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Caterpillar-induced nocturnal plant volatiles repel conspecific females

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Plants respond to insect herbivory by synthesizing and releasing complex blends of volatile compounds, which provide important host-location cues for insects that are natural enemies of herbivores[1-3]. The effects of these volatile blends on herbivore behaviour have been investigated to only a limited extent[4,5], in part because of the assumption that herbivore-induced volatile emissions occur mainly during the light phase of the photoperiod[6,7]. Because many moths—whose larvae are some of the most important insect herbivores—are nocturnal, herbivore-induced plant volatiles have not hitherto been considered to be temporally available as host-location cues for ovipositing females. Here we present chemical and behavioural assays showing that tobacco plants (Nicotiana tabacum) release herbivore-induced volatiles during both night and day. Moreover, several volatile compounds are released exclusively at night and are highly repellent to female moths (Heliothis virescens). The demonstration that tobacco plants release temporally different volatile blends and that lepidopteran herbivores use induced plant signals released during the dark phase to choose sites for oviposition adds a new dimension to our understanding of the role of chemical cues in mediating tritrophic interactions.

Feeding by insect herbivores induces plants to release chemical signals that serve as important foraging cues for parasitoids and predators, and thus enhance the plants' defence[1-3,8-10]. Synthesis and release of these chemical signals is an active physiological process triggered by substances in the oral secretion of herbivores[11,12]. The recent discovery that plant volatiles can transmit herbivore-specific information that allows natural enemies to identify particular herbivore species demonstrated that chemically mediated plant–insect interactions are more sophisticated and complex than was previously appreciated[13]. However, the role of chemical signals in plant–herbivore interactions remains largely unexplored. Some researchers have examined the effects of constitutive plant volatiles and herbivore-induced daytime volatiles on conspecific herbivores[4,14-17] including some lepidopterans[18], but the effect of herbivore-induced plant volatiles on moths that are active at night has been neglected. The fact that several major terpene components of herbivore-induced plant volatiles have high emissions during the periods of maximal photosynthesis[6,7] may explain why little attention has been paid to the importance of these volatiles to female moths searching for oviposition sites at night. To our knowledge, this study represents the first demonstration that plants emit herbivore-induced volatile blends that exhibit systematic temporal variation, that some volatile compounds are released exclusively at

night, and that female moths exploit these specific night-time signals to avoid oviposition on previously damaged plants.

Gas chromatographic analysis of volatiles collected in two-hour intervals continuously for seven days revealed consistent differences in the composition of volatile blends released by *H. virescens*-infested tobacco plants ($n = 6$) during the light and dark phases of the photoperiod (Fig. 1a). Visual and auditory observations confirmed that larvae fed during both the light and dark phases. Seven major compounds were consistently released during both light and dark phases, but usually in lesser amounts during the dark phase (Fig. 1a). In addition, five compounds ((Z)-3-hexenyl butyrate, (Z)-3-hexenyl isobutyrate, (Z)-3-hexenyl acetate, (Z)-3-hexenyl tiglate, and one unidentified compound) were produced only during the dark phase. Others—(E)-2-hexenal and three unidentified compounds—were produced in significantly larger amounts during the dark than the light period. Thus, the qualitative and quantitative composition of volatile blends emitted by tobacco plants in response to feeding by *H. virescens* larvae can differ significantly between night and day.

Repetition of our analysis using two other species of lepidopteran larvae ($n = 6$ per species), *Manduca sexta* and *Helicoverpa zea*, provided further evidence of induced volatile release from tobacco plants during the dark period (Fig. 1b). Although the volatile
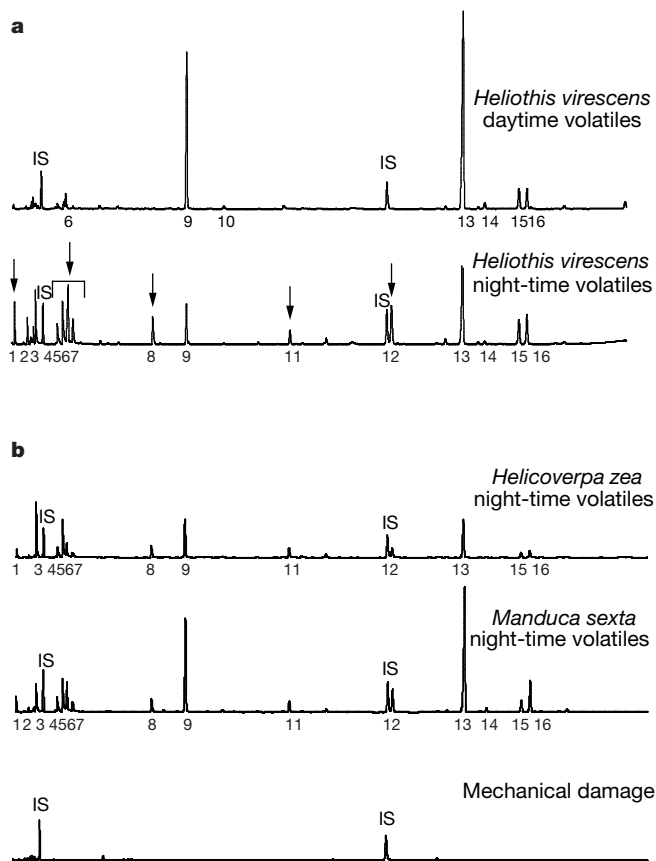

Figure 1 Gas chromatographic analysis of induced plant volatiles. **a**, Diurnal and nocturnal profiles of volatiles released from tobacco plants during a 2-h interval after 48 h of feeding by *H. virescens*. Arrows represent volatiles that are present only (or in significantly larger amounts) in the nocturnal profile. **b**, Nocturnal profiles of volatiles released from tobacco plants during a 2-h interval after 48 h of feeding by *H. virescens*, *H. zea* or *M. sexta* compared with mechanical damage. Represented are: 4, (E)-2-hexenal; 5, (Z)-3-hexen-1-ol; 8, (Z)-3-hexenyl acetate; 9, (E)-β-ocimene; 10, linalool; 11, (Z)-3-hexenyl butyrate; 12, (Z)-3-hexenyl tiglate; 13, β-caryophyllene; 14, α-humulene; 15, (E,E)-α-farnesene; 16, unidentified sesquiterpene; compounds 1–3, 6, 7 are unidentified compounds; IS, internal standards (*n*-octane and *n*-nonyl-acetate).

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profiles revealed quantitative differences in plant response to the three herbivores, all caterpillar species induced release of the same volatile compounds from tobacco during the night (Fig. 1). To determine whether plants continue nocturnal volatile production in the absence of continuous feeding by the caterpillars, *H. virescens* were removed from the plants at 15:00 after 48 hours of feeding. In this case, plants still emitted volatiles during the dark phase although in smaller amounts.

In behavioural trials, mated *H. virescens* females spent a significantly greater proportion of time (80% of the observed hour) in an area with only undamaged plants than in an area with both damaged and undamaged plants (Fig. 2A, a). Moths exhibited a tendency to fly south when first released; however, if the damaged plants were placed to the south, the moths would often turn and fly in the opposite direction. The same preference was displayed in oviposition. Females selected only non-infested plants for oviposition (Fig. 2A, b; $F_{1,10} = 427$, $P < 0.0001$) and also avoided uninfested plants close to infested plants.

Because the preference of *H. virescens* for uninfested plants might conceivably be explained by visual cues associated with plant damage, we repeated the behavioural assays using synthetic volatile blends. Synthetic blends of the major volatile compounds that were produced in significant amounts and emitted by the plants during the dark period were formulated on rubber septa[19,20] to release volatiles in approximately the same proportions and amounts as released by herbivore-damaged plants. In behavioural assays, *H. virescens* demonstrated a significant preference ($F_{1,10} = 134$,

$P < 0.0001$) for untreated tobacco plants over those with septa releasing the synthetic blends (Fig. 2B, b). In control trials, the response to plants with septa containing only solvent (hexane) was statistically indistinguishable ($F_{1,10} = 1.91$, $P > 0.05$) from that to untreated plants (Fig. 2B, a). Thus, female *H. virescens* are able to identify infested plants on the basis of chemical cues consisting of volatile compounds emitted by the plants at night.

Some of the volatiles in the nocturnal volatile profile were also present in the diurnal profile. However, others were released only at night. To determine the importance of volatiles produced at night, relative to those produced during the day, we repeated the behavioural assays using the diurnal blends. Although these daytime volatiles produced avoidance behaviour (Fig. 2B, c; $F_{1,10} = 55$, $P < 0.001$), they were significantly less repellent than the nocturnal volatiles (Fig. 2B, b), indicating that the moths are specifically repelled by the night-time volatile blends. To further examine the extent to which the repellence of the night-time volatiles is due to the presence of compounds produced exclusively at night, the experiment was repeated using a synthetic blend containing only these compounds. The presence of these exclusively nocturnal compounds alone was sufficient to explain the moth repellence effect (Fig. 2B, d; $F_{1,10} = 145$, $P < 0.0001$).

It is well established that induced plant volatiles function as signals between plants and the natural enemies of insect herbivores. The discovery that the herbivores themselves exploit the information present in night-time volatile blends to avoid oviposition on previously damaged plants reveals a new dimension of chemically
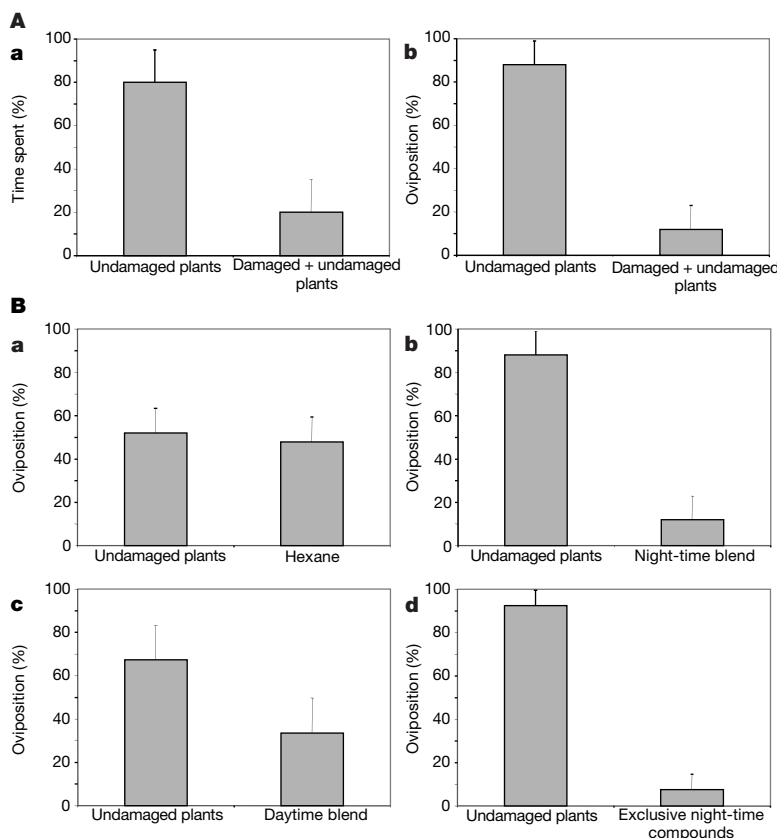

Figure 2 Response of female moths to plant volatiles. **A**, Nocturnal flight responses of three mated *H. virescens* females to tobacco plants. On one side of the cage, only undamaged plants (6) were used. On the other side, two plants that had been infested by ten third-instar *H. virescens* larvae were included. Larvae were removed from the plants before moths were introduced. **a**, Per cent of time during one hour of observation spent on each side of the cage, **b**, per cent of eggs oviposited on each side of the cage. **B**, Oviposition preference by *H. virescens* females for tobacco plants that had been treated with synthetic blend. On one side of the cage only undamaged plants (6) were used, on the other side two plants were treated (synthetic blends on rubber septa) placed among four undamaged and untreated plants. **a**, Solvent (hexane) used as control. **b**, A nocturnal blend compared to undamaged plants. **c**, A diurnal blend compared to undamaged plants. **d**, A blend containing compounds released exclusively at night compared to undamaged plants.

mediated plant–insect interactions and raises a number of issues. The fitness advantages to herbivores of avoiding oviposition on induced plants are obvious, as such plants are likely to host not only larvae that represent potential competitors for the moth's offspring but also potentially a population of natural enemies attracted by the volatile blend[21]. Moreover, the associated induction of direct defence mechanisms means that infested plants are likely to contain chemical toxins and to be of lower nutritional value than uninfested plants[22,23]. It is less clear whether plants benefit significantly from the release of nocturnal volatiles or whether such release is merely a physiological by-product associated with diurnal volatile production. The protection enjoyed by undamaged plants that reside near induced plants is interesting, but it is not certain whether this phenomenon would have been significant in the ancestral environment or represents only an effect of high-density agricultural cultivation. If plants do benefit from advertising their status to herbivores, it raises the question of whether herbivore-induced signals first evolved as parasitoid attractants or as herbivore repellents; alternatively, the dual functions of herbivore-induced plant signals may have evolved simultaneously.

The recognition that plant volatile signals, long known to be important in the mediation of plant–parasitoid interactions, also transmit information to herbivores expands our view of tritrophic systems and is significant with regard to our understanding of the selective pressures governing the evolution of such signals. We are currently exploring whether moths can interpret the herbivore-specific information that these signals convey to parasitoids[13] or rather use these signals only as generalized indicators of insect feeding. If moths can interpret the higher-order information content of these signals, then they may be making sophisticated choices based on the likely presence of particular larval competitors and perhaps even of particular predators and parasitoids. □

Methods

Volatile collection and analysis

All experiments were conducted in an insect-free greenhouse (temperature 29 ± 4 °C). Ten third-instar larvae of either *H. virescens*, *H. zea* or *M. sexta* were allowed to feed continuously on the leaves of an eight-week-old, greenhouse-grown tobacco plant (*Nicotiana tabacum* strain K326) enclosed in a volatile collection chamber[24], beginning the night before collection commenced.

Plant volatiles were collected 24-h a day in 2-h intervals by pulling 1 l of the air passing over the plant (5 l min^{-1}) through Super Q adsorbent (25 mg) traps at the base of the volatile-collection chamber; the remainder of the air vented out the bottom of the system[24]. Traps were rinsed with 150 μl methylene chloride, 400 ng of *n*-octane and nonyl acetate were added as internal standards, and samples were analysed by gas chromatography and mass spectrometry[13] (electron impact and chemical ionization with isobutane reagent gas). Volatile compounds were identified by comparison of chromatographic retention times and mass spectra with those of commercially available standards analysed on the same instruments. Quantification was based on peak area (flame ionization detector) relative to that of internal standards.

Average volatile released by plants from 11:00 to 13:00, 50 h after initial damage: (ng h^{-1}, s.d.): (*E*)-2-hexenal (190.93, 23.06); (*Z*)-3-hexen-1-ol (62.04, 40.08); (*E*)-β-ocimene (5510.46, 789.72); indole (212.67, 45.03); β-caryophyllene (7481.88, 823.40); α-humulene (221.88, 13.08); (*E,E*)-α-farnesene (1220.48, 152.81), unidentified sesquiterpene (1148.25,165.85).

Average volatile released by plants from 19:00 to 21:00, 58 h after initial damage: (ng h^{-1}, s.d.): (*E*)-2-hexenal (488.44, 36.78); (*Z*)-3-hexen-1-ol (792.70, 160.12); (*Z*)-3-hexenyl acetate (987.99, 97.12); (*E*)-β-ocimene (1610.46/349.32); (*Z*)-3-hexenyl isobutyrate (123.84, 23.76); (*Z*)-3-hexenyl butyrate (488.85, 121.67) indole (212.67, 45.03); (*Z*)-3-hexenyl tiglate (1328.87, 198.76), β-caryophyllene (2452.88, 223.46); α-humulene (83.48, 35.17); (*E,E*)-α-farnesene (888.88, 154.98), unidentified sesquiterpene (960.59, 200.91).

Behavioural assays

Twelve eight-week-old, potted, greenhouse-grown tobacco plants were placed in a screened cage (4 × 2 × 3 m) with six plants on each side of the cage (80 cm between plants). On one side only undamaged plants were used, on the other side two of the plants had been fed on by 10 laboratory-reared third-instar *H. virescens* (two larvae per leaf) for 48 h. Larvae were removed from plants before moth release. Three mated *H. virescens* females (18 females per treatment) were released in the central sector of the cage at dusk. To account for a possible directional preference by the moths, two cages were used so in one the damaged plants were on the north end and in the other they were on the south end of the cage. The moths' behaviour was visually observed for one hour (from approximately

19:00 to 20:00) and the time (min.) spent by moths on each side of the cage was recorded. Egg numbers per plant on each side of the cage were counted at 06:00 the next morning. Similar procedures were used for assays measuring oviposition and behaviour of female moths in response to plants with or adjacent to synthetic blends, but in this case plants on both sides of the cages were undamaged. For these treatments, pots of undamaged plants with volatile-releasing rubber septa on wooden sticks (three per plant) replaced the damaged plants. We evaluated the response of moths to three synthetic volatile blends (nocturnal volatiles, exclusively nocturnal volatiles, and diurnal volatiles). Rubber septa without the synthetic blend were placed on the control side to neutralize any visual preference. Each bioassay was conducted on three days (two repetitions each) to account for day-to-day variation. Preference between the undamaged and damaged plants by the herbivores was subjected to analysis of variance (an arc-sine square root transformation of the percentage data was used; SAS Institute, Cary, North Carolina).

Synthetic blends

Synthetic blends were formulated according to a method developed to predict the release ratio of volatile compounds from rubber septa[19,20]. Calculations of predicted release ratios are based on relative vapour pressures[19] of the components and the original amounts released in the natural blend. Blends were dissolved in hexane and 0.3 ml of a blend solution was pipetted onto a rubber septum. Volatiles released by the blend when formulated on rubber septa were sampled and analysed. The relative amounts were adjusted to correct for slight deviations from the predicted amount. Compounds used to prepare synthetic blends were obtained from Sigma-Aldrich or from Chemical Samples and analysed by gas chromatography and mass spectrometry to determine purity. All synthetic compounds were at least 98% pure except for ocimene where both isomers were present (60% *trans* and 40% *cis*).

Release rates of synthetic blends: daytime blend 1, (*E*)-2-hexenal (200 ng h^{-1}); 2, (*E*)-β-ocimene (5,500 ng h^{-1}); 3, β-caryophyllene (7,300 ng h^{-1}); 4, α-humulene (200 ng h^{-1}); 5, (*E,E*)-α-farnesene (1,180 ng h^{-1}). Night-time blend 1, (*E*)-2-hexenal (500 ng h^{-1}); 2, (*Z*)-3-hexen-1-ol (850 ng h^{-1}); 3, (*Z*)-3-hexenyl acetate (1,000 ng h^{-1}); 4, (*E*)-β-ocimene (1,500 ng h^{-1}); 5, (*Z*)-3-hexenyl isobutyrate (100 ng h^{-1}); 6, (*Z*)-3-hexenyl butyrate (500 ng h^{-1}); 7, (*Z*)-3 hexenyl tiglate (1,200 ng h^{-1}); 8, β-caryophyllene (2,200 ng h^{-1}); 9, (*E,E*)-α-farnesene (900 ng h^{-1}). Exclusive night-time blend 1, (*Z*)-3-hexen-1-ol (850 ng h^{-1}); 2, (*Z*)-3-hexenyl acetate (1,000 ng h^{-1}); 3, (*Z*)-3-hexenyl isobutyrate (100 ng h^{-1}); 4, (*Z*)-3-hexenyl butyrate (500 ng h^{-1}); 5, (*Z*)-3-hexenyl tiglate (1,200 ng h^{-1}).

1. Dicke, M. Local and systemic production of volatile herbivore-induced terpenoids: their role in plant-carnivore mutualism. *J. Plant Physiol.* **143,** 465–472 (1994).
2. Turlings, T. C. J., Tumlinson, J. H. & Lewis, W. J. Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250,** 1251–1253 (1990).
3. McCall, P. J., Turlings, T. C. J., Lewis, W. J. & Tumlinson, J. H. Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Cresson (Braconidae:Hymenoptera). *J. Insect Behav.* **6,** 625–639 (1993).
4. Pallini, A., Jansen, A. & Sabelis, M. Odour-mediated responses of phytophagous mites to conspecific and heterospecific competitors. *Oecologia* **110,** 179–185 (1997).
5. Karban, R. & Baldwin, I. T. *Induced Responses to Herbivory* (Univ. Chicago Press, Chicago, Illinois, 1997).
6. Paré, P. W. & Tumlinson, J. H. Induced synthesis of plant volatiles. *Nature* **385,** 30–31 (1997).
7. Loughrin, J. H., Manukian, A., Heath, R. R. & Tumlinson, J. H. Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *J. Chem. Ecol.* **21,** 1217–1227 (1994).
8. Turlings, T. C. J. et al. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl Acad. Sci. USA* **92,** 4169–4174 (1995).
9. Karban, R. Resistance against spider mites in cotton induced by mechanical abrasion. *Entomol. Exp. Appl.* **37,** 137–141 (1985).
10. Dicke, M. et al. Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. Involvement of host plant in its production. *J. Chem. Ecol.* **16,** 381–396 (1990).
11. Mattiacci, L., Dicke, M. & Posthumus, M. A. Induction of parasitoid attracting synomone in brussels sprouts plants by feeding of *Pieris brassicae* larvae: Role of mechanical damage and herbivore elicitor. *J. Chem. Ecol.* **20,** 2229–2247 (1994).
12. Alborn, H. T. et al. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* **276,** 945–948 (1997).
13. De Moraes, C. M. et al. Herbivore-infested plants selectively attract parasitoids. *Nature* **393,** 570–573 (1998).
14. Dicke, M. Volatile spider-mite pheromone and host plant kairomone, involved in spaced-out gregariousness in spider mite *Tetranychus urticae*. *Physiol. Entomol.* **11,** 251–262 (1986).
15. Schultz, S. et al. Host plant selection of the Colorado Potato Beetle as influenced by damage induced volatiles of the potato plant. *Naturwissenschaften* **84,** 212–217 (1997).
16. Harari, A. R. Ben-Yakir, D. & Rosen, D. Mechanisms of aggregation behavior in *Maladera matrida* Argaman (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* **20,** 361–371 (1994).
17. Landolt, P. J., Tumlinson, J. H. & Alborn, D. H. Attraction of Colorado potato beetle (Coleoptera : Chrysomelidae) to damaged and chemically induced potato plants. *Environ. Entomol.* **28,** 973–978 (1999).
18. Landolt, P. J. Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. *Entomol. Exp. Appl.* **67,** 79–85 (1993).
19. Heath, R. R. & Tumlinson, J. H. Prediction of release ratios of multicomponent pheromones from rubber septa. *J. Chem. Ecol.* **12,** 2081–2088 (1986).
20. Heath, R. R., Teal, P. E. A., Tumlinson, J. H. & Mengelkoch, L. J. Correlation of retention times on liquid crystal capillary column with reported vapor pressures and half-lives of compounds used in pheromone formulations. *J. Chem. Ecol.* **12,** 2133–2143 (1986).
21. Thaler, J. S. Jasmonate-inducible plant defenses cause increased parasitism of herbivores. *Nature* **399,** 686–588 (1999).

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22. Agrawal, A. A. Induced responses to herbivory and increased plant performance. *Science* **279**, 1201–1202 (1998).
23. Coley, P. D., Bryant, J. P. & Chapin, F. S. Resource availability and plant antiherbivore defense. *Science* **230**, 895–899 (1985).
24. Heath, R. R. & Manukian, A. An automated-system for use in collecting volatile chemicals released from plants. *J. Chem. Ecol.* **20**, 593–608 (1994).

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Unconscious priming eliminates automatic binding of colour and alphanumeric form in synaesthesia

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Synaesthesia is an unusual perceptual phenomenon in which events in one sensory modality induce vivid sensations in another[1,2]. Individuals may 'taste' shapes[3], 'hear' colours[4], or 'feel' sounds[5]. Synaesthesia was first described over a century ago[6], but little is known about its underlying causes or its effects on cognition. Most reports have been anecdotal or have focused on isolated unusual cases[3,7–9]. Here we report an investigation of 15 individuals with colour-graphemic synaesthesia, each of whom experiences idiosyncratic but highly consistent colours for letters and digits. Using a colour–form interference paradigm, we show that induced synaesthetic experiences cannot be consciously suppressed even when detrimental to task performance. In contrast, if letters and digits are presented briefly and masked, so that they are processed but unavailable for overt report, the synaesthesia is eliminated. These results show that synaesthetic experiences can be prevented despite substantial processing of the sensory stimuli that otherwise trigger them. We conclude that automatic binding of colour and alphanumeric form in synaesthesia arises after initial processes of letter and digit recognition are complete.

We studied 15 individuals with colour-graphemic synaesthesia and 15 non-synaesthetic controls. Each synaesthete reported vivid and immediate sensations of colour for specific letters and digits. All reported having had synaesthesia since childhood, and many had biological relatives with the phenomenon, consistent with previous reports[10]. Our study focused on colour-graphemic synaesthesia because it is the most common form[10], and because it has received considerable attention[11].

A test of consistency verified the presence of synaesthesia in our group[4]. Participants were each given a 150-item list containing letters (A–Z), digits (0–9) and words. They described their synaesthetic colour for each item (or an arbitrary colour in the case of non-synaesthetic controls). Three months later, without warning, the synaesthetes were given the same list and again asked to indicate their synaesthetic colour for each item. For controls, the retest was given just one month later, thus giving them a potential advantage. The synaesthetes were highly consistent in their responses overall, significantly more so than the controls ($F_{1,28} = 162.56$, $P < 0.0001$; Fig. 1). These findings show that the unusual sensations experienced

by synaesthetes, although idiosyncratic, remain highly stable over time[4].

Colour-graphemic synaesthesia may be triggered by automatic co-activation of independent brain areas responsible for processing colour and symbolic form[12,13]. This fits with synaesthetes' subjective accounts of the involuntary nature of their experiences. We therefore manipulated the physical colours of alphanumeric characters so that they differed from the synaesthetic colours induced. Our approach was based on the Stroop effect[14], in which naming the print colour of an incongruent colour word (for example, RED printed in blue) takes significantly longer than naming the print colour of a congruent colour word (for example, RED printed in red), or a colour patch. This slowing reflects interference arising from an involuntary word-reading response[15].

We began by comparing synaesthetes and controls on the standard Stroop task to check for any baseline differences in their susceptibility to interference. Colour words were displayed in either congruent or incongruent colours; solid colour patches were used in a baseline condition (see Methods). Participants named the colour of each stimulus aloud. As expected, both groups were significantly slower to name colours in the incongruent than the congruent condition ($F_{1,28} = 119.72$, $P < 0.001$). Critically, there was no overall difference between the groups ($F_{1,28} = 0.66$, $P > 0.10$) and no interaction ($F_{1,28} = 1.56$, $P > 0.10$; Fig. 2a), indicating equivalent interference for synaesthetes and controls. Moreover, their colour naming times in the baseline condition were the same (535 ms versus 536 ms; $t_{28} = 0.03$, $P > 0.10$).

If synaesthesia is an involuntary phenomenon, then having participants judge the physical colour of an alphanumeric character that elicits an incongruent synaesthetic colour should yield significant interference, and slow response times accordingly. We therefore constructed unique stimulus ensembles for each synaesthete, which
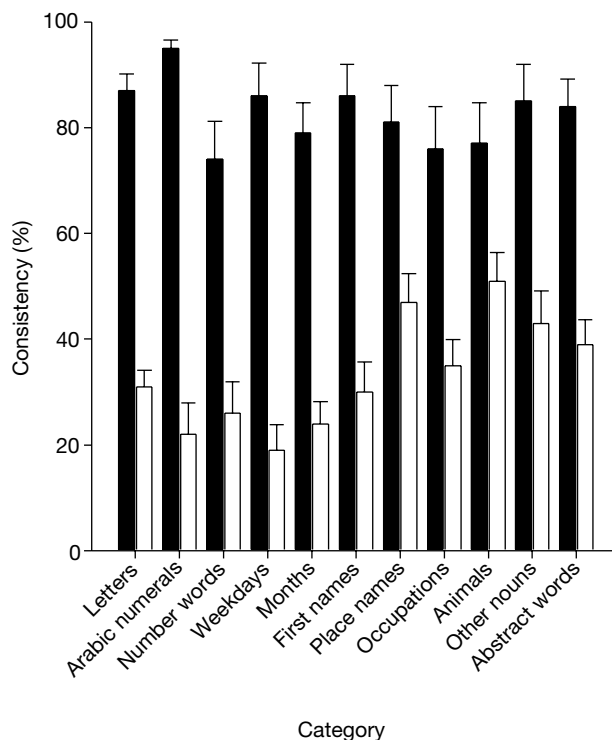

Figure 1 Mean (+1 s.e.) consistency of colour associations for 150 items (letters, arabic numerals and words), plotted separately for each of 11 categories tested. Results for synaesthetes (filled bars) represent performance with a 3-month retest interval; those for non-synaesthetic controls (open bars) represent performance with a 1-month retest interval. For every category tested synaesthetes were more consistent in their colour associations than non-synaesthetic controls.

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