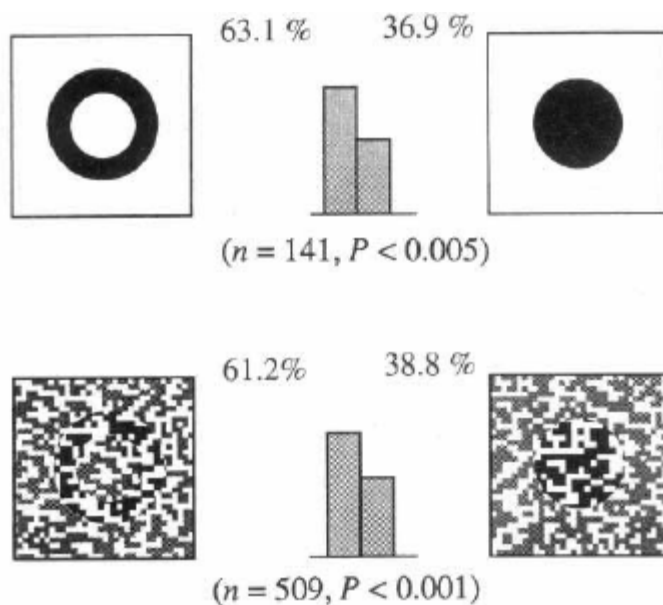


Figure 1: Résultats de l'expérience de Zhang et Srinivasan (1994). Les abeilles ont pu discriminer les deux stimuli camouflés du bas seulement après avoir été pré-entraînées avec les mêmes stimuli non camouflés (en haut).

Ainsi, si l'on définit les processus attentionnels comme étant à l'origine d'un traitement sélectif de l'information, les données présentées ci-dessus suggèrent leur existence chez l'abeille. Cela n'implique pas, néanmoins, que cela soit un mécanisme conscient, contrôlable par l'abeille. Les processus décrits ici seraient liés, de façon parcimonieuse, à une adaptation aux contraintes de l'apprentissage et des ressources mnésiques limitées. Même si les bases physiologiques restent encore à découvrir chez l'abeille, des études chez la drosophile mettent en évidence l'implication des corps pédonculés dans des phénomènes

similaires. En particulier, ils sont impliqués dans l'augmentation de la saillance d'un stimulus suite à un conditionnement ainsi que dans l'anticipation de la présence de ce stimulus régulièrement présenté, sous-tendue par (van Swinderen et Greenspan, 2003). Ces structures cérébrales particulières sont considérées comme sous-tendant les processus d'intégration et cognitifs de haut niveau car elles forment le centre de traitement multimodal de l'abeille (Giurfa, 2003).

Perspective : Il est possible de tester l'effet de processus attentionnels chez l'abeille en utilisant des stimuli composés présentant des symboles répétés (information locale) dont l'agencement spatial construit une forme géométrique (information globale) tels que ceux utilisés dans le chapitre V. Nous avons alors constaté une tendance naturelle de l'abeille à utiliser préférentiellement les formes globales pour choisir des stimuli. Serait-il possible de modifier cette préférence en colorant, par exemple, l'un des symboles locaux dans les premiers essais d'entraînement, ce qui aurait pour conséquence de focaliser l'attention des individus testés?

Théories de reconnaissance d'objets

Reconnaissance au niveau individuel

Les théories sur la reconnaissance d'objets peuvent se regrouper en deux sous-ensembles : les théories structuralistes qui prônent l'analyse des relations entre les différentes parties des objets et les théories basées sur l'image, pour lesquelles la reconnaissance se fait par comparaison avec des images stockées en mémoire (Palmeri et Gauthier, 2004). Ces deux ensembles de théories ont leurs partisans et il s'avère difficile de trancher entre les deux options car elles sont toutes deux soutenues par des arguments expérimentaux à la fois chez le primate (Palmeri et Gauthier, 2004) et le pigeon (Huber, 2001; Kirkpatrick, 2001).

Dans le premier cas, comme nous l'avons vu en introduction, ce seraient les configurations des objets qui permettraient leur identification. Selon la théorie de « reconnaissance par composants » (RBC) proposée par Biederman (1987), il serait même possible de composer toutes les classes d'objets avec seulement une trentaine de formes géométriques nommées geons (« geometric ions ») simplement en les agençant de façon différente (Figure 2A). L'information apportée par la configuration serait si robuste que seuls 2 à 3 geons dans une configuration appropriée (inter-relations spatiales et tailles relatives) suffiraient pour reconnaître un objet (Figure 2B). Cette théorie prônant l'analyse de la disposition des composants d'un stimulus visuel est appuyée par des résultats expérimentaux démontrant l'importance des relations spatiales dans la reconnaissance (Biederman, 1972; Biederman et al., 1973; Biederman et al., 1974). D'autres modèles de reconnaissance

structurale sont ensuite venus compléter cette théorie (Peterson et Rhodes, 2003; Palmeri et Gauthier, 2004). L'hypothèse d'une analyse structurale des objets est séduisante car elle permet d'expliquer les bons niveaux de reconnaissance atteints même lorsque l'on voit l'objet sous un autre éclairage, une autre position ou taille, d'un autre point de vue ou encore partiellement caché par un autre objet (Biederman, 1987; Kirkpatrick, 2001; Palmeri et Gauthier, 2004).

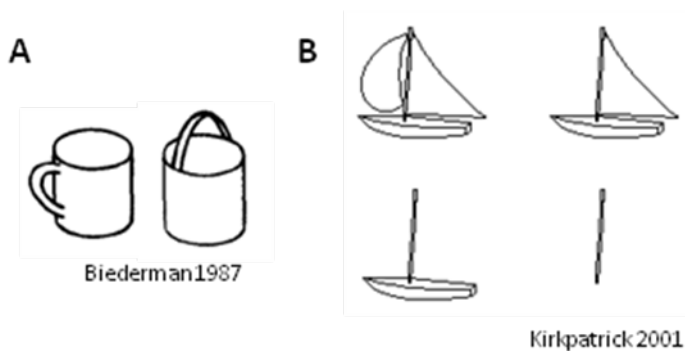


Figure 2: Théorie par « Reconnaissance de composants » (RBC) (Biederman, 1987). (A) Exemple de deux objets différents obtenus en changeant l'agencement des mêmes composants. (B) Objet composé de 4 éléments. La reconnaissance est toujours possible lorsque seuls 2 ou 3 éléments sont présents.

Chez l'animal, les données les plus abondantes concernent le pigeon. Le pigeon apparaît lui aussi sensible aux relations spatiales entre les éléments d'une image et présente de même une invariance de translation, de taille, de point de vue et une certaine résistance à l'obstruction partielle (Kirkpatrick, 2001). Par ailleurs, si les différents éléments d'une image sont intervertis de façon à détruire la configuration initiale, on observe une forte baisse des résultats. Enfin, si l'on dissocie les différents éléments d'une image en les écartant tout en maintenant leur disposition relative, la reconnaissance n'est alors pas altérée (Figure 3) (Kirkpatrick-Steger et al., 1998).



Figure 3: Stimuli utilisés pour étudier les performances des pigeons face à des configurations déconnectées ou mélangées (Kirkpatrick-Steger et al., 1998)

Dans le cas de reconnaissance par comparaison mentale avec des images apprises, l'invariance de reconnaissance dans le cas des changements de position ou de taille peut être obtenue par une simple transformation mentale de l'image perçue pour qu'elle s'apparie avec l'image stockée en mémoire. Cependant, la capacité de pouvoir reconnaître un objet sous différents points de vue ou éclairages implique le stockage de multiples vues de l'objet (Palmeri et Gauthier, 2004), avec de ce fait des besoins mnésiques très importants.

Concernant l'abeille, nos résultats présentés dans le chapitre II et surtout ceux du chapitre III semblent pencher en faveur d'une utilisation des configurations spatiales pour reconnaître des objets, du moins dans la recherche de nourriture. Dans les exemples du chapitre III, en effet, l'abeille ignore les détails de l'image dans une situation de conflit et suit plutôt la configuration générale présentée par l'image. Cependant, l'hypothèse de l'appariement avec une image stockée peut aussi permettre d'expliquer les résultats observés si on prend en compte une capacité de généralisation. Ainsi, le choix des dans la situation de conflit du chapitre III peut s'expliquer en termes de degré d'appariement avec le stimulus récompensé. En effet, le stimulus proposant la configuration apprise bien que composée des mauvais éléments locaux choisi par les abeilles présente une stimulation rétinienne plus proche du stimulus appris que la solution alternative grâce à une localisation identique des éléments locaux. A l'heure actuelle, nos résultats ne nous permettent donc pas de trancher façon certaine sur le type de représentation des objets particuliers chez l'abeille.

Reconnaissance de la catégorie d'appartenance

Si l'on s'intéresse maintenant à un autre niveau de reconnaissance, celui de la catégorie d'appartenance, on retrouve le même type de démarcation entre deux ensembles de théories : i) la théorie des exemplaires, selon laquelle les différents stimuli rencontrés sont mémorisés et les nouveaux stimuli sont classés selon leur degré de ressemblance avec les exemplaires de chaque catégorie ou avec un prototype correspondant à la moyenne des exemplaires ; ii) la théorie des paramètres, impliquant une extraction des paramètres communs aux exemplaires de la catégorie pour la définir (Zayan et Vauclair, 1998; Huber, 2001; Palmeri et Gauthier, 2004).

Chez l'abeille, il semble possible d'exclure la première théorie. Dans le cas de catégories basées sur la symétrie bilatérale par exemple (cf. Figure 6 de l'introduction) (Giurfa et al., 1996), les différents stimuli proposés diffèrent grandement d'un point de vue perceptif si ce n'est le partage d'un paramètre commun : la présence d'un axe de symétrie. Dans nos expériences montrant la capacité de former des catégories conceptuelles relationnelles (chapitre V), les abeilles ont pu classer des stimuli d'une autre modalité visuelle dans la bonne catégorie sur la base de la présence de la bonne relation (paramètre commun) malgré une importante distance perceptive. On peut néanmoins argumenter que ces stimuli

restent plus proches des stimuli de la catégorie récompensée que de ceux de la catégorie non récompensée. Cependant, les résultats de la troisième expérience de ce chapitre sont encore plus convaincants, les abeilles choisissant alors un stimulus présentant deux éléments différents dans la mauvaise relation spatiale au détriment d'un stimulus présentant deux éléments identiques dans la même relation. Les abeilles ont donc fait leur choix sur la base d'un paramètre définissant la catégorie (différence entre les éléments impliqués dans la relation) plutôt que sur la base d'une similarité avec les exemplaires des deux catégories. En effet, le stimulus choisi lors de ce test est un parfait exemple de la catégorie non récompensée et aurait donc dû être évité. Par contre les exemples de catégorisation des chapitres II et IV ne permettent pas de trancher entre les deux alternatives. Dans ces cas-là, en effet, les nouveaux stimuli présentés lors des tests de transfert restent plus proches des exemplaires de la catégorie récompensée que de l'autre, bien que l'utilisation d'un paramètre particulier, la configuration ou la relation spatiale, permette aussi d'expliquer les performances des abeilles.

Chez la primate, il a été mis en évidence chez le macaque l'existence de cellules dont l'activité se spécialise avec l'entraînement aux seuls paramètres utiles pour la classification d'images en deux catégories (Figure 4 ; Sigala et Logothetis, 2002; Miller et al., 2003; Op de Beeck et Baker, 2010). De plus, l'Homme s'avère particulièrement rapide dans la détection d'objets d'une certaine catégorie au sein d'images complexes (jusqu'à moins de 100 ms dans le cas de visages) ce qui s'accorde difficilement avec l'hypothèse d'une comparaison par le système nerveux avec des images mémorisées, à l'origine d'un délai avant de pouvoir reconnaître la catégorie d'appartenance (Kirchner et Thorpe, 2006; Crouzet et al., 2010).

Il est donc possible que la catégorisation, qui nécessite un traitement équivalent de stimuli parfois très différents serait médiée par un système basé sur l'extraction des paramètres utiles (des configurations par exemple) tandis que la reconnaissance individuelle des objets serait plus basée sur la mémorisation des différentes occurrences de l'objet permettant de le reconnaître facilement malgré des variations d'éclairage par exemple mais pas de couleur ou de texture (Logothetis et Sheinberg, 1996; Palmeri et Gauthier, 2004).

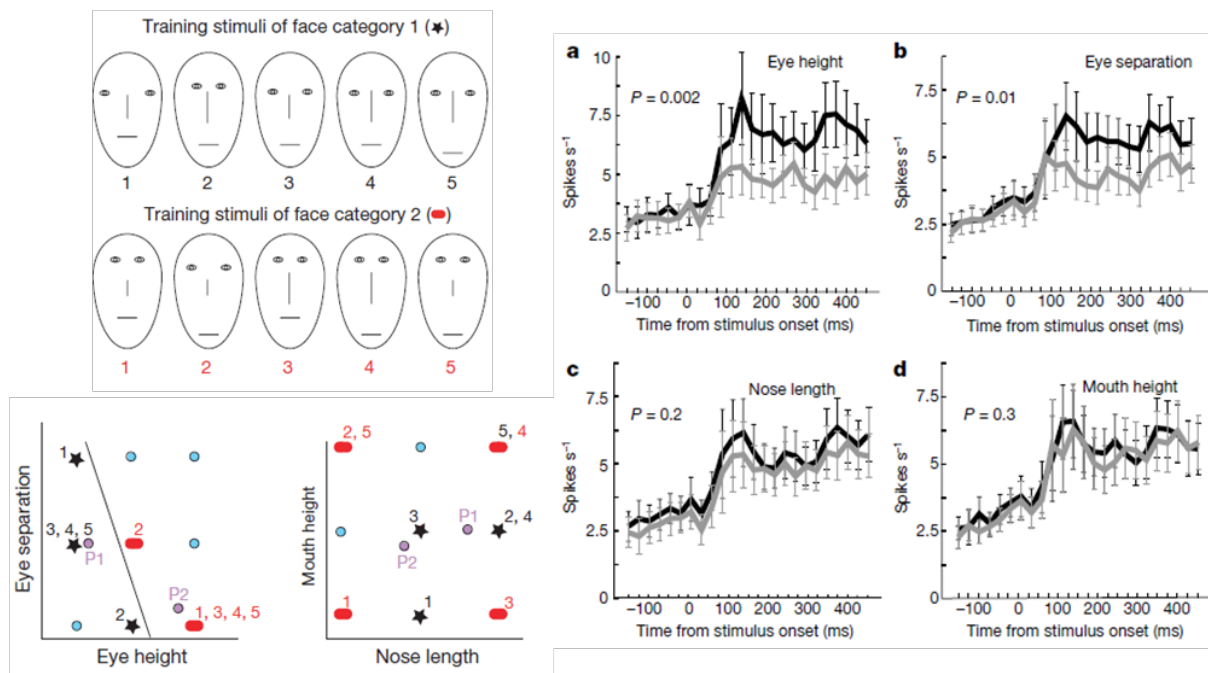


Figure 4: Expérience de Sigala et Logothetis (2002). A) Stimuli utilisés. Ils varient selon quatre paramètres. Les deux catégories peuvent être différenciées sur la base de la conjonction de deux paramètres: la hauteur des yeux et leur séparation et non sur la base de la longueur du nez ou la hauteur de la bouche (voir B) les étoiles représentent une catégorie tandis que les traits rouges représentent l'autre catégorie). C) Moyenne des réponses spécifique des neurones enregistrés pour chaque paramètre avant l'apprentissage (gris) et après (noir).

Influence de l'expertise

Avec l'acquisition d'une expertise pour une catégorie d'objets (par exemple l'exposition répétée à des visages pour l'ensemble des Êtres humains ; les voitures, les oiseaux, les empreintes digitales, etc. pour les spécialistes), il semble que l'on ait tendance à analyser ces objets d'une façon holistique. Il devient ainsi plus difficile d'analyser séparément les parties composant ces objets (Kanwisher, 2000; Maurer et al., 2002; Palmeri et Gauthier, 2004). Il est ainsi plus facile de reconnaître un élément d'un visage (par exemple le nez) dans le contexte du visage entier que s'il est présenté isolé (Tanaka et Farah, 1993; Tanaka et Sengco, 1997). Cette spécificité est perdue si le visage est présenté à l'envers. Il est de même plus difficile de reconnaître la moitié supérieure d'un visage par exemple, si elle est associée à la moitié inférieure d'un autre visage. Le visage composé apparaît alors comme un nouveau visage (Figure 5A ; Young et al., 1987). Ce n'est pas le cas si on utilise des visages à l'envers ou si l'on décale latéralement une moitié par rapport à l'autre. La même conclusion s'applique si l'on change la partie externe du visage (Figure 5B ; Sinha et Poggio, 1996). Par ailleurs, l'illusion de Tatcher (Figure 5C) montre que le changement de l'orientation des éléments d'un

visage (changement de configuration) apparaît presque inaperçu sur des visages à l'envers par comparaison aux mêmes visages modifiés présentés à l'endroit (Thompson, 1980). Une étude plus poussée de ce phénomène montre un changement brutal de notre perception de l'illusion à partir d'une rotation de 90° (Murray et al., 2000 ; à tester sur la Figure 5C). Un tel effet de l'orientation n'apparaît pas si les composants de l'image sont totalement changés (par exemple ajout de dents noircies ou blanchissement des yeux) au lieu d'être retournés (Murray et al., 2000).

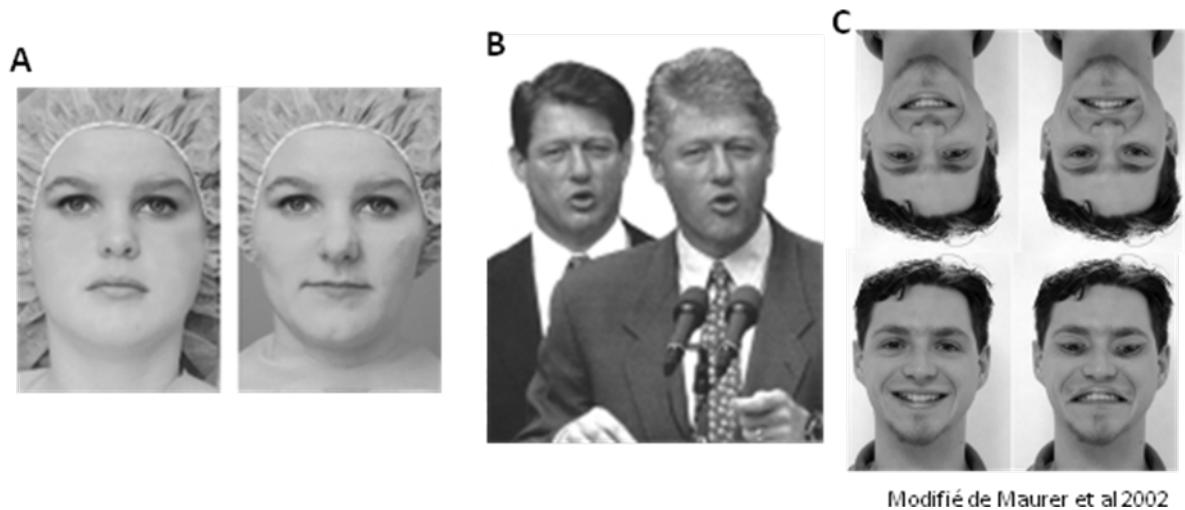


Figure 5: Manipulations démontrant le traitement holistique des visages. (A) Il est difficile de reconnaître que les moitiés supérieures des deux visages sont identiques lorsqu'on les combine à des moitiés inférieures différentes. (B) Il est difficile de reconnaître le visage de Bill Clinton lorsque l'on change la partie extérieure du visage. (C) L'illusion de Thatcher montre que des changements d'orientation d'éléments du visage apparaissent bien plus importants quand le visage est à l'endroit plutôt que retourné.

De façon conjointe, on observe l'existence de neurones dans le cortex inférotemporal, structure impliquée dans les phénomènes de reconnaissance visuelle, qui s'activent fortement à la présence d'un visage complet quelque soient les caractéristiques physiques de ce visage, mais pas à la présentation des parties d'un visage ni à d'autres stimuli (Kanwisher, 2000). Chez les primates, des neurones répondant à différents exemplaires de la catégorie « arbre », « poisson » ou « chien » ont pu être mis en évidence avec l'entraînement (Miller et al., 2003; Op de Beeck et Baker, 2010). De même, l'étude de Gauthier et Tarr (1997) montre l'acquisition d'un traitement holistique avec l'expertise. Après avoir entraîné de façon intensive des sujets à identifier et reconnaître des « greebles », représentations 3D d'objets qui peuvent être regroupés en famille et genre sur la base de caractéristiques communes (Figure 6), les auteurs ont pu analyser l'effet de l'expertise sur le traitement holistique. Les sujets ont alors montré des difficultés à reconnaître des parties de greebles isolés ou fusionnées avec un autre greeble pour en reconstituer un entier. Des données similaires ont été trouvées chez des experts en races canines, des ornithologues (Gauthier et al., 2000), des experts de voitures (Gauthier et al., 2000) ou encore d'empreintes digitales (Busey et Vanderkolk, 2004).

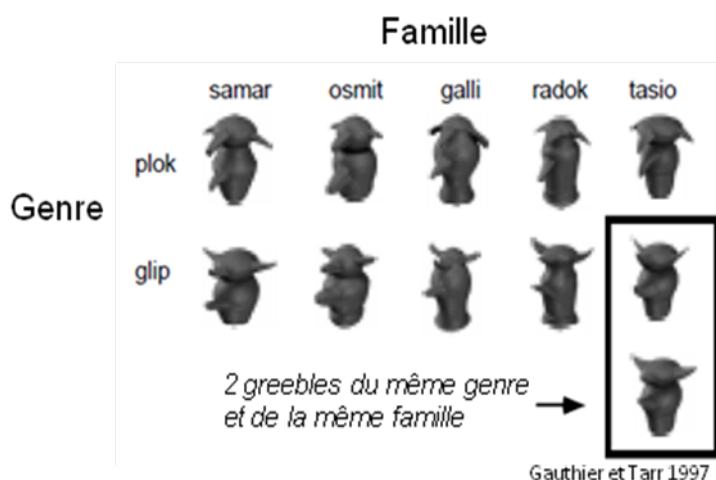


Figure 6: Exemples de « Greebles » utilisés dans l'étude de Gauthier et Tarr (Gauthier et Tarr, 1997) sur l'apparition d'un traitement holistique de stimuli du fait de l'expertise.

Cependant, les mécanismes neuronaux sous-jacents à l'acquisition d'un traitement holistique ne sont pas déterminés (Palmeri et Gauthier, 2004). En effet, une simple détection de la co-activation des unités neuronales associées aux différents éléments d'un visage, ne permettent pas d'expliquer de façon précise l'effet composite observé Figure 5A et B par exemple. Néanmoins, le traitement holistique est à l'origine d'une rapidité et d'une précision de discrimination plus importante entre les membres d'une catégorie. Cela peut s'expliquer par une meilleure perception des différences subtiles entre les stimuli, par exemple des différences spatiales dites de second ordre, c'est-à-dire impliquant des variations fines de distance entre les différents éléments (Maurer et al., 2002; Palmeri et Gauthier, 2004). Le traitement holistique permettant des discriminations plus fines, il pourrait être sollicité pour toute classe de stimuli présentant des variations subtiles entre eux et pour lesquels nous avons atteint un niveau d'expertise nous permettant de les différencier aisément. Cependant, l'hypothèse d'une utilisation d'un traitement holistique pour des objets chez l'Homme est controversée car de nombreux arguments vont plutôt dans le sens d'une spécificité du traitement holistique pour les visages (Kanwisher, 2000; Maurer et al., 2002).

Chez l'abeille, il n'existe pas d'études spécifiques sur la possibilité d'acquérir un traitement holistique avec l'expérience. Cependant, différents éléments nous laissent supposer qu'il ne sera pas déraisonnable de l'envisager. Avec l'entraînement, les abeilles se mettent à traiter des stimuli composés de deux disques de couleurs comme étant différents de la somme des deux couleurs. Bref, d'un traitement analytique du stimulus, où celui-ci est perçu comme la somme linéaire de ses deux composantes, on passe avec l'entraînement, à une situation où le stimulus est perçu comme une image unique, différente de la somme des composantes (Giurfa et al., 2003). De plus, les abeilles peuvent faire preuves de capacités de discrimination étonnantes entre des stimuli complexes appartenant à une même classe d'objet. Cette capacité pourrait être éventuellement sous-tendue par un traitement holistique (Figure 7 ; Dyer et al., 2005; Dyer et al., 2008).

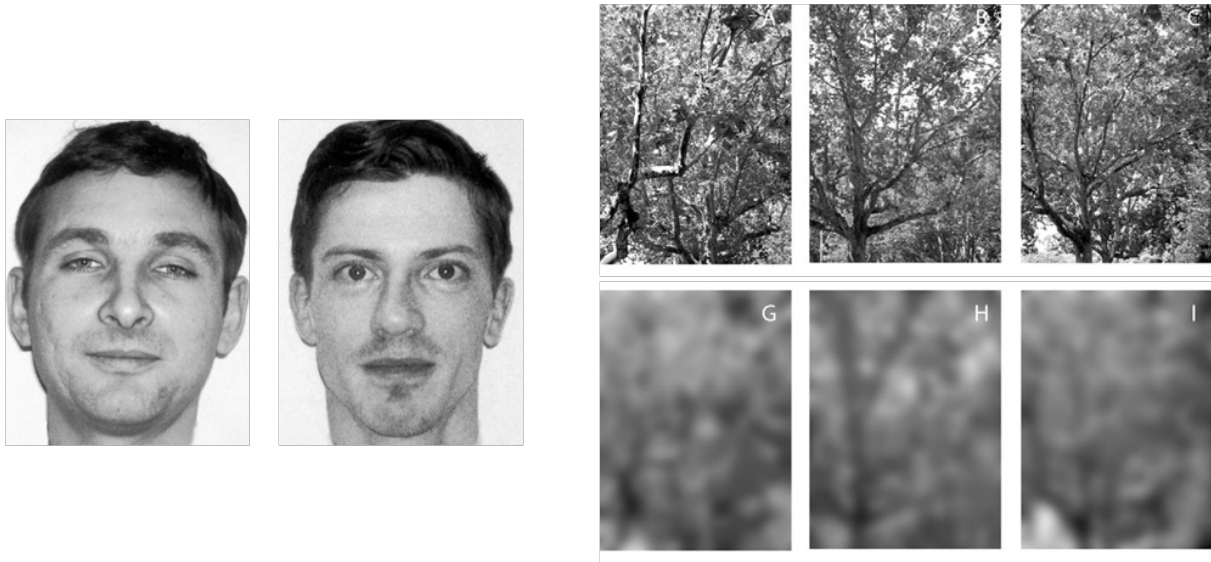


Figure 7: Stimuli utilisés dans les expériences ayant mis en évidence une discrimination fine chez l'abeille entre des visages humains (Dyer et al., 2005) ou des paysages de forêt (Dyer et al., 2008). En bas, les images ont été traitées de façon à simuler l'acuité visuelle de l'abeille et donc sa perception des paysages présentés au-dessus.

Perspective : Dans ce contexte, il serait intéressant de tester l'acquisition d'un traitement holistique chez l'abeille suite à un entraînement poussé. En utilisant des stimuli composés, il est possible de tester les réponses des abeilles dans des situations de discrimination entre deux éléments, suivants qu'ils soient présentés en isolation ou dans le contexte d'un stimulus complet. Enfin, les abeilles peuvent être testées dans leur capacité à reconnaître un élément d'une image complexe placé dans un contexte différent.

Bases physiologiques de la catégorisation visuelle

Catégorisation perceptive

La formation de catégories perceptives implique la détection des caractéristiques partagées par des stimuli biologiquement ou fonctionnellement corrélés. L'animal peut ensuite produire un comportement adapté face à de nouvelles occurrences de la catégorie créée grâce à un transfert de ses connaissances accumulées sur les stimuli connus de la catégorie.

Cette faculté apparaît donc comme une preuve d'apprentissage non-élémentaire dans le sens où l'animal apprend à répondre de façon appropriée à des stimuli n'ayant pas fait directement l'objet d'association pavlovienne simple de type stimulus-récompense.

Néanmoins, si on s'intéresse aux bases neurales permettant cette catégorisation, il apparaît que des phénomènes simples, voire pavloviens, peuvent permettre d'expliquer le traitement identique des différents éléments d'une catégorie.

Prenons le cas d'une catégorie basée sur un paramètre commun comme l'orientation. Les travaux de van Hateren (1990) présentés dans l'introduction montrent que les abeilles apprennent à classer différents types de motifs rayés selon l'orientation des rayures par rapport à la verticale. Une telle information est facilement détectable par le système visuel de l'abeille car celui-ci possède des neurones détecteurs d'orientation comparables à ceux identifiés dans la première aire visuelle V1 des mammifères (Hubel et Weisel, 1968; Op de Beeck et Baker, 2010). Ainsi, des patterns présentant une orientation définie vont induire l'activation spécifique de neurones particuliers présents dans les lobes optiques (Yang et Maddess, 1997). Quelque soient les différences de motifs, deux stimuli présentant la même orientation induiront le même schéma d'activation de ces détecteurs. Grâce à ces détecteurs, les performances de l'abeille peuvent s'expliquer en impliquant une synchronisation temporelle d'activation de ces neurones avec ceux impliqués dans la détection de la récompense sucrée. Un tel neurone, VUM_{mx1} , est connu chez l'abeille mais est spécifique du circuit olfactif (Hammer, 1993). Ce neurone a pour propriété fonctionnelle de coder la présence de sucre dans le cerveau de l'abeille. Le fait qu'il contacte le circuit olfactif dans les endroits où l'on observe des phénomènes de plasticité dépendante de l'apprentissage fournit le substrat pour les associations entre représentation neurale de l'odeur et celle du sucre. D'autres neurones de type VUM ont été décrits anatomiquement dans le cerveau de l'abeille sans que l'on sache quelle est leur fonction actuellement (Schröter et Menzel, 2007). Certains d'entre eux pourraient fournir un signal de renforcement à des neurones spécifiques du circuit visuel et ainsi permettre des associations comme celle entre orientation et récompense de sucre. A l'heure actuelle, seuls des détecteurs d'orientation ont été décrits chez l'abeille mais il est probable que d'autres types de détecteurs sensibles par exemple à différentes fréquences spatiales où à des configurations radiales ou annulaires soient présents.

Cependant, dans le cas où des paramètres plus complexes doivent être extraits afin de définir la catégorie (par exemple dans le cas d'une configuration d'éléments définissant la catégorie), il est difficile d'imaginer l'implication seule de détecteurs spécifiques naturellement présents dans les aires visuelles du cerveau de l'abeille. Ici, les résultats de modélisation obtenus par Masquelier et Thorpe (2007) permettant d'expliquer la détection des éléments utiles d'une image dans la reconnaissance de sa catégorie d'appartenance par le système visuel humain semblent particulièrement intéressants dans la mesure ils ne présupposent pas de 'supervision' dans l'extraction des éléments communs aux images d'une classe. Leur modèle se base sur la STDP (spike timing dependant plasticity), qui induit une modification de l'importance relative de chaque synapse d'un neurone en fonction du timing d'arrivée des potentiels d'activation (Song et al., 2000; Guyonneau et al., 2005). Ainsi, lorsqu'un neurone est systématiquement soumis à des inputs similaires, la STDP permet de réduire l'information aux éléments les plus utiles en donnant plus de poids aux synapses correspondant aux afférences qui s'activent en premier. Les auteurs montrent que grâce à ce

procédé, des réseaux neuronaux peuvent se spécialiser rapidement dans la détection des éléments saillants et présents de façon constante dans un ensemble d'images naturelles, ce qui permet une reconnaissance efficace des objets. L'existence de ce mécanisme chez l'abeille permettrait d'expliquer leur capacité à créer de nouvelles catégories basées sur des paramètres complexes malgré un système nerveux aux capacités computationnelles limitées.

Nous constatons donc que, même si d'importants travaux restent encore à faire pour découvrir les bases physiologiques des catégorisations perceptives complexes chez l'abeille, il semble raisonnable de faire l'hypothèse que les mécanismes impliqués sont relativement simples et donc envisageables en routine dans un cerveau d'invertébré. Cependant, la formation et la manipulation de concepts tels que démontrés dans les chapitres III et IV exigent un traitement cognitif plus élaboré car elles ne dépendent pas de similarité perceptives et reposent donc sur un niveau d'abstraction plus élevé.

Formation de concepts relationnels

La formation et l'utilisation de concepts relationnels est un véritable défi pour un système nerveux. En effet, la formation de catégories conceptuelles repose sur l'existence d'un substrat neuronal activé spécifiquement par la présence de la relation et ce, indépendamment des éléments la composant. Par exemple, le concept « au-dessus » doit présenter le même schéma d'activation quelque soit l'objet situé au-dessus d'un autre objet (Doumas et al., 2008; Halford et al., 2010). Cependant, il est impossible d'observer dans notre environnement un exemple de relation « au-dessus » sans avoir un objet spécifique au dessus d'un autre. Il n'est donc pas trivial *à priori* pour un système nerveux de coder la relation d'une façon totalement indépendante des éléments la composant. Chez le primate, la conceptualisation implique le cortex préfrontal, dernier centre d'intégration et de traitement cognitif (Miller et al., 2003; Halford et al., 2010). L'abeille ne possède évidemment pas de cortex préfrontal mais possède-t-elle néanmoins les structures cérébrales permettant l'abstraction de relations ?

Pour permettre un traitement équivalent de toutes les instances appartenant au concept, nous avons vu dans le paragraphe précédent que le cerveau doit détecter et isoler l'invariance entre les différents stimuli présentant la relation et coder de façon fiable cette relation en termes d'activité neurale. Dans le cas de relations de nature spatiale comme celles étudiées dans les chapitres IV et V (au dessus de ou au dessous de ; a gauche de ou a droite de), le cerveau de l'abeille doit tout d'abord conserver l'information spatiale issue des stimuli visuels. Ceci est possible grâce à l'organisation rétinotopique des circuits visuels dans le cerveau de l'abeille (voir Introduction). Ainsi on peut imaginer que la stimulation simultanée et systématique des champs visuels supérieurs et inférieurs (dans le cas d'une relation au dessus/au dessous) aura un corrélat neural sous forme d'activité régionalisée dans différentes aires du cerveau de l'abeille.

Le deuxième critère nécessaire à l'établissement d'un concept spatial est la possibilité d'isoler la relation des caractéristiques perceptives particulières des éléments la composant ainsi que la taille globale du stimulus, sa position dans le champ visuel, etc. Comme nous l'avons discuté plus haut, il est démontré que l'abeille est capable d'extraire les paramètres communs d'un jeu d'images (Benard et al., 2006). Bien que les mécanismes physiologiques soient encore inconnus chez l'abeille dans le cas de paramètres complexes, il semble que des expositions répétées à divers stimuli de la catégorie puissent conduire à une potentialisation de réponse des réseaux neuronaux pour les paramètres saillants et partagés par les différents stimuli. L'entraînement avec des stimuli variés est donc essentiel pour la conceptualisation d'une relation. Ainsi dans l'exemple de la relation « au-dessus/en-dessous » du chapitre V, l'entraînement doit conduire à l'activation d'unités neuronales capables de détecter la réponse simultanée de neurones signalisant 'en haut' d'un côté et 'en bas' de l'autre côté (Doumas et al., 2008). L'existence de telles unités n'est pas connue chez l'abeille, néanmoins il est tentant de suggérer que les corps pédonculés puissent jouer ce rôle. Ces structures cérébrales sont en effet un centre de traitement de l'information visuelle traditionnellement associé à des tâches cognitives d'ordre supérieur (Menzel et Giurfa, 1999; 2001; Giurfa, 2003; Devaud et al., 2007; Giurfa, 2007). On peut d'ailleurs en gardant les distances nécessaires et en prenant compte des différentes histoires évolutives, faire le parallèle entre ces structures qui présentent un développement au cours de l'évolution des arthropodes pour atteindre la complexité de structure et de connexion la plus importante chez les insectes sociaux, et le cortex préfrontal qui représente une part de plus en plus importante du cerveau chez les mammifères, des rongeurs aux primates avec le développement maximal chez l'Homme (Farris, 2008; Strausfeld, 2009). De plus, au cours de la vie de l'abeille, les corps pédonculés présentent un important développement lors du dernier stade, celui de butineuse, correspondant à une augmentation des besoins d'apprentissage et de plasticité cognitive (Fahrbach et al., 2003).

Perspective : Tester l'implication des corps pédonculés dans la formation de concepts est à priori possible en bloquant sélectivement le fonctionnement des corps pédonculés, ce qui peut être fait de façon réversible, par exemple par injection localisée de procaine, un anesthésique local (Devaud et al., 2007). Cependant, il existe un risque important que le blocage des corps pédonculé interfère avec le comportement de navigation de l'abeille en libre-vol entre la ruche et la table d'entraînement, rendant le conditionnement impossible. Ainsi, il semble indispensable pour étudier les bases neurales sous-tendant les processus cognitif visuels de mettre en point des procédures de conditionnement qui permettent d'observer chez des abeilles fixées une sophistication d'apprentissage similaire à celle des abeilles en libre vol.

Conclusion

Ce travail de thèse a donc permis de montrer que malgré les limitations apparentes de son système nerveux l'abeille est néanmoins capable d'utiliser les configurations spatiales et des concepts relationnels dans l'analyse et la compréhension de son environnement visuel.

On peut s'interroger de l'utilité de posséder des structures cognitives si élaborée pour un insecte. Cependant, comme nous l'avons vu, l'activité de l'abeille butineuse requière une plasticité comportementale et des niveaux d'apprentissage et de mémorisation élevés pour sa recherche de nourriture. En effet, celle-ci implique des capacités de navigations exceptionnelles ainsi qu'une mémorisation des caractéristiques florales. Or, les procédés cognitifs étudiés dans ce travail de thèse sont connus comme facilitant la reconnaissance de nouveaux objets et situations rencontrés, et ce, avec une économie cognitive importante (Biederman, 1987; Zentall et al., 2008). Posséder des capacités de catégorisation basées sur des configurations spatiales apparaît donc comme potentiellement très utile dans les phénomènes de reconnaissance pour l'abeille. En particulier, son acuité visuelle étant réduite (cf. Figure 4) (Srinivasan et Lehrer, 1988; Giurfa et Vorobyev, 1997; 1998), la détection des configurations au détriment des détails d'un objet visuel peut apparaître plus aisé en vol. Quant aux capacités de catégorisation conceptuelle spatiale, il est tout à fait envisageable qu'elles soient un épiphénomène issu de la conjonction de l'existence d'une plasticité neuronale permettant de détecter les paramètres communs, à la base de la catégorisation perceptive, et un entraînement spécifique poussé. Il est néanmoins aisé d'imaginer que la manipulation de concepts de relations spatiales puissent faciliter la navigation de l'abeille par exemple en lui permettant l'élaboration de pseudo-cartes cognitives notamment (Menzel et al., 2005).

Cependant, même si de telles facultés cognitives sont utiles, n'impliquent-elles pas de posséder des structures cérébrales complexes aux multiples niveaux de traitement ? Il semble donc impossible qu'un cerveau d'insecte de 1 mm^3 , contenant moins d'un million de neurones à comparer aux cent milliards chez l'Homme, puisse générer de telles capacités. L'abeille nous oblige donc à rejeter certaines préconceptions attribuant seulement à des vertébrés (et à quelques-uns d'entre eux seulement) des capacités cognitives sophistiquées. Les arguments développés ci-dessus voudraient que dans certains cas, les phénomènes observés ne soient pas finalement si complexes (voir discussion sur la catégorisation ci-dessus) et donc à la portée du cerveau de l'abeille (Chittka et Niven, 2009). Les concepts relationnels requièrent, cependant, des traitements plus sophistiqués et leur acquisition par les abeilles pose des questions sur l'architecture neurale minimale pour achever une telle tâche. La caractérisation des structures et mécanismes impliqués est donc un grand défi pour la poursuite des recherches cognitives chez l'abeille.

Ce modèle invertébré est donc particulièrement adapté à la compréhension de l'architecture cérébrale minimale nécessaire dans ces processus de cognition visuelle. La mise au point de protocoles permettant l'observation des niveaux cognitifs similaires à ceux mis en

évidence chez l'abeille en libre-vol chez des abeilles fixées est, dans ce but, particulièrement attractive. Enfin, de part sa distance évolutive avec les vertébrés et les primates en particulier, les études comparatives impliquant l'abeille permettent de comparer les réponses apportées de façon indépendante par l'évolution aux problèmes de reconnaissance visuelle complexes.

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Annexes

Avarguès-Weber, A., Deisig, N. et Giurfa, M. (2011). Visual cognition in social insects. *Annu Rev Entomol* **56**, 423-443.

Giurfa, M., **Avarguès-Weber, A.** et Menzel, R. (2011). Complex abilities of simple nervous systems. Dans *Encyclopedia of animal behavior*, (eds. M. Breed et J. Moore): Elsevier.

Visual cognition in social insects

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ABSTRACT

Visual learning admits different levels of complexity, from the formation of a simple associative link between a visual stimulus and its outcome, to more sophisticated performances such as object categorization or rules learning, which allow flexible responses beyond simple forms of learning. Not surprisingly, higher-order forms of visual learning have been mainly studied in vertebrates with larger brains, while simple visual learning has been the focus in animals with small brains such as insects. This dichotomy has recently changed as studies on visual learning in social insects have shown that these animals can master extremely sophisticated tasks. Here we review a spectrum of visual learning forms in social insects, from color and pattern learning, visual attention, top-down image recognition, to inter-individual recognition, conditional discrimination, category learning and rule extraction. We discuss the necessity and sufficiency of simple associations to account for complex visual learning in Hymenoptera and discuss possible neural mechanisms underlying these visual performances.

KEY WORDS

Cognition, Learning, Non-elemental Learning, Social Insects, Hymenoptera, Vision

INTRODUCTION

Visual learning refers to an individual's capacity of acquiring experience-based information pertaining to visual stimuli so that adaptive responses can be produced when viewing such stimuli again. This capacity admits different levels of complexity as it varies from the establishment of a simple associative link connecting a visual stimulus (e.g. a specific color) and its outcome (e.g. a reward or a punishment) to more sophisticated performances such as learning to categorize distinct objects (e.g. animal vs. non-animal) or apprehending abstract rules applicable to unknown visual objects (e.g. "larger than", "on top of" or "inside of").

The first situation, the establishment of univocal, unambiguous links between a visual target and its outcome, constitutes a case of elemental learning. For instance, what is learned for a color is valid only for that color and not for different ones. In contrast, learning about categories or rules constitutes a case of non-elemental learning as appropriate responses can be transferred to unknown stimuli for which the subject has no personal experience, as long as they satisfy the learned category or rule. In these cases, the subject's response is flexible and relatively independent of the physical nature of the stimuli considered.

Social Hymenoptera, particularly bees (*Apis* sp. and *Bombus* sp.), ants and wasps (several genera), which are at the center of this article, are interesting models for the study of visual learning because in their natural context they have to solve a diversity of visual problems of varying complexity. For instance, these insects learn and memorize the local cues characterizing the places of interest, which are essentially the hive and the food sources (28, 29, 69, 72, 86, 113). Honeybees, and to a minor extent bumblebees, are 'flower constant' which means that they forage on a unique floral species as long as it offers profitable nectar and/or pollen reward (15, 41, 47). This capacity is partly based on visual cues provided by flowers such as colors or patterns. Learning and memorizing the visual cues of the exploited flower through their association with nectar and/or pollen reward is what allows a bee forager tracking a particular species in the field (69). Similarly, learning abilities for landmark constellations, complex natural scenes and for celestial cues used in navigation (azimuthal position of the sun, polarized light pattern of the blue sky) ensure a safe return to the nest and enhance foraging efficiency (16, 17, 25, 75, 104).

Visual capacities are highly developed in social Hymenoptera. It has been shown that bees, wasps, and some ant species see the world in color (3, 8, 9, 13, 22, 61, 62, 64, 71), perceive shapes and patterns (24, 34, 61, 62, 85, 103) and resolve movements with a high temporal resolution (87). One of the reasons why bees, ants and wasps constitute an attractive model for the study of visual learning resides precisely in the existence of controlled experimental methods for the study of these capacities in the laboratory.

VISUAL CONDITIONING OF BEES

Visual conditioning of honeybees (99) has allowed uncovering perceptual capabilities of these insects and has been used to this end for more than nine decades. This protocol exploits the fact that free-flying honeybees learn visual cues such as colors, shapes and patterns, depth and motion contrast, among others (34, 35, 60, 99, 103) when these are presented together with a reward of sucrose solution. Each bee is individually marked by means of a color spot on the thorax or the abdomen so that individual performances can be recorded. The marked bee is generally displaced by the experimenter towards the experimental site where it is rewarded with sucrose solution to promote its regular return. Such pre-training is performed without presenting the training stimuli in order to avoid uncontrolled learning. When the bee starts visiting the experimental place actively (i.e., without being displaced by the experimenter), the training stimuli are presented and the choice of the appropriate visual target rewarded with sucrose. This basic protocol has been used to study visual learning in other bee species such as bumblebees (22, 25, 64, 83), solitary bees (9, 73), stingless bees (6,98) and wasps (3, 61).

In studies on pattern vision, the plane of stimulus presentation is extremely important. Early works (48-50, 99) presented stimuli on the horizontal plane of an experimental table and the position of the stimuli were varied to verify that bees were indeed choosing a rewarded pattern rather than using position information. In this situation it is difficult to determine which specific information contained in the patterns is used by the insects to make their choices because they can approach the patterns from any possible direction. Later, vertical presentation was preferred because it constrained the approach direction to the patterns (100), thereby forcing a frontal approach and perception. In this way, it was possible to determine whether bees are sensitive to different pattern cues such as orientation, bilateral and radial symmetry, center of gravity, etc.

In all cases, insects have to be trained and tested *individually* to achieve a precise control of the experience of each subject. It is also important to control the distance at which a choice is made because visual orientation and choice are mediated by different visual cues at different distances or angles subtended by the target (34, 35, 38). The time between visits to the experimental place has also to be recorded as it reflects the appetitive motivation of the bee (76) and thus its motivation to learn. The associations built in this context can be either classical, operant or both, i.e. they may link a visual stimulus (conditioned stimulus or CS) and sucrose reward (unconditioned stimulus or US), the response of the animal (e.g. landing) and the US, or both, respectively. The experimental framework is nevertheless mainly operant as the bee's behavior is determinant for obtaining or not the sucrose reinforcement.

Visual conditioning of freely-flying insects does not allow studying visual learning at the cellular level. Because bees freely fly during the experiment, studying simultaneously neural activity in visual centers in the brain remains, so far, impossible. Recently, however, a protocol for visual conditioning of harnessed bees has been developed (51, 52). This protocol, based on pioneer studies by Kuwabara (57), consists in training a harnessed bee to extend its

proboscis to colors (51) or motion cues (52) paired with sucrose solution. Hungry bees reflexively extend the proboscis when their antennae are touched with sucrose solution, the equivalent of nectar reward. In this protocol, colors or patterns are paired with sucrose reward to create a pavlovian association in which the visual stimuli are the conditioned stimuli (CS) and sucrose the unconditioned stimulus (US). Learning is, however, poor in this protocol. It takes two days to reach an acquisition level that is around 40%, and this is only possible if bees have their antennae previously cut. The reasons for the apparent interference of the antennae on visual learning remain unknown. Cutting the antennae may affect the general motivation of the bee so that the sucrose reward is not as attractive as expected by the experimenter (20). Improving this protocol is a priority for future research on visual learning as it will allow combining behavioral quantification with access to the nervous system.

VISUAL CONDITIONING OF ANTS

Visual learning in ants has been mostly studied in the context of insect navigation. Experiments with the desert ant *Cataglyphis bicolor* have paved the way to understand navigation strategies based on celestial cues and landmarks (see 104 for review). In other ant species such as *Melophorus bagotti* (11, 75), *Gigantiops destructor* (65) or *Formica rufa* (40, 46), among others, similar questions have been investigated, thus putting the accent on the question of how ants use visual cues to negotiate their spatial environment. These works will not be reviewed here because they rarely focus on the learning process itself, which is the main framework of our review. Although learning of visual cues definitely underlies navigation processes studied in these ants, learning curves or memory retention tests are generally absent from these works, thus making difficult any analysis in terms of the associative links established during spatial learning in ants. It is therefore difficult to determine what is and what is not elemental in these performances.

A recent work on *Cataglyphis aenescens* and *Formica cunicularia* has used, nevertheless, an experimental design which reproduces basic features of visual training in bees (8), as it focus on color learning and discrimination. These ants were trained in a Y-maze to choose and discriminate monochromatic lights of constant intensity associated with a food reward. Using this kind of design could help dissecting in a more controlled way the nature of associative learning in ants.

ATTENTIONAL AND EXPERIENCE-DEPENDENT MODULATION OF VISUAL LEARNING

The first study on bee learning and memory that used controlled protocols for characterizing individual learning and memory employed colors as rewarding stimuli (67). Free-flying honeybees were trained to choose a rewarded monochromatic light and were then presented in dual choice situations with the rewarded light vs. an alternative color on a horizontal plane. This study reported learning curves for different wavelengths and showed that, under these experimental conditions, bees learned all wavelengths after few learning trials. Some wavelengths, particularly 413 nm, were learned faster than others, requiring only one to three acquisition trials (67; but see below). This result argued in favor of innate biases in color learning, probably reflecting the intrinsic biological relevance of the color signals that are learned faster (67). Indeed, color-naïve honeybees in their first foraging flight prefer those colors that experienced bees learn faster (36) and those colors seem to correspond to floral colors that are highly associated with profitable nectar reward (36).

Visual learning, as studied in these color conditioning experiments, is elemental as bees are just presented with a single color target paired with sucrose solution. It was supposed to be a fast form of learning (67; see above), compared, for instance, to learning of visual patterns which usually takes longer (twenty or more trials). Recent studies on bumblebee and honeybee color learning (22, 30) have, nevertheless, introduced a new twist to these conclusions. It was long thought that what an animal sees and visually learns is constrained by its perceptual machinery with no or less place for experience-dependent modulations of perception. Studies on honeybees (23, 30) and on bumblebees (22) have shown that this idea is wrong: in some cases, learning one and the same color may occur after few trials but in other cases it may take more than twenty trials (Fig. 1a). The critical feature is *how* the bees learn the task. *Absolute conditioning*, in which a subject is trained with a single color rewarded with sugar water, yields generally fast learning. *Differential conditioning*, in which the same subject has to learn to discriminate a rewarded from a non-rewarded color, takes more trials, even if the rewarded color is the same as in absolute conditioning. When these animals are asked to discriminate colors in a test, their performance differs dramatically. While bees trained in differential conditioning discriminate colors that are very similar (Fig. 1c), bees trained in absolute conditioning do not discriminate the same pair of colors (Fig. 1b; 22, 30). Similar results were obtained in ants trained to discriminate colors in a Y-maze (8).

Comparable results were obtained in studies on pattern learning and discrimination by honeybees (33, 90). While differential conditioning results in a visual recognition strategy that uses the cues present in the whole pattern, absolute conditioning results in a recognition strategy that restricts cue sampling mainly to the lower half of the pattern (33). In other words, bees recognize a pattern differently, depending on the kind of learning implicit to the conditioning task. In both cases (color and pattern learning), however, differential conditioning increases the demands imposed to the perceptual system of the bees, which has not only to go where a rewarded stimulus is presented (absolute conditioning) but to

discriminate it from a non-rewarding alternative (differential conditioning). The difference in performance suggests, therefore, that attentional processes are involved as in differential conditioning the bee has to focus on the difference and not on the mere presence of a visual target, thus making learning slower. In any case, the result goes against the idea that the difference between two colors is an immutable property constrained by the visual machinery.

At the time at which Menzel characterized color learning (67, 68; see above) studies on pattern perception were simultaneously performed by Wehner (100-102) and others (e.g. 1), continuing the tradition started by von Frisch's students (48-50, 106-108). In contrast to Menzel's work, these studies did not focus on learning but on the perceptual capabilities of bees confronted with pattern discrimination tasks. Certainly, visual conditioning was also used in these and in later works on pattern perception (for review see 60, 85, 103) but a quantification of acquisition curves and/or a characterization of pattern memory were absent from these works. This tradition was continued in the 70's, 80's and even 90's as visual learning was mainly used as a tool to answer questions on visual perception and discrimination. Yet, some experiments showed that, in pattern vision, as in color vision, what a bee perceives depends on its previous visual experience and of possible attentional processes. Zhang and Srinivasan (118) showed, for instance, that the previous visual experience of a bee can speed up the analysis of the retinal image when a familiar object or scene is encountered. They first attempted to train bees to distinguish between a ring and a disk when each shape was presented as a textured figure placed a few cm in front of a similarly textured background. The figures are, in principle, detectable through the relative motion that occurs at the figure borders, which are at a different distance than the background when bees fly towards the targets. Despite intensive training, the bees were incapable of learning the difference between a ring and a disk, a discrimination that usually poses no problems when the bees experience these stimuli as plain (non-textured) shapes. Zhang and Srinivasan (118) trained then a group of bees to this 'easy' problem, presenting a plain black disk and ring few cm in front of a white background. The bees could, as expected, easily learn the task. They were then confronted with the difficult problem of learning the textured disk vs. the ring and this time, they solved immediately the discrimination. Thus, pre-training with plain stimuli primed the pattern recognition system in such a way that it was able to detect shapes that otherwise could not be distinguished. It may be that such pre-training triggers attentional processes that allow better focusing on the targets that have to be discriminated.

Uncovering how attentional processes and learning modulate visual perception constitutes an unexplored and promising research field. The existence of attentional processes in insect brains is not far-fetched and recent research has been able to locate such processes in precise structures of the insect brain. In the fruit fly *Drosophila melanogaster*, attention can be demonstrated and characterized at the physiological level (97). A fruit fly fixed stationary within a circular arena, and tracking a visual moving object (a vertical black bar) moving at a constant frequency around it, exhibits anticipatory behavior consistent with attention for the bar tracked. Such an anticipatory tracking has a neural correlate in the form of a transient

increase in a 20-30 Hz local-field potential recorded in a region of the brain called the medial protocerebrum (Fig. 1d; 97). In other words, the 20-30 Hz response in the fly brain is correlated with transitions to behavioral tracking. This response is not only anticipatory, but also selective to the stimulus presented, increased by novelty and salience and reduced when the fly is in a sleep-like state (97). Moreover, the use of mutants showed that a subset of neurons of the mushroom bodies, which are a higher-order structure of the insect brain (Fig. 1d,e), are required for both the tracking response and the 20-30 Hz response (97). This result is consistent with the finding that mushroom bodies are required for choice behavior of *Drosophila* facing contradictory visual cues (91). In this case, individually tethered flies flying stationary are trained in a circular arena in which one kind of visual stimulus (say, a T pattern) represents a permitted flight direction, while another kind of visual stimulus (say, an inverted T pattern) represents a forbidden flight direction associated with a displeasing heat beam on the thorax. Tang and Guo (91) conditioned flies to choose one of two directions in response to color and shape cues; after the training, flies were tested with contradictory cues. Wild-type flies made a discrete choice that switched from one alternative to the other as the relative salience of color and shape cues gradually changed, but this ability was greatly diminished in mutant flies with miniature mushroom bodies or with chemically ablated mushroom bodies. In other words, mushroom bodies mediate the assessment of the relative saliency of conflicting visual cues (91, 111) and are also involved in improving the extraction of visual cues after pre-training in *Drosophila* (78). The mushroom bodies of hymenopterans may play similar roles (Fig. 1e), thus favoring attention and better problem solving and discrimination.

Yet, visual learning and the neural circuits mediating it are still poorly understood in the fruit fly. The mushroom bodies, which are the main site for olfactory memories, are not directly involved in visual learning because in *Drosophila*, contrary to hymenopterans, there is no direct input from the visual areas of the brain to these structures (109). Recent studies have succeeded in identifying the precise neuronal substrates of two forms of visual memory in the *Drosophila* brain, outside the mushroom bodies (63). Memory for pattern elevation and orientation were retraced to different neuronal layouts of the central complex, a median structure of the insect brain (Fig. 1d). Liu et al. (63) showed that two neuronal layers of the central complex are required to achieve visual discriminations based on pattern elevation or orientation respectively. In all cases, visual short-term memory was studied, thus leaving the question of the localization of visual long-term memory open. In bees and wasps, the localization of visual memories may differ with respect to *Drosophila*. In contrast to the fruit fly, visual areas of the hymenopteran brain provide direct input to the mushroom bodies (27), thus making of these structures a candidate for the localization of visual memories in addition of the central complex (Fig. 1d).

COMPLEX FORMS OF VISUAL LEARNING (THAT MAY NOT BE SO COMPLEX)

Only in the 90's, researchers became interested by the existence of cognitive processing in insects and the honeybee was the model chosen to address most of the works performed in that direction. Such a delay with respect to the "cognitive revolution" which flourished at the end of the 70's and begin of the 80's (74), can only be explained by the reluctance to view invertebrates, and therefore insects, as organisms capable of non-elemental, higher-order forms of learning. For instance, the main idea with respect to visual pattern learning, which is still sometimes defended, was that insects can only view isolated spots, blobs and bars without the capacity of integrating them in a given configuration (53-56). Even a basic capacity of recognition systems such as generalization, the ability of individuals to respond to stimuli that despite being different from a trained target are nevertheless perceptually similar to it (82, 84), was and is considered by some researchers as being too high-level for a honeybee (55, 56). Yet, dozens of works have already shown that honeybees generalize their choice of visual patterns to novel figures that have some similarity with those that have been trained (e.g. 1, 101). This refusal of generalization capacities is consistent with the preconception that insects have limited plasticity and should be rather viewed as reflex machines reacting to specific features in the environment to which they are tuned.

In the last decade, however, researchers have found evidence showing that bees are not robots and that they exhibit visual learning capabilities that were, so far, only suspected in some vertebrates. Some of these capacities are surprising and may be viewed as non-elemental. However, an alternative view could argue that it is possible to explain them based on simple, elemental associations. These experiments, reviewed in the next section, were not conceived to address these opposite views so that we are currently unable to determine whether these performances are forms of elemental or higher-order learning.

Visually-based individual recognition in wasps

The capacity of individuals to recognize their distinctive identity has long been dismissed in social insects due to the cognitive requirements that such performance may impose in colonies made of thousand of individuals. For instance, Wilson (105) stated that "...insect societies are, for the most part, impersonal...The sheer size of the colonies and the short life of the members make it inefficient, if not impossible, to establish individual bonds". Not all social insects live, however in huge, overcrowded societies. Small, relatively primitive colonies of bumble bees, wasps and some ant species are based on dominance hierarchies where individual recognition may be crucial for responding appropriately to a conspecific. Indeed, recent studies have shown that ant queens *Pachycondyla villosa* recognize each other using olfactory, cuticular cues (21). In the visual domain, studies on the paper wasp *Polistes fuscatus* have shown that individual recognition is achieved through learning the yellow-black patterns of the wasp faces and/or abdomens (93). In another species, *Polistes dominulus*, more variable patterns with larger black components were found to be carried by individuals

ranking higher in the nest hierarchy. Altering these facial and/or abdominal color patterns induced aggression against such animals, irrespective of whether their patterns were made to signal higher or lower ranking. These results were, however, challenged by another study (10), which could not find evidences supporting the hypothesis that the facial patterns of *P. dominulus* act as hierarchy or quality signals. Size, on the contrary, was highly correlated with social dominance in this wasp (10). Although these results question the validity of the hypothesis that posits that visual facial patterns that contain more black areas or more black spots are associated with dominant wasps, they do not exclude the possibility that visual patterns are used as individual identity markers rather than as status markers. In this scenario, wasps would recognize each other on the basis of their facial features and each individual mask, irrespective of its amount of black areas or spots, would have an unambiguous outcome in terms of its ranking in the social structure (i.e. mask A \rightarrow \square individual; mask B \rightarrow \square individual, etc.). Wasps would learn a series of elemental associations between mask patterns and social ranking. Given the small size of colonies in which five to ten individuals can coexist, storing several memories, one for each individual, seems plausible. If this were the case, a fundamental goal would be to characterize the storage capacity of the visual memory as related to colony size.

Observatory learning in bumblebees

Recent studies on bumblebees (58, 59, 110) have shown that these insects copy other bees' learnt foraging preferences by observing their choices of visual, rewarded targets. Bumblebees, *Bombus terrestris*, are influenced by other conspecifics when sampling unfamiliar flowers so that they land on unknown flowers where other bees are (58). This occurs even when naïve bees are separated from experienced foragers by a transparent screen so that they can neither sample the flowers by themselves nor interact with their foraging conspecifics (110). Similarly, naïve bees abandon an unrewarding species and switch to a more rewarding alternative more quickly if accompanied by experienced foragers (59).

As surprising as this performance may appear, it can be accounted for by an elemental form of associative learning called second-order conditioning (77), which involves two connected associations. In this scenario, an animal first learns an association between a conditioned stimulus (CS) and an unconditioned stimulus (US) (CS1 + US) and then experiences a pairing between a new conditioned stimulus CS2 and CS1 (CS1 + CS2). In this way, CS2 becomes meaningful, through its association with CS1, and indirectly, with the US. How would this apply to the observational learning of bumblebees? One could propose that naïve bumblebees would first associate the presence of a conspecific with reward (CS1 + US) simply by foraging close to experienced foragers. Afterwards, observing a conspecific landing on a given color may allow establishing an association between color and conspecific (CS2 + CS1) (59). These connected elemental links may thus underlie the observational learning of bees. This hypothesis is supported by the fact that honeybees can learn 2nd-order associations while searching for food. They learn to connect both two odors (Odor 1 + Sucrose Reward; Odor 2 + Odor 1; 7) and one odor and one color (Odor + Sucrose Reward; Color + Odor; 43).

Symbolic matching to sample and other forms of conditional discrimination in bees and wasps

Symbolic matching to sample is a term used to describe an experimental situation in which the correct response to a problem depends on a specific background or condition. In other words, animals have to learn, for instance, that given condition A, response C is correct while in condition B, response D is correct. Symbolic matching to sample is a form of *conditional discrimination* because a given stimulus, the sample (also called the ‘occasion setter’), sets the condition for the next choice. Using this design, Zhang et al. (117) trained honeybees to fly through a compound Y-maze consisting of a series of interconnected cylinders. The first cylinder carried the sample stimulus (e.g. a vertical or a horizontal black-and-white grating). The second and third cylinders each had two exits. Each exit presented a visual stimulus so that the bee had to choose between them. In the second cylinder, bees had to choose between a blue and a green square. In the third cylinder, they had to choose between a radial sector pattern and a ring pattern. Correct sequences of choices were ‘*Vertical – Green – Ring*’ and ‘*Horizontal – Blue – Radial*’. Only after making a succession of correct choices (i.e. both in the second and in the third cylinder) a bee could reach a feeder with sucrose solution. The bees learned to master these successive associations between different kinds of visual cues (117). This finding was also extended to other sensory modalities as the same principle applied when visual cues were combined with odors in a similar protocol (88).

Conditional learning admits other variants that, depending on the number of occasion setters and discriminations involved, have received different names. For instance, another form of conditional discrimination involving two occasion setters is the so-called *transwitching problem*. In this problem, an animal is trained differentially with two stimuli, A and B, and with two different occasion setters C and D. When C is available, stimulus A is rewarded while stimulus B is not (A+ vs. B-), while the opposite occurs (A- vs. B+) when D is available. The transwitching problem is also considered a form of contextual learning because the occasion setters C1 and C2 can be viewed as contexts determining the appropriateness of each choice. Bumblebees have been trained in a transwitching problem to choose a 45° grating and to avoid a 135° grating to reach a feeder, and to do the opposite to reach their nest (29). Here, the nest and the feeder provide the appropriate contexts defining what has to be chosen. Bumblebees can also learn that an annular or a radial disc must be chosen, depending on the disc’s association with a 45° or a 135° grating either at the feeder or the nest entrance: at the nest, access was allowed by the combinations 45° + radial disc and 135° + annular disc, but not by the combinations 45° + annular disc and 135° + radial disc; at the feeder, the opposite applied (28). In both cases, the potentially competing visuo-motor associations were insulated from each other because they were set in different contexts.

Solving this kind of problem can be viewed as a form of non-elemental learning and thus as a sophisticated form of cognitive visual processing. Indeed, as for other forms of conditional discrimination, one could describe this protocol as CA+, CB- (if C then A but not

B), and DA-, DB+ (if D then B but not A). Each stimulus, A, B, C, and D, is therefore as often rewarded as non-rewarded so that solutions cannot be based on the mere outcome of A, B, C or D. A higher-order solution would be then to learn the outcome of each particular configuration CA, CB, DA, DB. However, an alternative explanation could argue that what the insects do is to establish hierarchical simple associations as the ones underlying 2nd-order conditioning (see above). Indeed, one could imagine that bees learn to associate a radial disc with sucrose reward and that they then learn to associate a 45° grating with the radial disc. This is a relatively simple strategy that is probably used by bees for navigational purposes (115, 117) when they are confronted with successions of different landmarks en route to the goal.

A critical factor determining one strategy or the other may be therefore the temporal order of stimulus presentation. If these are presented *serially*, learning chains of simple associations could be primed while *simultaneous* presentation of stimuli may prime learning of configurations and their specific outcome. An example of the latter is the case of honeybees trained to solve a *biconditional discrimination* AC+, BD+, AD-, BC- in which all four stimuli were presented simultaneously, and were as often rewarded as non-rewarded (81). Four different gratings combining one color (yellow or violet = A or B) with one orientation (horizontal or vertical = C or D) were used in such a way that bees had to learn that, for instance, yellow-horizontal (AC) and violet-vertical (BD) were rewarded while yellow-vertical (AD) and violet-horizontal (BC) were non-rewarded. Bees learned to choose the rewarded stimuli despite the fact that colors and orientations were ambiguous when considered alone. They thus learned the configurations and not the specific outcome of the elements (81). Again, cumulative experience is a critical factor promoting configural learning (37). While few learning trials promote processing of a compound color stimulus made of A and B elements as the sum of A and B, increasing number of trials result in bees treating the compound AB as a unique entity, different from its composing elements (37).

NON-ELEMENTAL VISUAL LEARNING

The visual performances of the previous section could be accounted for by elemental associations despite their sophistication. A higher level of complexity is, however, reached when animals respond in an adaptive manner to novel stimuli *that they have never encountered before and that do not predict a specific outcome per se based on the animals' past experience*. Such a positive transfer of learning (79) is therefore different from elemental forms of learning, which link known stimuli or actions to specific rewards (or punishments). In the cases considered in this section, the insects' responses have in common the transfer to novel stimuli, which cannot be explained based on the previous 'knowledge' that the animal has of these stimuli.

Categorization of visual stimuli in honeybees

Visual categorization refers to the classification of visual stimuli into defined functional groups (45). It can be defined as the ability to group distinguishable objects or events on the basis of a common feature or set of features, and therefore to respond similarly to them (95, 114). A typical categorization experiment trains an animal to extract the basic attributes of a category and then tests it with novel stimuli that were never encountered before and that may present or not the attributes of the category learned. If the animal chooses the novel stimuli based on these attributes it classifies them as belonging to the category and exhibits therefore positive transfer of learning.

Several studies have shown recently the ability of visual categorization in free-flying honeybees trained to discriminate different patterns and shapes. For instance, van Hateren et al. (96) trained bees to discriminate two given gratings presented vertically and differently oriented (e.g. 45° vs. 135°) by rewarding one of these gratings with sucrose solution and the other not. Each bee was trained with a changing succession of pairs of different gratings, one of which was always rewarded and the other not. Despite the difference in pattern quality, all the rewarded patterns had the same edge orientation and all the non-rewarded patterns had also a common orientation, perpendicular to the rewarded one. Under these circumstances, the bees had to extract and learn the orientation that was common to all rewarded patterns to solve the task. This was the only cue predicting reward delivery. In the tests, bees were presented with novel patterns, which they were never exposed to before, which were all non-rewarded, but which exhibited the same stripe orientations as the rewarding and non-rewarding patterns employed during the training. In such transfer tests, bees chose the appropriate orientation despite the novelty of the structural details of the stimuli. Thus, bees could categorize visual stimuli on the basis of their global orientation.

They can also categorize visual patterns based on their bilateral symmetry. When trained with a succession of changing patterns to discriminate bilateral symmetry from asymmetry, they learn to extract this information from very different figures and transfer it to novel symmetrical and asymmetrical patterns (32). Similar conclusions apply to other visual features such as radial symmetry, concentric pattern organization and pattern disruption (see 5 for review) and even photographs belonging to a given class (e.g. radial flower, landscape, plant stem) (119).

How could bees classify appropriately different photographs of radial flowers if these vary in color, size, outline, etc.? An explanation was provided by Stach et al. (89, 90) who showed that different coexisting orientations can be considered at a time, and can be integrated in a global stimulus representation that is the basis for the category (89). Thus, a radial flower would be, in fact, the conjunction of five or more radiating edges. Besides focusing on a single orientation, honeybees were shown to assemble different features to build a generic pattern representation, which could be used to respond appropriately to novel

stimuli sharing such a basic layout. Honeybees trained with a series of complex patterns sharing a common layout comprising four edge orientations remembered these orientations simultaneously in their appropriate positions, and transferred their response to novel stimuli that preserved the trained layout. These results show that honeybees extract regularities in their visual environment and establish correspondences among correlated features. They may, therefore, generate a large set of object descriptions from a finite set of elements.

This capacity can explain recent findings showing that honeybees learn to recognize human faces if trained to do so (24). In this case, bees were rewarded with sugar water to choose and distinguish a photograph of a person's face from other persons' photographs. Bees were able to choose the appropriate photograph thus showing a capacity to discriminate this particular kind of stimuli. Does this mean that bees realize that two different persons are behind two discriminated photographs? Not really. For the bees rewarded on the photographs, these were just strange flowers. The question would then be which information of the photographs was used for recognizing the right stimulus. This question was recently tackled by a work that studied whether bees can bind the features of a face-like stimulus (two dots in the upper part as the eyes, a vertical line below as the nose, and a horizontal line in the lower part as the mouth) and recognize faces using this basic configuration (2). Bees did indeed distinguish between different variants of the face-like stimuli, thus showing that they discriminate between these options, but grouped together and reacted, therefore, similarly to faces if trained to do so. Stimuli made of the same elements (two dots, a vertical and a horizontal line) but not preserving the configuration of a face were not recognized as positive, thus showing that bees learn that the rewarded stimulus consists of a series of elements arranged in the specific spatial configuration of a face (2). Furthermore, if trained with real faces, bees can learn to recognize novel views of a face by interpolating between or 'averaging' views they have experienced (26).

In any case, honeybees show positive transfer of learning from a trained to a novel set of stimuli, and their performances are consistent with the definition of categorization. Visual stimulus categorization is not, therefore, a prerogative of certain vertebrates. However, this result might not be surprising as it admits (again) an elemental learning interpretation. To understand this elemental interpretation, the possible neural mechanisms underlying categorization should be considered. If we admit that visual stimuli are categorized on the basis of specific features such as orientation, the neural implementation of category recognition could be relatively simple. The feature(s) allowing stimulus classification would activate specific neuronal detectors in the optic lobes, the visual areas of the bee brain. Examples of such feature detectors are the orientation detectors whose orientation and tuning have been already characterized by means of electrophysiological recordings in the honeybee optic lobes (112). Thus, responding to different gratings having a common orientation of, say, 60°, is simple as all these gratings will elicit the same neural activation in the same set of orientation detectors despite their different structural quality. In the case of category learning, the activation of an additional neural element is needed. Such element would be a 'reward neuron' whose activity would substitute for sucrose reward. Such neuron has been identified

in the honeybee brain and it is called VUM_{mx1} (from ‘ventral unpaired median’ neuron located in the maxillar neuromere 1; 44). VUM_{mx1} mediates olfactory learning in the honeybee as it contacts the olfactory circuit at its key processing stages in the brain. In other words, when an odor activates the olfactory circuit, concomitant sucrose stimulation activates VUM_{mx1} thus providing the basis for neural coincidence between odor and reward. The branching of VUM_{mx1} makes it specific for the olfactory circuit and thus for olfactory learning (44). Other VUM neurons whose function is still unknown are present in the bee brain (80). Some of them could provide the neural basis of reward in associative visual learning. Category learning could be thus reduced to the progressive reinforcement of an associative neural circuit relating visual-coding and reward-coding neurons, similar to that underlying simple associative (e.g. Pavlovian) conditioning. From this perspective, even if categorization is viewed as a non-elemental learning form because it involves positive transfer of learning, it may simply rely on elemental links between conditioned and unconditioned stimuli.

An even simpler alternative may account for this performance. The mechanism explained above could be viewed as a form of supervised learning, in which a visual network is instructed by the external signal of the reinforcement neuron to respond to the right combination of features. Recent modeling work on the vertebrate visual system has shown that visual networks can learn to extract the distinctive features of a category without any kind of supervision (66). The model relies on spike timing dependent plasticity (STDP), which is a learning rule that modifies synaptic strength as a function of the relative timing of pre- and postsynaptic spikes. When a neuron is repeatedly presented with similar inputs, STDP is known to have the effect of concentrating high synaptic weights on afferents that systematically fire early, while postsynaptic spike latencies decrease. Masquelier et al. (66) showed that a network that exhibits STDP and is repeatedly presented with natural images of a given category becomes progressively tuned to respond better to the features which correspond to prototypical patterns of the category. Those features that are both salient and consistently present in the images are highly informative and enable robust object recognition. Testing whether similar neural mechanisms underlie object categorization in the insect visual system would be a fascinating endeavor.

Rule learning in honeybees

In rule learning, the animal learns relations between objects and not the objects themselves. Typical examples are the so-called rules of *sameness* and of *difference*. These rules are demonstrated through the protocols of delayed matching to sample (DMTS) and delayed non-matching to sample (DNMTS), respectively. In DMTS, animals are presented with a sample and then with a set of stimuli, one of which is identical to the sample and which is reinforced. Since the sample is regularly changed, animals must learn the sameness rule, i.e. ‘*always choose what is shown to you (the sample), independent of what else is shown to you*’. In DNMTS, the animal has to learn the opposite, i.e. ‘*always choose the opposite of what is shown to you (the sample)*’. Honeybees foraging in a Y-maze learn both rules (39). Bees were

trained in a DMTS problem in which they were presented with a changing non-rewarded sample (i.e. one of two different color disks or one of two different black-and-white gratings, vertical or horizontal) at the entrance of a maze (Fig. 2). The bees were rewarded only if they chose the stimulus identical to the sample once within the maze. Bees trained with colors and presented in transfer tests with unknown black-and-white gratings solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained with the gratings and tested with colors in transfer tests also solved the problem and chose the novel color corresponding to that of the sample grating at the maze entrance (39). Transfer was not limited to modalities within the visual domain (pattern vs. color), but could also operate between different domains such as olfaction and vision (39). Furthermore, bees also mastered a DNMTS task, thus showing that they also learn a rule of difference between stimuli (39). The capacity of honeybees to solve a DMTS task has recently been analyzed with respect to the working memory underlying it (116). It was found that the sample is stored for approximately 5s (116), a period that coincides with the duration of other visual and olfactory short-term memories characterized in simpler forms of associative learning in honeybees (70; see above). Moreover, bees trained in a DMTS task can learn to pay attention to one of two different samples presented successively in a flight tunnel (either to the first or to the second) and can transfer the learning of this sequence weight to novel samples (116).

Despite the honeybees' evident capacity to solve relational problems such as the DMTS or the DNMTS tasks, such capacities are not unlimited. In some cases, biological constraints may impede the solving of a particular problem for which rule extraction is necessary. It is therefore interesting to focus on a different example of rule learning which bees could not master, the *transitive inference problem* (4). In this problem, animals have to learn a transitive rule, i.e. $A > B$, $B > C$, then $A > C$. Preference for A over C in this context can be explained by two strategies: 1) deductive reasoning in which the experimental subjects construct and manipulate a unitary and linear representation of the implicit hierarchy $A > B > C$; or 2) responding as a function of reinforced and not reinforced experiences (92), in which animals choose among stimuli based on the effective number of reinforced and non-reinforced experiences (A is always reinforced while C is always non-reinforced).

To determine whether bees learn a transitive rule, they were trained using five different visual stimuli A, B, C, D, and E in a multiple discrimination task: A+ vs. B-, B+ vs. C-, C+ vs. D-, D+ vs. E- (4). Training involved overlapping of adjacent premise pairs ($A > B$, $B > C$, $C > D$, $D > E$), which underlie a linear hierarchy $A > B > C > D > E$. After training, bees were tested with B vs. D, a non-adjacent pair of stimuli that were never explicitly trained together. In theory, B and D have equivalent associative strengths because they are, in principle, equally associated with reinforcement or absence of it during training. Thus, if bees were guided by the stimulus' associative strength, they should choose randomly between B and D. If, however, bees used a transitive rule, they should prefer B to D. Honeybees learned the premise pairs as long as these were trained as uninterrupted, consecutive blocks of trials (4). But if shorter and interspersed blocks of trials were used, such that bees had to master all pairs practically simultaneously, performance collapsed and bees did not learn the premise pairs.

The bees' choice was significantly influenced by their experience with the last pair of stimuli (D+ vs. E-) such that they preferred D and avoided E. In the tests, no preference for B to D was found. Although this result agrees with an evaluation of stimuli in terms of their associative strength (see above), during training bees visited more B when it was rewarding than D, such that a preference for B should have been expected if only the associative strength were guiding the bees' choices. It was then concluded that bees do not establish transitive inferences between stimuli but rather guide their choices by the joint action of a recency effect (preference of the last rewarded stimulus, D) and by an evaluation of the associative strength of the stimuli (in which case preference for B should be evident). As the former supports choice of D while the latter supports choice of B, equal choice of B and D in the tests could be explained (4). In any case, memory constraints (in this case the fact that simultaneous mastering of the different premise pairs was not possible and the fact that the last excitatory memory seems to predominate over previous memories) impeded learning the transitive rule. Recently, Cheng and Wignall (12) demonstrated that failure to master several consecutive visual discriminations is due to response competition occurring when animals are tested. This may explain why bees in the transitive inference protocol were unable to master the successive short blocks of training with different premise pairs.

Counting

Counting could be useful in navigation tasks where the number of landmarks encountered during a foraging trip or near the hive may contribute to efficient orientation of free-flying bees. Furthermore, it could also improve foraging through evaluation of food source profitability (e.g. number of flowers in a patch). Whether or not honeybees estimate numerosity has been addressed recently by two different works which reached similar conclusions (19, 42).

Dacke and Srinivasan (19) were inspired by Chittka and Geiger's (14) pioneer work, suggesting that bees may count landmarks *en route* to the feeder. In Dacke and Srinivasan's protocol, bees were trained to fly into a tunnel to find a food reward after a given number of landmarks. The shape, size and positions of the landmarks were changed in the different testing conditions in order to avoid any confounding factor. Bees showed a stronger preference to land after the correct number of landmark in non-rewarded tests. This behavior was observed when bees were trained to collect reward after 1, 2, 3 or 4 landmarks but not further, thus indicating a limit in their counting capacity.

A similar limit was found in a DMTS protocol (42; see above), in which bees had to choose the stimulus containing the same number of items as a sample. The authors controlled for low-level cues such as cumulated area and edge length, configuration identity and illusionary shape similarity formed by the elements. Their results showed that honeybees have the capacity to match visual stimuli in a DMTS task based on the number of items as long as this number does not exceed four. Together with Dacke and Srinivasan's work (19), this result

indicates that the bee brain can deal with a real numerosity concept, even if it is limited to a number of four. Interestingly, the same limit was found in Humans when time exposure of items to be counted is limited (18).

CONCLUSION

Almost hundred years of research on visual learning in bees and other social Hymenoptera, starting with Karl von Frisch's (99) first demonstrations on color and pattern learning in bees, have yielded an impressive amount of information about how bees, bumblebees and wasps see the world and learn about visual cues in their environment. New discoveries in this field have shown that besides simple forms of visual learning, social Hymenoptera also master complex forms of visual learning going from conditional discriminations and observational learning to rule learning. Although the cognitive capabilities of bees and wasps may surprise due to their sophistication, limitations related to natural life seem inescapable. For instance, in the case of wasps learning facial mask patterns of conspecifics, one could imagine that interindividual recognition is certainly possible but has probably limitations in terms of the number of individuals that can be learned and remembered. Similarly, mastering simultaneously several different associations would be facilitated if these are organized *serially or hierarchically* in chains of associations that can mediate successful navigation in a complex environment. But if these associations have to be mastered *simultaneously* at the same place, learning them would be probably difficult given the bees' biological specialization as a serial forager. In this case, learning configurations of stimuli may be more adaptive than learning each component separately.

If bees and wasps exhibit such a high degree of complex forms of visual learning which kind of limitation do they present as a model for unraveling the mechanisms of these phenomena? Clearly, the main limitation resides so far in the impossibility of addressing questions related to the cellular and molecular mechanisms underlying these learning forms. Learning protocols have exploited the advantage of not restraining the animals' movements so that the behaviors recorded express all the potential of the insect brain. However, they are limiting because no access to the brain is so far possible in a flying bee. As mentioned above, new protocols in which bees learn color – reward and motion cues– reward associations under restrained conditions (51, 52) are promising because they allow accessing the neural circuits involved in these learning forms (31). The critical question would be then to what extent restraining conditions limit the expression of more complex forms of visual learning.

Why bees and wasps should continue to be attractive for research on visual cognition despite this technical limitation? The answer is simple: because they exhibit sophisticated visual performances that could not be uncovered so far in a fruit fly. Future research in social insects should benefit from a comparative analysis between the visual performances and mechanisms of bees and flies and overcome the historic burden of not having a window open

to the neural and molecular basis of visual learning, irrespective of the level of complexity considered.

SUMMARY POINTS

1. Visual learning admits different levels of complexity, from the formation of a simple associative link between a visual stimulus and its outcome (elemental learning), to more sophisticated performances such as object categorization or rules learning, which allow flexible responses beyond simple forms of learning (non-elemental learning). Social insects excel at visual learning in a foraging and navigation context.
2. Honeybees, ants and bumblebees learn to associate different kinds of visual cues such as color or patterns with food reward. Even if these associations remain elemental, performance can be modulated by the complexity of the task, thus suggesting attentional processes in these insects. Attention and experience-dependent changes in visual discrimination can be traced to the neural level. Studies in the fruit fly suggest that these processes can be located at the level of the mushroom bodies, a structure of the insect brain involved in learning and memory.
3. Social insects exhibit sophisticated visual abilities. Wasps recognize each other based on facial marks, bumblebees learn to choose profitable food sources by observing the choice of other bees, and honeybees and bumblebees learn to solve different kinds of conditional discriminations. These performances admit both elemental and non-elemental explanations.
4. Based on their past experience, honeybees respond in an adaptive manner to novel stimuli that they have never encountered before. Such a positive transfer of learning, characteristic of non-elemental forms of learning, has been shown in studies demonstrating categorization, learning of rules such as sameness or difference, and basic counting abilities in bees.

DISCLOSURE STATEMENT

The authors are not aware of any affiliation, membership, funding, or financial holding that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank the support of the French National Research Agency (ANR; Project APICOLOR), the French Research Council (CNRS) and the University Paul Sabatier.

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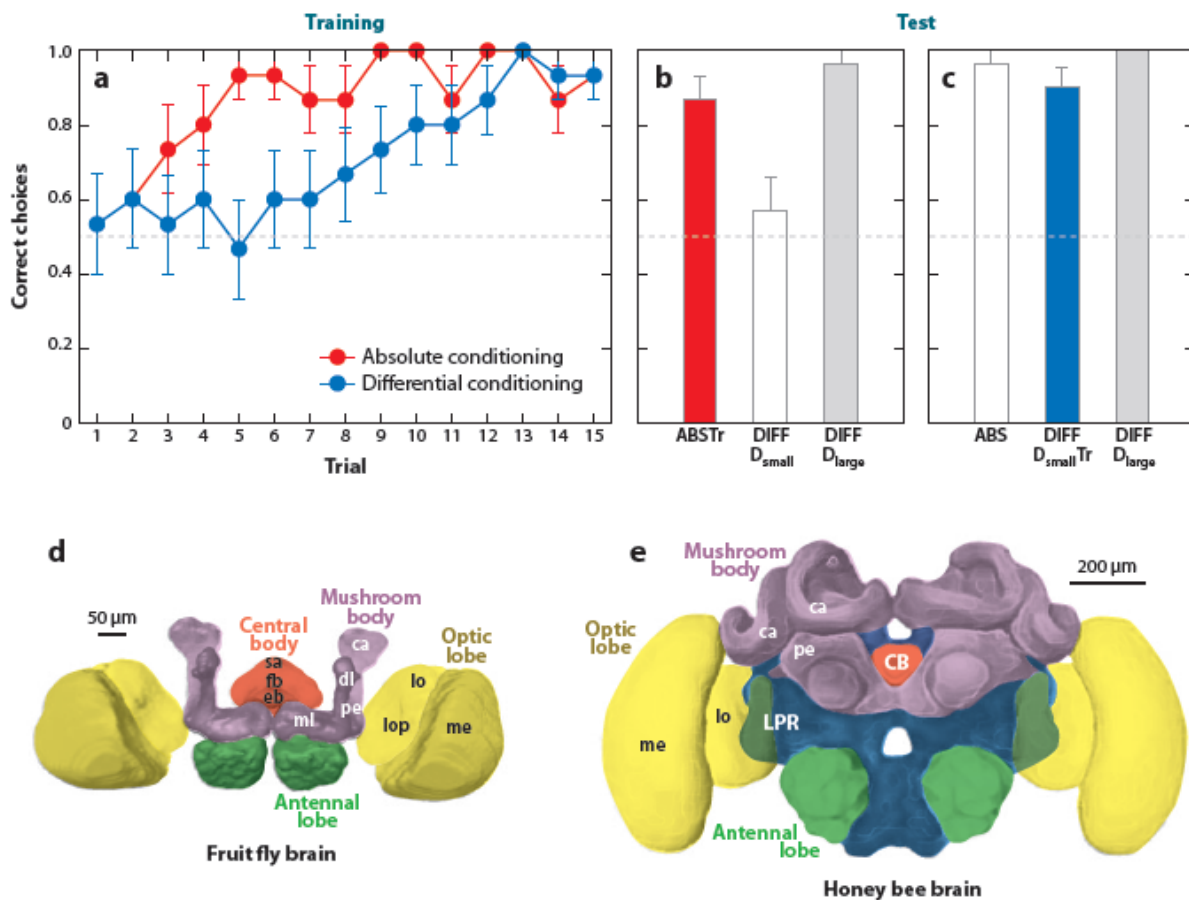


Figure 1: a,b,c) attention-like processes in honeybees. Performance of the free-flying bees trained with colors under absolute and differential conditioning. Adapted from (30) **a) Acquisition** along 15 trials (mean \pm S.E.; $n = 15$ bees for each curve). Red circles: absolute conditioning; blue circles: differential conditioning. **b) Tests of the group trained with absolute conditioning.** **Red Bar:** test presenting the trained situation (ABSTr), i.e. the single color that was previously rewarded. **White Bar:** test presenting a novel differential situation (DIFF D_{small}), i.e. the color that was previously rewarded vs. a new color that was very similar to the trained one. **Grey Bar:** test presenting a novel differential situation (DIFF D_{large}), i.e. the color that was previously rewarded vs. a new color that was very different from the trained one. **c) Tests of the group trained with differential conditioning.** **Blue Bar:** test presenting the trained situation (DIFF D_{small}Tr), i.e. the previously rewarded and the non-rewarded colors which were very similar. **White Bar:** test presenting just the previously rewarded color (ABS). **Grey Bar:** Test presenting the previously rewarded color vs. a novel color very different from the rewarded one (DIFF D_{large}). **d) Three-dimensional reconstruction of a fruit fly brain (courtesy of Arnim Jenett);** AL: antennal lobe; OL: optic lobe; Me: medulla;

Lo: lobula; Lop: lobula plate; MB: mushroom body; Ca: calyx; dl: dorsal lobe; ml: medial lobe; pe: peduncle; CB: central body; eb: ellipsoid body; fb: frontal bridge; fb: fan-shaped body; sa: superior arch. **e) *Three-dimensional reconstruction of a honeybee brain (courtesy of Randolph Menzel)***; LPR: lateral protocerebrum.

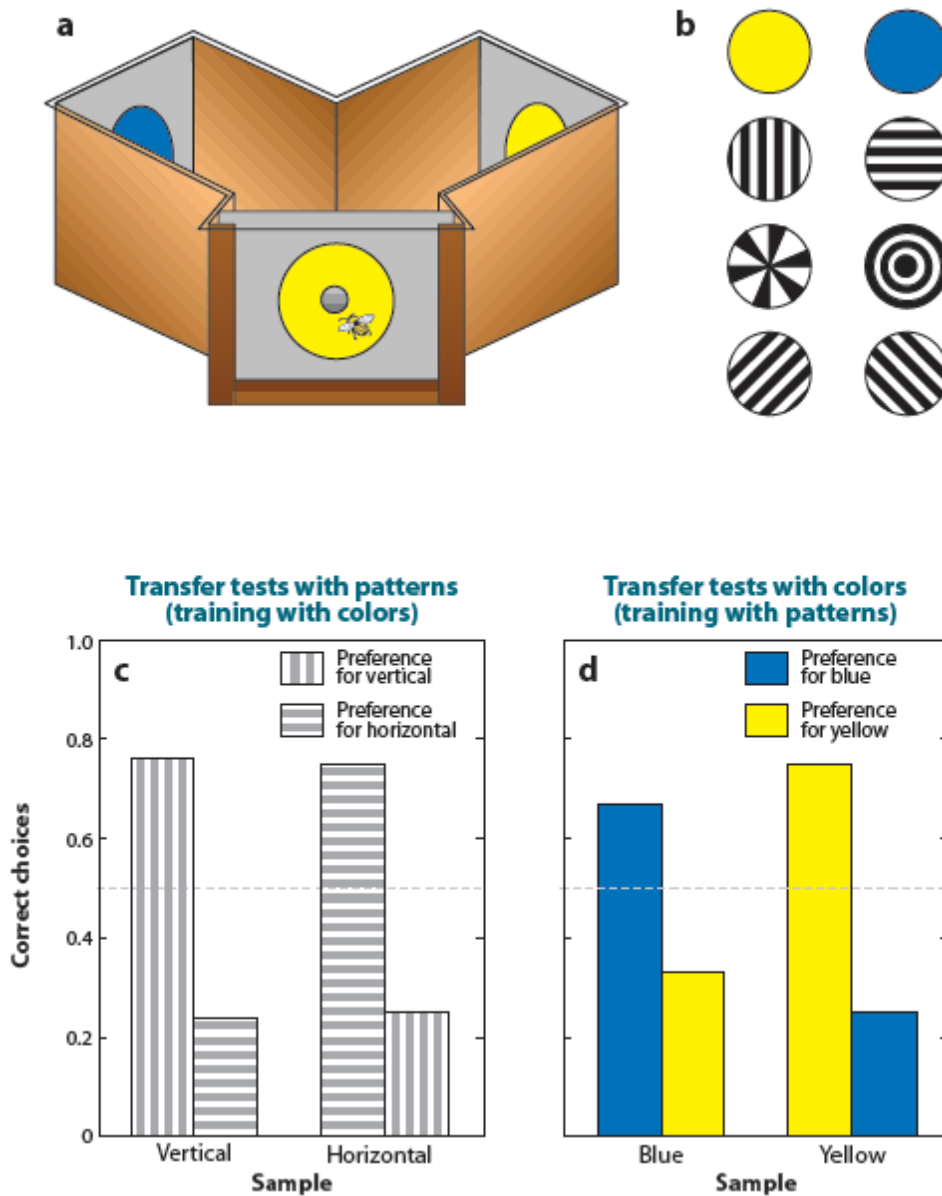


Figure 2: Rule learning in honeybees. Adapted from (39). Honeybees trained in a delayed matching-to-sample task to collect sugar solution in a Y-maze **a**) on a series of patterns or colors **b**) learn a rule of sameness. **c**, **d**) Transfer tests with novel stimuli. **c**) In Experiment 1, bees trained on the colors were tested on the gratings. **d**) In Experiment 2, bees trained on the gratings were tested on the colors. In both cases bees chose the novel stimuli corresponding to the sample although they had no experience with such test stimuli. n: number of choices.

Complex abilities of simple nervous systems

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Keywords: Invertebrate, Cognition, Learning, Non-Elemental learning, Contextual Learning, Rule Learning

Introduction

Cognitive science provides a fresh look at animal behavior, and its merge with neuroscience overcomes the conceptual limitations of traditional experimental psychology and ethology. Despite the multitude of approaches in cognitive neuroscience and the respective attempts to define these approaches a general definition for the term ‘cognition’ remains elusive probably because key terms are understood differently depending on the conceptual traditions to which the scientists relate themselves, the behaviors in question, and the considered complexity of the neural substrates underlying them. A key term is “representation”, the understanding that the brain is actively involved in perceiving the world and creating motor patterns by recruiting memories, expecting outcomes and making decisions between neural instantiations of behavioral options. Gaining information by learning and by storing it in multiple forms of memory, as a fundamental form of representation, is an essential and most likely a basic property of any neural system of some complexity. Here we shall focus on *non-elemental forms of associative learning*, i.e. on learning forms in which simple, unambiguous links between specific events in an animal’s environment cannot account for experience-dependent changes in behavior, and which require operations on remote and recent memories. In this respect *non-elemental associative learning* transcends *elemental forms of associative learning*, in which animals learn univocal connections between specific events in their environment. In particular we shall ask whether animals with small brains like mollusks and insects are capable of performing *non-elemental associative learning*.

Elemental forms of associative learning in invertebrates

Associative learning allows extracting the logical structure of the world by evaluating the sequential order of events. Two major forms of associative learning are usually recognized: in *classical conditioning*, animals learn to associate an originally neutral stimulus (conditioned stimulus, CS) with a biologically relevant stimulus (unconditioned stimulus, US); in *operant conditioning*, they learn to associate their own behavior with a reinforcer and relate this connection to the context conditions of the environment. In their most simple version, both learning forms rely on the establishment of associative links connecting two (or

more) specific and unambiguous events in the animal's world. For instance, in *absolute classical conditioning* (A+), a direct link between an event (A) and reinforcement (+) is learned while in *differential classical conditioning* (A+ vs. B-), simple, unambiguous links between A and reinforcement and between B and the absence of reinforcement are simultaneously learned.

Multiple cases of these simple learning forms have been described for invertebrates. For instance, in the honeybee *Apis mellifera*, olfactory conditioning of the proboscis extension response (PER) has been repeatedly used for the study of *elemental classical conditioning* and its neural substrates. Individually harnessed hungry bees that do not respond to an odor presentation with an extension of their proboscis do so when their antennae are stimulated with sucrose solution (the US). If the odor (the CS) is forward paired with sugar, the bees learn an association between odor and sugar reward so that they exhibit conditioned PER to future presentations of the odor alone (Fig. 1). An example of *elemental operant conditioning* is provided by the aquatic mollusk *Lymnaea stagnalis*, which can be trained to suppress the opening of its pneumostome, a small respiratory orifice, when the animal surfaces and attempts to breathe. This is achieved by an aversive and repeated mechanical stimulation of the pneumostome, which determines that the mollusk learns to reduce its attempts to open the pneumostome as training progresses. In both examples, the neural networks mediating associative learning are relatively simple and well-studied, thus underlining the advantages of invertebrates as model systems for the understanding the neural mechanisms of simple forms of learning.

Non-elemental forms of associative learning in invertebrates

In the higher-order forms of learning on which we focus here, simple links connecting specific events are generally not useful because ambiguity characterizes the events under consideration. For instance, in the discrimination termed *negative patterning discrimination*, an animal has to learn to differentiate a non-reinforced binary compound AB- from its reinforced elements (A+, B+). This situation is particularly challenging as each element A and B appears as often reinforced as non-reinforced. Relying on elemental links between A (or B)

and reinforcement (or absence of reinforcement) is useless to solve this problem. Another example of non-elemental learning is the so-called *biconditional discrimination* where the subject learns to respond to the compounds AB and CD and not to the compounds AC and BD (AB+, CD+, AC-, BD-). As in negative patterning, each element, A, B, C and D, appears reinforced as often as non-reinforced so that it is impossible to rely only on the associative strength of a single element to solve the task. In both examples, animals have to suppress linear processing of compounds and learn that a compound is an entity different from its components.

A second form of non-elemental learning is *contextual learning*, in which animals learn to produce adaptive responses that can be linked to a specific context. They learn that, given a certain stimulus or condition, a particular response is appropriate whereas, given a different stimulus or condition, the same response is no longer appropriate. This form of learning, usually referred as conditional learning or occasion setting, cannot be viewed as elemental learning because a given stimulus may or not be predictive of a certain outcome, depending on the particular environment.

A third form of non-elemental rule is provided by *rule learning* in which animals respond to novel stimuli that they have never encountered before or can generate novel responses that are adaptive given the context in which they are produced. In doing this, the animals exhibit a positive transfer of learning, a capacity that cannot be referred to as an elemental learning because the responses are aimed toward stimuli that do not predict a specific outcome per se based on the animals' past experience.

One of the first works adopting a non-elemental learning perspective in invertebrates was performed on lobsters. These animals normally exhibit exploratory behavior when placed in an aquarium. They can be aversively conditioned to stop searching by pairing an olfactory stimulus delivered in water with a mechanosensory disturbance produced by the experimenter. Lobsters were trained in this way with an olfactory compound AX reinforced by the aversive mechanosensory stimulation (AX+). Conditioning was either absolute (AX+) or differential, using a second compound AY (AX+ vs. AY-). After absolute conditioning, lobsters inhibited their search behavior when presented with AX as expected, but still searched when presented with A, X or with a novel odor Y. This result is consistent with learning the compound AX as an entity different from its components A and X, as proposed

by the configural theory (Pearce 1994). After differential conditioning, lobsters again inhibited their searching behavior when presented with AX but not with AY. Interestingly, they also inhibited search when presented with the element X but not with the element Y. A was not useful as it was common to the reinforced and the non-reinforced compounds AX+ and AY-, respectively. In this case, lobsters seemed to have learned the compounds AX and AY in elemental terms, thus being able to fully generalize their respective responses to X and Y. This work shows that depending on the conditioning protocol, lobsters treat and learn an olfactory compound differently so that either elemental or non-elemental associations with the negative mechanosensory reinforcer are built.

In the honeybee, several studies have addressed the issue of elemental vs. non-elemental learning, using visual conditioning of free-flying animals or olfactory PER conditioning of harnessed animals. In the first protocol, bees flying between the hive and a feeding site are trained to discriminate different kinds of visual targets (colors, shapes, motion cues, etc.) at the food source. Correct choices are rewarded with a drop of sucrose solution. In the second protocol, described above, harnessed bees learn a Pavlovian association between odor and sucrose reward. In both experimental protocols, bees were shown to solve a biconditional discrimination (AB+, CD+, AC-, BD-). In the visual modality, free flying bees had to discriminate complex patterns that were arranged to fulfill the principles of this discrimination problem. In the olfactory modality, olfactory compounds were used and bees learned to respond appropriately to each compound, independently of the ambiguity inherent to the components.

Bees proved also to be able to solve a negative patterning discrimination (A+, B+, AB-) in the olfactory domain. It was shown that in situations in which ambiguity is created at the level of the odorants integrating a compound, olfactory processing is consistent with the *unique cue theory*, a form of processing in which animals detect to some extent the presence of the components in the compound but in which they also assign a unique identity to the compound (the unique cue), resulting from the interaction between its components.

Neural bases of non-elemental learning in invertebrates

The interest in non-elemental olfactory learning protocols in insects relates to the possibility to correlate the behavior with the plasticity of the underlying neural circuits. The olfactory circuit is relatively well known. In the case of the honeybee (Fig. 2), peripheral processing of odor molecules occurs at ~60.000 olfactory receptor neurons (ORNs) and in 160 glomeruli of the antennal lobe (AL). ORNs and glomeruli in the AL have broad, overlapping and combinatorial responses to a range of odors. Processed olfactory information is conveyed by ~800 projection neurons (PNs) to higher-order brain centers (mushroom bodies or lateral protocerebrum). Mushroom bodies (MBs) are particularly interesting from the perspective of non-elemental learning since they receive segregated information of different sensory modalities (visual, olfactory, mechanosensory) and provide multimodal output that reflects the integration of information between modalities at the level of the neurons that constitute them, the Kenyon cells and the mushroom body output neurons.

In honeybees, bilateral olfactory input to both antennae is required to solve a negative patterning discrimination. Given that the olfactory circuit remains practically unconnected between hemispheres until the mushroom bodies, this result suggests that the reading of a unique cue, arising from odorant interactions within the mixture, occurs upstream the antennal lobes, i.e. at the level of the mushroom bodies. Mushroom body-ablated honeybees were used to determine whether these structures are necessary to solve non-elemental olfactory discriminations. Bees were conditioned in a side-specific discrimination so that when odorants were delivered to one antenna, the contingency was A+ vs. B- while it was reversed (A- vs. B+) when they were delivered to other antenna. Bees without lesions could solve this non-elemental problem (each odor is as often rewarded as non-rewarded) while bees with unilateral lesions of the mushroom bodies were impaired in this problem solving but not in elemental discriminations. It was therefore proposed that mushroom bodies are required for solving non-elemental discriminations.

It thus appears that at least lobsters and honeybees are capable of non-elemental learning in the strict sense and that, in insects, mushroom bodies are involved in such kind of problem solving. Such forms of learning are highly dependent on the way in which animals are trained, on the number of trials and on the similarity between elements in a compound. Further research should ask whether other invertebrates particularly *Drosophila* solve non-

linear discrimination problems. Neurogenetic tools available in this insect could be a most useful tool for identifying in a more precise way the neuronal circuits involved in non-linear discriminations.

Contextual learning in invertebrates

Contextual learning can be subsumed in the so-called occasion setting problem. In this problem, a given stimulus, the occasion setter, informs the animal about the outcome of its choice (for instance, given stimulus C, the occasion setter, the animal has to choose A and not B because the former but not the latter is rewarded). This basic form of conditional learning admits different variants depending on the number of occasion setters and discriminations involved, which have received different names. For instance, another form of occasion setting involving two occasion setters is the so-called *transwitching problem*. In this problem, an animal is trained differentially with two stimuli, A and B, and with two different occasion setters C1 and C2. When C1 is available, stimulus A is reinforced while stimulus B is not (A+ vs. B-), while it is the opposite (A- vs. B+) with C2. This problem does not admit lineal solutions based on elemental learning of A or B with reinforcement as each element (A, B) and each occasion setter C1, C2 appears equally as often reinforced and non-reinforced. Animals have, therefore, to learn that C1 and C2 define the valid contingency.

In the mollusc *Aplysia californica*, exposure to two different contexts (a smooth, round bowl containing lemon-flavored seawater and a rectangular chamber with a ridged surface containing unscented seawater that was gently vibrated by an aerator located in one corner) and experiencing a series of moderate electric shocks (US) in one of these two contexts leads to the establishment of an association between the context and the shock. The context alone elicited a defensive reaction which was exclusive for the reinforced context.

Crickets *Gryllus bimaculatus* and cockroaches *Periplaneta Americana* also exhibit contextual learning as they solve a typical version of the transwitching problem (see above). Both crickets and cockroaches associate one odorant with water reward (appetitive US) and another odorant with saline solution (aversive US) under illumination, and learn the reversed contingency in the dark. Thus, the visual context affected learning performance only when

crickets were requested to use it to disambiguate the meaning of stimuli and to predict the nature of reinforcement.

Bumblebees *Bombus terrestris* have been also trained in a transwitching problem to choose a 45° grating and to avoid a 135° grating to reach a feeder, and to do the opposite to reach their nest. They also learn that an annular or a radial disc must be chosen, depending on the disc's association with a 45° or a 135° grating either at the feeder or the nest entrance: in one context (the nest) access was allowed by the combinations 45° + radial disc and 135° + annular disc, but not by the combinations 45° + annular disc and 135° + radial disc; at the feeder, the opposite was true. In both cases, the potentially competing visuo-motor associations were insulated from each other because they were set in different contexts. Comparable behavior was found in honeybees where distinct odors or times of the day were the occasion setters for a given flight vector or rewarded color. Further examples for contextual learning could be provided but they would be redundant for the main conclusion of this section which is that invertebrates are capable of different forms of conditional learning. Despite this cumulative body of evidences, the nature of the associations underlying this kind of learning and the neural substrates underlying this form of learning remain unclear.

Studies on decision making in the fruit fly *Drosophila melanogaster* indicate that mushroom bodies are of fundamental importance for this behavior. In this case, an individual fly suspended at a torque meter from a copper wire glued to its thorax beats its wings when hanging in the middle of a cylindrical arena displaying a visual panorama with identifiable landmarks (Fig. 3). An unpleasant heat-beam is focused on the fly's thorax and switched on whenever the insect fly towards a given landmark on the cylinder. The fly controls the reinforcer delivery as its flight maneuvers determine the on/off switching of the heat beam if the appropriate flight directions (i.e. landmarks) are chosen. In studies on decision-making in *Drosophila*, flies were conditioned to choose one of two flight paths in response to color and shape cues; after the training, they were tested with contradictory cues. Normal flies made a discrete choice that switched from one alternative to the other as the relative salience of color and shape cues gradually changed, but this ability was greatly diminished in mutant flies with miniature mushroom bodies or with hydroxyurea ablation of mushroom bodies. Although this protocol does not provide a formalized non-linear discrimination problem such as those presented above (e.g. negative patterning), it has the merit of moving from the traditional elemental learning protocols applied so far in *Drosophila* to a more sophisticated problem in

which the cognitive richness of fly behavior could be revealed and related to the mushroom bodies. Furthermore, it was shown that salience-dependent choice behavior consists of early and late phases; the former requires activation of the dopaminergic system and mushroom bodies, whereas the latter is independent of these activities. Immunohistological analysis showed that mushroom bodies are densely innervated by dopaminergic axons, thus suggesting that the circuit from the dopamine system to mushroom bodies is crucial for choice behavior in *Drosophila*

Positive transfer in rule learning by invertebrates

Non-elemental associative learning also underlies problem solving in which animals respond to novel stimuli that they have never encountered before or can generate novel responses that are adaptive given the context in which they are produced. In doing this, the animals exhibit a positive transfer of learning, a capacity that cannot be referred to as an elemental learning because the responses are aimed toward stimuli that do not predict a specific outcome per se based on the animals' past experience.

A typical example of rule learning is the acquisition of the sameness or difference principle. These rules are demonstrated through the protocols of delayed matching to sample (DMTS) and delayed non-matching to sample (DNMTS), respectively. In DMTS, animals are presented with a sample and then with a set of stimuli, one of which is identical to the sample and which is reinforced. Since the sample is regularly changed, animals must learn the sameness rule, i.e. *'always choose what is shown to you (the sample), independent of what else is shown to you'*. In DNMTS, the animal has to learn the opposite. Honeybees foraging in a Y-maze learn both rules. Bees were trained in a DMTS problem in which they were presented with a changing non-rewarded sample (i.e. one of two different color disks or one of two different black-and-white gratings, vertical or horizontal) at the entrance of a maze (Fig. 4). The bees were rewarded only if they chose the stimulus identical to the sample once within the maze. Bees trained with colors and presented in transfer tests with black-and-white gratings that they have not experienced before solved the problem and chose the grating identical to the sample at the entrance of the maze. Similarly, bees trained with the gratings

and tested with colors in transfer tests also solved the problem and chose the novel color corresponding to that of the sample grating at the maze entrance. Transfer was not limited to different kinds of modalities (pattern vs. color) within the visual domain, but could also operate between drastically different domains such as olfaction and vision. Furthermore, bees also mastered a DNMTS task, thus showing that they also learn a rule of difference between stimuli. These results document that bees learn rules relating stimuli in their environment. The capacity of honeybees to solve a DMTS task has recently been verified and studied with respect to the working memory underlying it. It was found that the working memory for the sample underlying the solving of DMTS is around 5 s and thus coincides with the duration of other visual and olfactory short-term memories characterized in simpler forms of associative learning in honeybees (Menzel 1999). Moreover, bees trained in a DMTS task can learn to pay attention to one of two different samples presented successively in a flight tunnel (either to the first or to the second) and can transfer the learning of this sequence weight to novel samples.

The neural basis of rule extraction has not been addressed yet in invertebrates. The potentials offered by *Drosophila* with respect to molecular genetics and by the bee with respect to the recording of neural correlates will certainly be used in the near future to establish closer links to the neural substrates.

Conclusion

Here we focused on a particular basic cognitive faculty that relates to the ability of animals to process sequences of associative connections such that structures of interrelatedness are derived which are not housed in the elemental associations. In some cases rules are learned and applied across sensory modalities, in others temporal relations are acquired. Learning under natural conditions will be much richer than implied here because bees, for example, are known to navigate along novel routes according to the expected outcome of the navigational choices, and *Drosophila* decides between flight goals by integrating multiple stimulus conditions. These and the examples discussed here require brain functions best conceptualized as representations, since the relations established during learning cannot reside in basic cellular modules of associative connections as they were so

successfully studied in invertebrates. Rather they must be represented in network properties composed of multiple cellular association modules which incorporate new information into already stored information by some self-organization process, retrieve appropriate information from remote stores and allow decisions to be made according to the current conditions, the internal status of the animal and the evaluated expected outcomes. Hints for memory processing both during memory storage and retrieval come from multiple observations. For example, consolidation of earlier forms of memory into later and stable forms change the content of the memory and are accompanied by transfer between structures, e.g. between the gamma lobe and the alpha/beta lobe neurons in the mushroom body of *Drosophila*. Memory retrieval initiates processes describes as reconsolidation, and decisions between simultaneously activated memories are being made without access to stimuli according to the expected outcome. In this respect, memory processing during storage and retrieval in invertebrates resembles basic features described for mammals and humans, and it is conceivable that analog network processes may be responsible despite the large differences in the structure and functional organization between e.g. insect and mammalian brains. How are we to discover these processes? A fundamental requirement for any experimental approach is that the working of the neural nets are monitored at the level of multiple but single neurons under conditions in which the animal learns, retrieves and processes memory. Ideally these neurons should be identifiable anatomically, aiming to establish a close relationship between structure and function. These strict requirements are not met by any animal although recent advances in optical and electrical recordings from neurons in the *Drosophila* and the bee brain come close.

Two streams of new developments have to meet in an attempt to take advantage of invertebrates as models for a cognitive neuroscience approach, a conceptual shift in addressing the phenomena of learning and memory, and a major methodological advance. Methodological advances are already on the verge. Calcium and voltage sensitive dyes as well as light driven dyes for controlling neural excitation can be expressed in defined neurons of the *Drosophila* brain while recordings from multiple neurons in the bee brain can be performed for several days when the animals learn and perform. A more fundamental achievement will be the conceptual shift, which relates to the necessity to include invertebrates into the cognitive view of behavior. It is the combination of stereotypical and

highly flexible behavior of invertebrates which makes them such attractive study objects for a cognitive approach. Evidence presented and discussed in this article aims at promoting this cognitive framework to understand invertebrate behavior.

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Glossary

Classical conditioning: A process of behavior modification by which a subject comes to respond in a desired manner to a previously neutral stimulus (conditioned stimulus or CS) that has been repeatedly presented along with an unconditioned stimulus (US) that elicits the desired response.

Non-elemental Learning: Associative forms of learning in which individual events are ambiguous and only logical combinations of them can be used to solve a discrimination problem.

Occasion setting: A learning situation in which a stimulus, the occasion setter, sets the occasion for when or where a predictive relationship applies. Contextual learning is closely related to occasion setting.

Operant conditioning: Associative forms of learning in which an individual learns the consequences of its own behavior. It is a form of conditioning in which the desired behavior or increasingly closer approximations to it are followed by a rewarding or reinforcing stimulus.

Rule learning: The ability to infer rule information from a number of different examples connected by a logical operation 'if \rightarrow then'

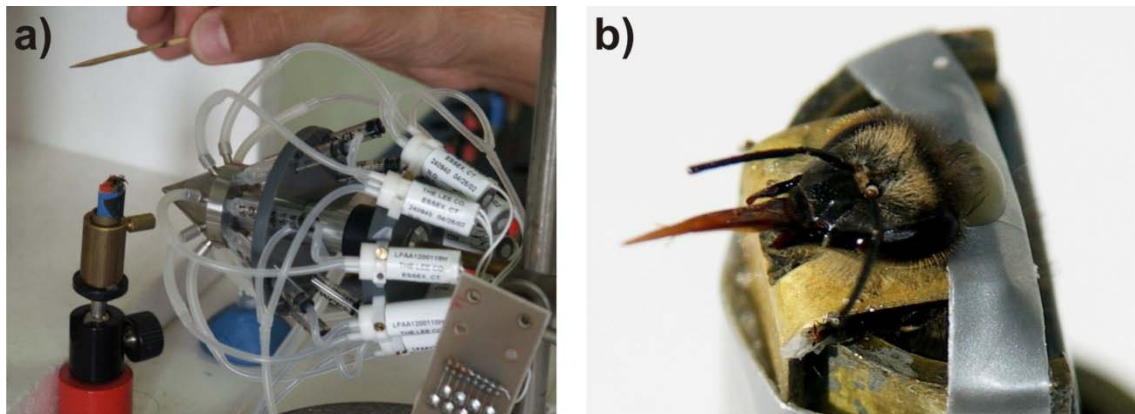


Figure 1: Olfactory conditioning of the proboscis extension reflex. **a)** An individual bee is immobilized in a metal tube so that only the antennae and mouth parts (the proboscis) are free to move. The bee is set in front of an odorant stimulation setup which is controlled by a computer and which sends a constant flow of clean air to the bee. The air flow can be rerouted through cartridges presenting chemicals used for olfactory stimulation (conditioned stimuli or CS). A toothpick soaked in sucrose solution (unconditioned stimulus or US) is delivered to the antennae and the proboscis. In this appetitive classical conditioning, the bee learns to associate odorants (CS) and sucrose solution (US). **b)** The proboscis extension reflex of the honeybee. Bees exhibit this reflex when their antennae are touched with sucrose solution (US). After successful conditioning, bees extend the proboscis to the odorant (CS) which predicts the US.

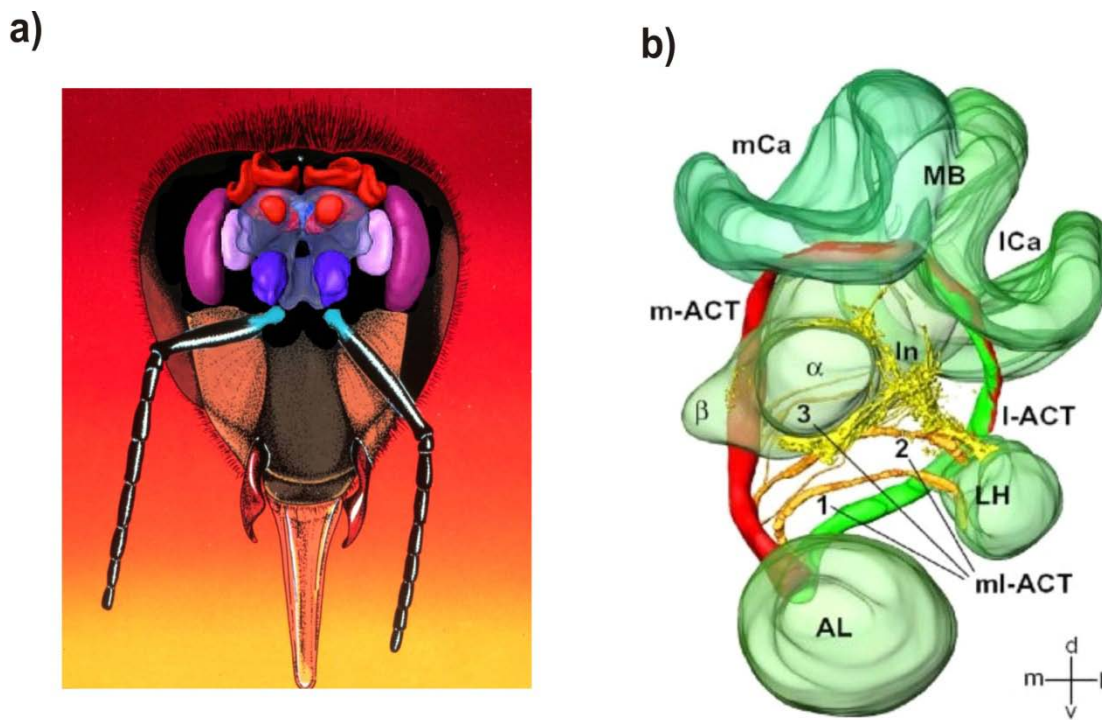


Figure 2: The basic organization of the honeybee olfactory system. a) Frontal view of the brain with the main olfactory centers; b) Three-dimensional reconstruction of the olfactory circuit based on confocal microscopy (*kind courtesy of Wolfgang Roessler*); AL: antennal lobe; LH: lateral horn; MB: mushroom body; m-ACT: medial antenno-cerebral tract; l-ACT: lateral antenno-cerebral tract; mCa: medial calyx; lCa: lateral calyx; alpha and beta: alpha and beta lobes of the mushroom body.

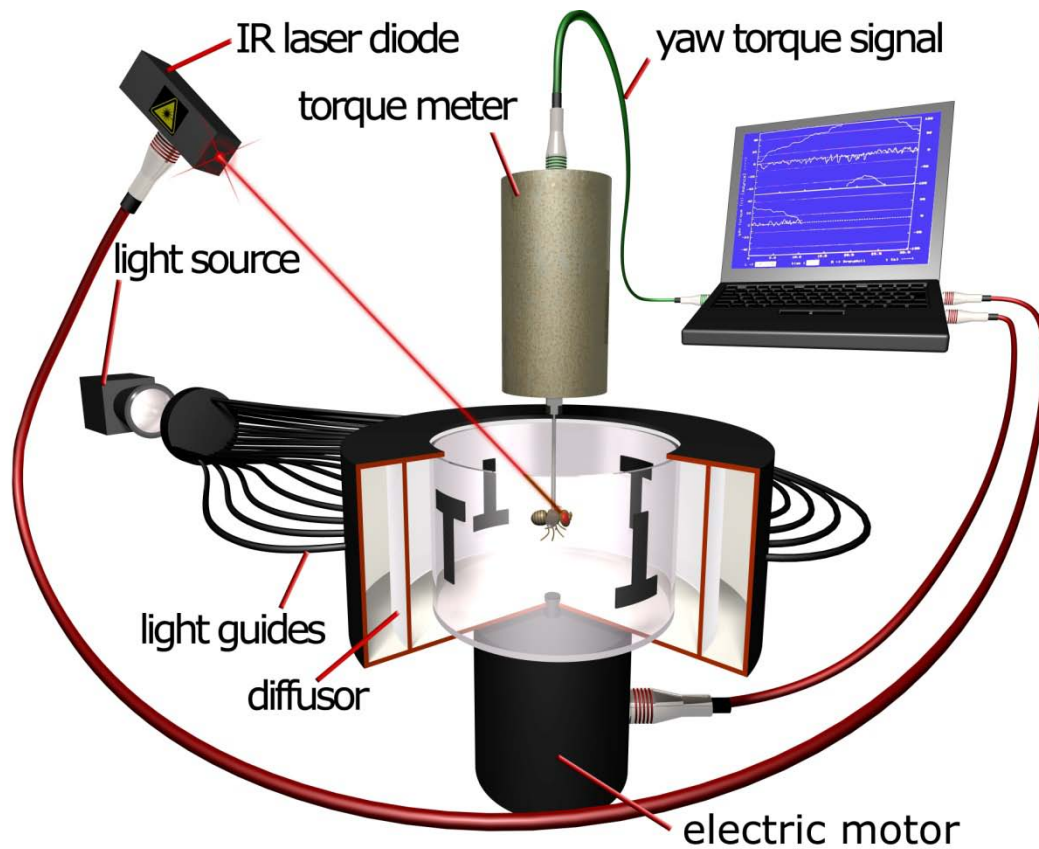


Figure 3: The flight simulator used for visual conditioning of a tethered fruit fly. A *Drosophila* is flying stationarily in a cylindrical arena homogeneously illuminated from behind. The fly's tendency to perform left or right turns (yaw torque) is measured continuously and fed into the computer. In closed-loop, the computer controls arena rotation. On the screen four 'landmarks', two Ts and two inverted Ts, are displayed in order to provide a referential frame for flight direction choice. The illumination of the arena can be changed using color filters. A heat beam focused on the fly's thorax is used as an aversive reinforcer. The reinforcer is switched on whenever the fly flies towards a prohibitive direction. The fly controls therefore reinforcer delivery by means of its flight direction so that operant conditioning mediates the performance observed. However, classical associations between landmarks and reinforcer (or its absence) can also be established in this protocol (courtesy of B. Brembs).

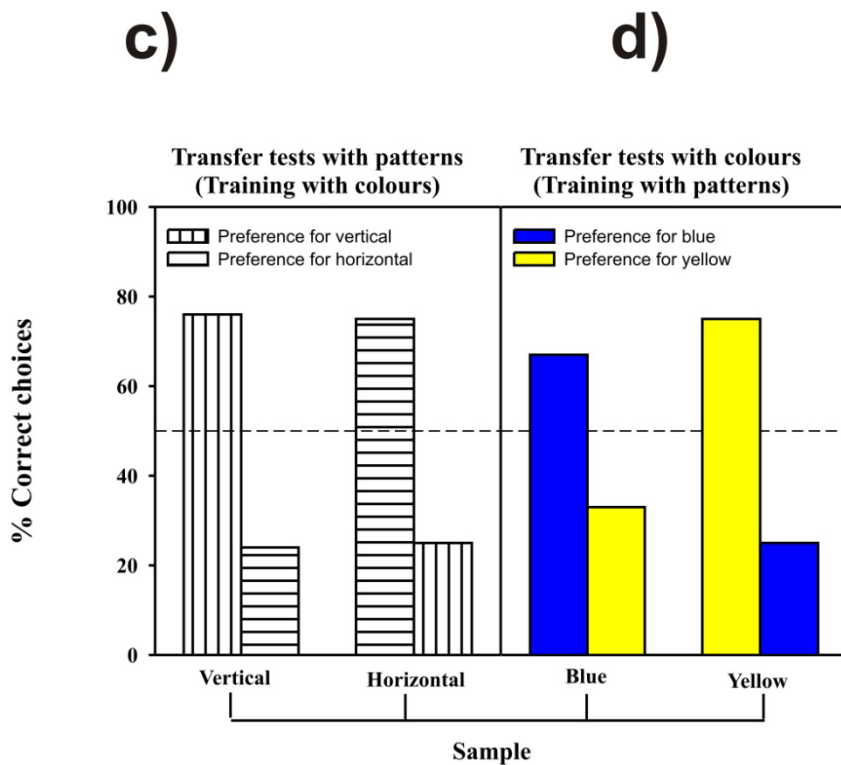
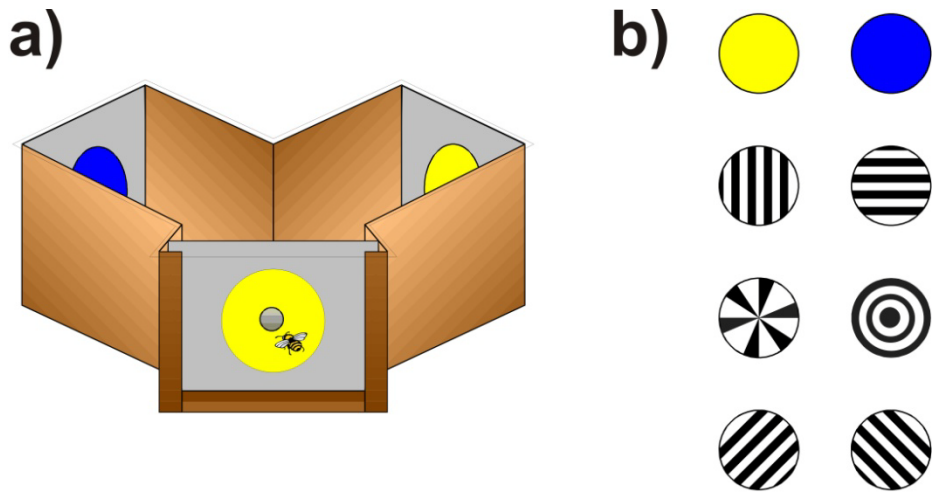


Figure 4: Rule learning in honeybees. Honeybees trained to collect sugar solution in a Y-maze (a) on a series of different patterns (b) learn a rule of sameness. Learning and transfer performance of bees in a delayed matching-to-sample task in which they were

trained to colors (Experiment 1) or to black-and-white, vertical and horizontal gratings (Experiment 2). **c, d**) Transfer tests with novel stimuli. **c**) In Experiment 1, bees trained on the colors were tested on the gratings. **d**) In Experiment 2, bees trained on the gratings were tested on the colors. In both cases bees chose the novel stimuli corresponding to the sample although they had no experience with such test stimuli. n denotes number of choices evaluated (from Giurfa et al. 2001).

Abstract

In this work we studied the cognitive sophistication reached by the honeybee *Apis mellifera* when analysing its visual environment. Thanks to a new-designed learning protocol allowing better performance of bees' visual discrimination, we studied visual stimuli classification by categorization and concept formation. In the first case, bees grouped visual objects into classes defined by perceptual similarity; in the second case, bees extract abstract rules from visual stimuli (e.g. 'bigger than') instead of their specific physical properties. We studied in particular stimuli categorization based on a "face-like" configuration. We show that this insect can extract relationships between the elements of a schematic face and combine them to define a category. Thus, novel stimuli presenting this configuration would be processed as member of the category of interest. Moreover, bees seem to naturally use configuration to recognize visual objects. This processing is thus not only induced by our training procedure. We also studied the bees' acquisition of spatial relational concepts such as "above" or "below", regardless of the elements involved in these relationships. The bee has, in addition, shown its ability to combine two different concepts (spatial relationship and difference between the elements involved in the relationship) in a rule in order to obtain a reward. This rule is transferable to novel physically different stimuli. These results demonstrate an unsuspected level of analysis and abstraction in an invertebrate and open debate on the neural minimum architecture required to achieve such cognitive complexity.

Key-words: Vision, cognition, configuration, relational concept, categorization, comparative cognition, insect, honeybee, *Apis mellifera*.

Résumé

Dans ce travail nous avons étudié la sophistication cognitive dont est capable l'abeille domestique *Apis mellifera* dans l'analyse de son environnement visuel. Grâce à la mise en place d'une procédure expérimentale d'apprentissage permettant de mettre en évidence les performances fines de discrimination visuelle des abeilles, nous avons étudié la classification de stimuli visuels par catégorisation et formation de concepts. Dans le premier cas, les abeilles regroupent des objets visuels en fonction de leur appartenance à une catégorie définie par une similarité perceptive; dans le deuxième cas, les abeilles regroupent les stimuli visuels à partir de règles abstraites (ex: 'plus grand que') et non de leurs propriétés physiques. Nous avons étudié en particulier la catégorisation de stimuli sur la base d'une configuration de type « visage ». Nous montrons que cet insecte peut extraire les relations entre les éléments d'un visage schématique et les combiner de façon à définir une catégorie. Ainsi, la présence de cette configuration permet de traiter de nouveaux stimuli comme appartenant à la catégorie d'intérêt. L'utilisation de configuration pour reconnaître des objets visuels semble être naturellement utilisée par l'abeille et n'est donc pas seulement induite par un entraînement spécifique. Nous avons par ailleurs étudié l'acquisition par l'abeille de concepts relationnels de nature spatiale tels que « au-dessus » ou « en-dessous », indépendamment des éléments impliqués dans ces relations. L'abeille s'est de plus montrée capable d'associer deux concepts différents (relation spatiale et différence entre les éléments impliqués dans la relation) dans une règle permettant d'obtenir une récompense, transférable à de nouveaux stimuli physiquement très différents. Ces résultats mettent en évidence un niveau d'analyse et d'abstraction insoupçonné pour un invertébré et ouvrent le débat sur l'architecture neurale minimale requise pour atteindre une telle sophistication cognitive.

Mots-clés : Vision, cognition, configuration, concept relationnel, catégorisation, cognition comparative, insecte, abeille, *Apis mellifera*.

Titre : Cognition visuelle chez l'abeille *Apis mellifera*: Catégorisation par extraction de configurations spatiales et de concepts relationnels

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Lieu et date de soutenance : Université Paul Sabatier - Toulouse III, le 13 décembre 2010

Résumé

Dans ce travail nous avons étudié la sophistication cognitive dont est capable l'abeille domestique *Apis mellifera* dans l'analyse de son environnement visuel. Grâce à la mise en place d'une procédure expérimentale d'apprentissage permettant de mettre en évidence les performances fines de discrimination visuelle des abeilles, nous avons étudié la classification de stimuli visuels par catégorisation et formation de concepts. Dans le premier cas, les abeilles regroupent des objets visuels en fonction de leur appartenance à une catégorie définie par une similarité perceptive; dans le deuxième cas, les abeilles regroupent les stimuli visuels à partir de règles abstraites (ex: 'plus grand que') et non de leurs propriétés physiques. Nous avons étudié en particulier la catégorisation de stimuli sur la base d'une configuration de type « visage ». Nous montrons que cet insecte peut extraire les relations entre les éléments d'un visage schématique et les combiner de façon à définir une catégorie. Ainsi, la présence de cette configuration permet de traiter de nouveaux stimuli comme appartenant à la catégorie d'intérêt. L'utilisation de configuration pour reconnaître des objets visuels semble être naturellement utilisée par l'abeille et n'est donc pas seulement induite par un entraînement spécifique. Nous avons par ailleurs étudié l'acquisition par l'abeille de concepts relationnels de nature spatiale tels que « au-dessus » ou « en-dessous », indépendamment des éléments impliqués dans ces relations. L'abeille s'est de plus montrée capable d'associer deux concepts différents (relation spatiale et différence entre les éléments impliqués dans la relation) dans une règle permettant d'obtenir une récompense, transférable à de nouveaux stimuli physiquement très différents. Ces résultats mettent en évidence un niveau d'analyse et d'abstraction insoupçonné pour un invertébré et ouvrent le débat sur l'architecture neurale minimale requise pour atteindre une telle sophistication cognitive.

Mots-clés : Vision, cognition, configuration, concept relationnel, catégorisation, cognition comparative, insecte, abeille, *Apis mellifera*.

Discipline : Neurosciences cognitives

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