Animals do not always ‘reflexively’ associate a conditioned stimulus (CS) with an unconditioned stimulus (US). In recent years, theoretical accounts of learning have focused on several paradigms that demonstrate that animals can regulate whether a CS enters into an association even though it may be properly paired with reinforcement (Kamin, 1968, 1969; Rescorla and Wagner, 1972; Macintosh, 1974, 1983; Pearce and Hall, 1980; Rescorla and Holland, 1982; Rescorla, 1988; Pearce, 1994). This flexible association system probably reflects the need to extract pertinent sensory stimuli from a confusing, stimulus-rich environment every time that an animal learns an association (Smith, 1996). Since animals do not have unlimited sensory capacities, they have evolved strategies for focusing sensory processing capacities on the most useful stimuli. In most instances, this involves tuning sensory systems to stimuli that are predictive, and several central nervous system (CNS) tactics have evolved to facilitate this tuning.

One such tactic regards a learning phenomenon called blocking, in which a CS that has been previously learned (e.g. odor A) will substantially overshadow a second CS (e.g. odor X) that is added to it. The association of X with reinforcement is largely blocked despite the fact that it is paired with reinforcement in a way that would produce robust associative learning if A were not present. Blocking is a widespread phenomenon and is central to understanding associative learning of complex mixtures of stimuli. It was first described by Kamin (1968, 1969) in rat associative conditioning, but has been found in a variety of other animals, including invertebrates such as honeybees (Smith and Cobey, 1994; Smith, 1996, 1997) and slugs (Sahley et al. 1981), and blocking is even found in spinal reflexes (Illich et al. 1994). It may thus represent a basic and widespread tactic for experience-dependent biasing of sensory processing. Furthermore, blocking is independent of the type of US, occurring in both appetitive (Kamin, 1968, 1969) and aversive (Ross, 1985) conditioning, and, at least in the honeybee, it may be more robustly expressed among conditioned stimuli from the same sensory modality (Bitterman, 1996).

Little is known about the neuroanatomical substrates of blocking. Several studies of vertebrates have found that hippocampal lesions disrupt blocking (Solomon, 1977; Rickett et al. 1978), presumably by affecting memory of pretraining. Holland and Gallagher (1993a,b) have produced specific blocking deficits with neuroanatomical lesions in the rat.
Materials and methods

Honeybee (Apis mellifera L.) workers were obtained either from indoor colonies (during the months of February and March) or from colonies maintained outdoors (April–May). We specifically used foragers for all of our training to minimize any variability due to age or behavioral caste. The indoor colonies were maintained in a flight room on a 16h:8h light:dark cycle and fed unscented sucrose solutions. ‘Foraging’ honeybees from the indoor colonies typically flew towards the overhead lights and briefly alighted on the net. Subjects from outdoor colonies were collected as they returned to their colonies from foraging trips. Only pollen foragers were captured, and their pollen packets were analyzed to ascertain the type of flower that they had visited. Of the outdoor honeybees, only those that had foraged at the same type of flowers, as indicated by similar pollen types, were used and compared in each experiment.

Conditioning protocols

Honeybees were odor-trained using the restrained bee preparation as described elsewhere (Kuwabara, 1957; Bitterman et al., 1983; Menzel, 1990). In brief, individual subjects were mounted in a harness that allowed them to move their head, antennae and mouthparts. Each was trained to associate a brief 4s pulse of odor in a moving airstream with a touch of 2.0 mol l⁻¹ sucrose solution to the antenna. The timing of odor delivery was controlled via computer. The sucrose acts as a US, releasing a motor pattern called the proboscis extension reflex (PER) that bees use to ingest nectar. PER was reinforced with a 0.4μl drop of a 2.0 mol l⁻¹ sucrose solution in each conditioning trial. The timing of US delivery was signaled to begin 3s after odor onset and, given the time it takes to consume the 0.4μl droplet, would extend slightly beyond odor delivery. A subject that has learned the CS–US association will frequently exhibit PER in response to the odor alone, or prior to US onset, after as few as 1–2 conditioning trials (Menzel, 1990).

The conditioning protocol for odor blocking consisted of three phases of training conducted in parallel on two different groups of honeybees using the procedure developed for honeybees by Smith and Cobey (1994; Table 1). In the pretraining phase, subjects were conditioned in a cluster of four trials with a 10 min intertrial interval (ITI) (this interval was constant across all phases). All subjects experienced one of two pure odors as the CS during this phase. Group BLOCK was exposed to the blocking odorant (A), while the control group NOVEL received the other odorant (N). In the mixture phase, each subject in both groups was conditioned with a mixture of two odorants as the new odor CS for a block of four trials. One of the odors was A and the other was a new odor X. This AX compound odor was used to train both the groups NOVEL and BLOCK; however, subjects in group BLOCK were the only ones to have experienced one of the mixture components (i.e. A) during pretraining. A 0.4μl droplet of 2.0 mol l⁻¹ sucrose was again used as reinforcement in both phases.

In the final test phase, each subject was presented with a series of four consecutive unreinforced (extinction) trials consisting of exposure to a 4s pulse of odorant X on its own. If pretraining significantly blocked acquisition of X during the mixture phase, then subjects that had experienced A in the pretraining phase (i.e. group BLOCK; Table 1) would be expected to show significantly fewer PER responses on average to X across the four trials in this phase than subjects that had experienced odor N (i.e. group NOVEL; Table 1). In Table 1. Summary of treatment groups used in blocking experiments

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Pretraining</th>
<th>Mixture training</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOVEL</td>
<td>N → sucrose</td>
<td>AX → sucrose</td>
<td>X1, X2, X3, X4</td>
</tr>
<tr>
<td>BLOCK</td>
<td>A → sucrose</td>
<td>AX → sucrose</td>
<td>X1, X2, X3, X4</td>
</tr>
</tbody>
</table>

A, N and AX refer to odorants or a mixture. X1–X4 refer to four sequential extinction trials in the test phase.
all figures, we use line graphs to depict response probability across acquisition (reinforced) trials, whereas frequency histograms depict the frequency of responses within a treatment population across the four test trials.

The pure odorants geraniol and 1-hexanol were counterbalanced as A and X throughout all experiments, although the results are separated to demonstrate that the identity of A or X did not qualitatively affect the conclusions. 2-Octanone was always used as N. For a generalization test, 1-octanol was used because it elicits stronger generalization responses from subjects conditioned to 1-hexanol (Smith and Menzel, 1989). Geraniol, 1-hexanol and 2-octanone have each been found to stimulate different classes of antennal receptors (Vareschi, 1971). Only geraniol has been identified as a component of honeybee pheromones: it is a part of the Nasonov gland pheromone (Pickett et al. 1980). 1-Hexanol and 1-octanol are found in some flower aromas (Knudsen et al. 1993). To make up odor cartridges, 3μl of pure odorant was placed onto a strip of filter paper, which was then inserted into a 1 ml glass syringe (see Smith, 1997, for details of odor delivery). In the case of mixtures, 1.5μl of each odorant in the mixture was placed onto the filter paper strip.

The training regimen to assess stimulus generalization consisted of a block of five consecutive training trials with a 10 min ITI followed by a wait of 1 h, after which subjects were tested with a pseudorandomized series of three odors. The three test odorants included the conditioned odor (CS), an odor similar to the conditioned odor and a dissimilar odor. In all trials, subjects were conditioned to either 1-hexanol or 1-octanol, then tested with 1-hexanol, 1-octanol and geraniol. Previous experiments have shown that animals display a decreasing gradient of generalization across these odors, responding best to their conditioned odor, less to the similar odor and least to the dissimilar odor (Smith and Menzel, 1989).

Antennal manipulations
To interfere with processing of odors in the antennal system, we disrupted the processing capability of one antenna by either covering it or removing it. Removal consisted of cutting off the antenna at the base and allowing the wound to heal for at least 24 h. To cover an antenna, a small polyethylene tube (inner diameter 0.25 mm, outer diameter 0.76 mm) was cut to a length just longer than an antenna and was then slid over the target antenna. The open end was sealed with Vaseline or warm sealing wax, while the end at the base of the antenna was sealed with Vaseline. This ‘sleeve’ did not physically harm the antenna and could subsequently be removed at different stages of the experiment. Experiments in which the sleeve was switched between antennae demonstrated that the sleeve was a very effective barrier to odors. Sleeves were discarded after a single use.

Statistical analyses
Statistical analyses were performed on the number of responses across the four extinction trials performed during the test phase. Each subject was ranked on the basis of this sum (0–4 depending upon how often they demonstrated PER to the test odorant X). The rankings of subjects in the NOVEL and BLOCK groups in each experiment were compared using single-classification analysis of variance (ANOVA) for two groups (Sokal and Rohlff, 1981). The results from this test are reported as a F-statistics and always have one degree of freedom. A Wilcoxon non-parametric test was also performed in parallel to each ANOVA, which confirmed the significance of the F-statistic in every case. Statistical analyses of generalization results were performed on the response trials to the three different odorants and are compared using χ²-tests.

Results
Using a protocol developed by Smith and Cobey (1994), we were able reliably to reproduce blocking of odor learning in honeybees (Fig. 1). Subjects were trained with the PER procedure to associate the odorant 1-hexanol (group BLOCK) or 2-octanone (group NOVEL) with sucrose reinforcement during pretraining. Both groups received identical conditioning to the AX mixture, which contained geraniol and 1-hexanol. Groups show patterns of acquisition during both phases that are typical of blocking experiments (Smith and Cobey, 1994; Smith, 1996). During the mixture phase, group BLOCK, which received pretraining with odor A (1-hexanol; Table 1), shows strong generalization to the mixture on the first mixture training trial. Group NOVEL generalizes to a lesser degree but reaches approximately the same asymptotic level of responding by trial 3 of mixture training.

All subjects were then identically tested with geraniol, which was odor X in this experiment (Fig. 1B; Table 1). As can be seen in the test responses, subjects that were initially trained to 1-hexanol in group BLOCK showed on average fewer responses to geraniol in the test series than did subjects in group NOVEL (F=6.4, P<0.05, d.f.=1,53). That is, the frequency distribution of the response classes (0–4) is shifted to the left in group BLOCK (open columns) relative to NOVEL (hatched columns). Therefore, pretraining with 1-hexanol blocked, or at least significantly hindered, complete association of reinforcement with geraniol during mixture training. Note that in this experiment the identities of A and X were actually counterbalanced (see Fig. 2A,B below), but are reported here for one pair only in order to demonstrate the blocking effect.

The rest of the Results section is organized into two subsections. In the first, we present evidence that removing input from one antenna prevents or at least attenuates blocking. In the second subsection, we show that bilateral input is crucial at several different stages of the blocking process and that it extends to some other facets of odor learning.

Attenuation of blocking by removal of bilateral input
As expected, when both antennae were left exposed, blocking was evident regardless of whether geraniol (Fig. 2A; F=24.9, P<0.01, d.f.=1,28) or 1-hexanol (Fig. 2B; same data and statistics as in Fig. 1) was used as odor A (Table 1) during
pretraining. In contrast, when one antenna is either removed (Fig. 2C, $F=0.1$, NS, d.f.=1,24; Fig. 2D, $F=0.05$, NS, d.f.=1,31) or completely covered with an odor-impermeable plastic sleeve (Fig. 2E, $F=0.3$, NS, d.f.=1,28; Fig. 2F, $F=0.9$, NS, d.f.=1,38), subjects in groups BLOCK and NOVEL learned to recognize odorant X equally well. In the last four cases, the lack of significance is not due simply to sample size. There were still no significant differences between BLOCK and NOVEL when data from Fig. 2C,D were combined ($F=0.2$, NS, d.f.=1,57) or when data from Fig. 2E,F were combined ($F=1.1$, NS, d.f.=1,68). Since cutting and covering the antenna were indistinguishable in their effects on blocking, antennal covering was used in all subsequent experiments.

It is conceivable that subjects with only a single usable antenna displayed reduced blocking because of an impairment in perception of the pretraining odorant (i.e. A or N). Therefore, in a subsequent experiment, the length of pretraining was doubled to eight trials. However, blocking was still attenuated in this experiment (Fig. 3). For groups NOVEL and BLOCK in which subjects did not have one antenna covered, the normal blocking effect was evident during test trials (Fig. 3A; $F=3.7$, $P=0.05$, d.f.=1,25). In comparison, for groups NOVEL and BLOCK in which subjects had one antenna covered throughout training and testing, the blocking effect was attenuated and not significant (Fig. 3B; $F=0.2$, NS, d.f.=1,30). Therefore, it is unlikely that covering one antenna...
slowed the ability to learn the characteristics of odor A during pretraining, at least in as much as it would have impaired its ability to block X during mixture training.

Furthermore, attenuation of blocking was specific to manipulations that silenced one complete antenna, since blocking occurred as normal when only half of each antenna was covered (Fig. 4). Groups NOVEL and BLOCK showed the blocking effect when both antennae were exposed (Fig. 4A; \( F=10.6, P<0.01, \text{d.f.}=1,44 \)) and when both distal halves were exposed (Fig. 4C; \( F=7.9, P<0.01, \text{d.f.}=1,49 \)), but not when one complete antenna was covered (Fig. 4B; \( F=0.8, \text{NS}, \text{d.f.}=1,56 \)). This manipulation resulted in comparable areas of exposed sensory epithelium in subjects with two half-antennae and those with one whole antenna. The result specifically suggests that it is the loss of bilateral input that is crucial and not just the general decline in sensory input levels.

Finally, attenuation of blocking in subjects with a single antenna could have been due to a reduced discrimination of X presented during the test phase. The reduced surface area of a single antenna could fail to provide enough information about the pretraining odor so that the animal generalized too broadly between the odors A and X. That is, X might be perceived as more similar to A by single-antenna subjects than by subjects able to use both antennae. However, generalization tests do not necessarily support this explanation (Fig. 5). In those tests, generalization to geraniol, which was the dissimilar odor, provided a gauge of generalization between odors used as A and X in blocking experiments (i.e. 1-hexanol and 1-octanol were training odorants). In this case, generalization was not different across the three antennal treatment groups (dissimilar:...


\( \chi^2 = 2.2, \text{NS} \). Response levels to the CS itself did not differ across the three groups (training: \( \chi^2 = 6.6, \text{NS} \)).

In contrast to these two test conditions, the antenna-covered group did tend to generalize more in the similar test condition (i.e. 1-hexanol was tested in groups conditioned to 1-octanol, and vice-versa). The test statistic in this case was barely non-significant (similar: \( \chi^2 = 5.8, 0.1 > P > 0.05 \)). Thus, covering one antenna has a slight affect on the ability to differentiate similar odors, but this is not likely to have had a significant impact upon our blocking experiments, in which we used dissimilar training and test odors.

**Bilateral input is specifically necessary during testing**

It is possible that the attenuation effect caused by covering an antenna might be restricted to covering during one of the three phases of the training procedure. Therefore, several experiments were performed during which antennal covers were moved or removed during one or two of the three training phases (see Table 1).

If the cover is briefly removed and then replaced over the same antenna between the pretraining and mixture training phases, blocking still fails to occur (Fig. 6B; \( F = 0.7, \text{NS, d.f.} = 1.36 \)). Blocking is still attenuated if different antennae are covered during pretraining and mixture training/testing (Fig. 6D; \( F = 0.7, \text{NS, d.f.} = 1.52 \)). Interestingly, under these conditions, subjects in group BLOCK do not show as much generalization from A to AX on the first mixture training trial (Fig. 6C) as do subjects with one antennae covered (Fig. 6A) or with both antennae exposed (Fig. 1A). A comparison of the response probability to AX on trial one of mixture training between group BLOCK in Fig. 6A,C yields a significant difference (\( \chi^2 = 49.6, P < 0.001 \)). Even generalization from the NOVEL odorant to AX is affected by the switch in covering the antennae (\( \chi^2 = 6.9, P < 0.01 \)). These results support earlier reports that information about A is not shared between the two antennal lobes even when one is covered and unstimulated (Masuhr and Menzel, 1972; Mercer and Menzel, 1982). The depression of AX learning during the initial trials of mixture training in animals with the cover switched between antennae further suggests that the US presentation alone could act to build up a resistance to learning 'new' odors using the naive antenna. This pattern defines a classic US–pre-exposure effect, which has been documented in honeybee odor training (Abramson and Bitterman, 1986).

It was thus important to know when both antennae were necessary for blocking. To dissect out this sensitive period, one antenna was covered or left exposed during different phases of training and testing in five groups of subjects (Fig. 7). Blocking was attenuated if the antenna was covered during pretraining (Fig. 7B; \( F = 0.3, \text{NS, d.f.} = 1.34 \)), but was still evident if the antenna was covered only during mixture training (Fig. 7C; \( F = 7.1, P < 0.05, \text{d.f.} = 1.26 \)). Covering an antenna only during the test phase also prevented blocking (Fig. 7D; \( F = 0.1, \text{NS, d.f.} = 1.42 \)). Covering an antenna during pretraining and mixture training with subsequent uncovering during the test phase did not attenuate blocking (Fig. 7E; \( F = 5.8, P < 0.05, \text{d.f.} = 1.43 \)), but covering during the mixture training and test phases did attenuate blocking (Fig. 7F; \( F = 2.0, \text{NS, d.f.} = 1.31 \)). Taken together, these results suggest that bilateral antennal input is essential during the test phase for blocking to occur, but its absence during other phases does not always attenuate blocking. This pattern of results is perplexing and suggests that blocking may involve a complex interaction between brain hemispheres, an idea that we will return to in the Discussion.
Fig. 7. Effects of covering one antenna at different times during the pretraining, mixture training and testing. The three honeybee diagrams in each figure section represent antennal treatment for NOVEL and BLOCK groups during each of the three phases. Antennae were covered as follows: (A) not covered; (B) pretraining only; (C) mixture training only; (D) testing only; (E) pretraining and mixture training; (F) mixture training and testing. Note how the right-hand column of experiments (B,D,F) shows attenuation of blocking, while the left-hand column (A,C,E) does not. Sample sizes for groups NOVEL and BLOCK are, respectively: (A) 14, 9; (B) 16, 20; (C) 12, 16; (D) 15, 29; (E) 16, 29; (F) 15, 18.

Discussion

We have used the proboscis extension reflex in the honeybee (Bitterman et al. 1983; Menzel, 1990) to explore how the honeybee brain might be organized to accomplish blocking (Smith and Cobey, 1994). Perhaps the most important overall finding is that bilateral input is necessary for one odor to block a second. This result suggests that the two hemispheres of the honeybee brain interact in the production of blocking, even though excitatory information about a learned odorant does not transfer between the hemispheres at the level of the antenna or antennal lobe (Masuhr and Menzel, 1972; Fig. 6A,C). Since cooling experiments by Erber et al. (1980) show that this type of information can be shared between mushroom bodies of different hemispheres, it is possible that the structures mediating odor blocking span the hemispheres at this level.

These results must now be placed into the broader context of their meaning for olfactory blocking. One consideration concerns the ecological role of a phenomenon such as blocking. Smith (1996) has argued that blocking acts in a sense like a filter, such that odors that are less reliably present in a floral bouquet might be less well represented in the memory (and thus blocked more readily). Why should this phenomenon of inter-hemispheric inhibition be important? The answer must await further research, but it might play a role in the detection of spatially restricted odor gradients between antennae, as described by Kramer (1976). The mechanism we describe here might function to enhance the signal-to-noise ratio embedded in such small-scale gradients, perhaps by highlighting odors present at different concentrations at each antenna during odor learning. To answer this question, we must consider the mechanism for olfactory blocking, towards which we now orient the Discussion.

Blocking is a phenomenon of the central nervous system

Blocking and related processes in vertebrates are localized to specific regions of the brain; for example, the hippocampus and amygdala (Holland and Gallagher, 1993a,b; Han et al. 1995; Hatfield et al. 1996). Indeed, most higher-order information processing in vertebrates occurs beyond the sensory epithelium. However, recent work on odor and odor mixture processing in such invertebrates as lobsters (Derby et al. 1994), honeybees (Getz and Akers, 1994; Bhagavan and Smith, 1997) and cockroaches (Getz and Akers, 1996) suggests that several non-linear interaction effects in odor coding can occur in the sensory cells of the antenna. Other work on honeybees suggests that neighboring sensory cells may influence one another (Akers and Getz, 1993; Getz and Akers, 1994), and the intercellular messenger nitric oxide may be capable of mediating this interaction (Breer and Shephard, 1993). Therefore, it must be considered possible that blocking in olfactory mixtures could occur at the level of the sensory epithelium in the antennae.

The present results argue against such a peripheral site for blocking and support other lines of evidence that olfactory blocking does not arise in the sensory cells of the antennal system (Smith and Cobey, 1994; Smith, 1996). Since covering one antenna, but not two half-antennae, can attenuate blocking, it seems unlikely that the antennal sensillae contribute substantially to blocking. If the sensillae played a significant role in inducing blocking, then blocking should occur regardless of whether one whole antenna or two half-antennae are covered. Furthermore, Smith and Cobey (1994) have shown that other kinds of presentation of odor A during pretraining do not produce blocking; that is, the blocking effect is limited to forward pairing of odor A with reinforcement during pretraining. This result is consistent with blocking due to associative mechanisms (Pearce and Hall, 1980; Rescorla, 1988) but not to non-associative mechanisms (Smith, 1996). Therefore, the search for the physiological mechanisms
underlying blocking must turn to more central areas of the brain and account for several characteristics revealed in our present analyses.

**Olfactory blocking must involve inter-hemispheric communication**

The need for interhemispheric communication is surprising, particularly since an antenna, its associated antennal lobe and mushroom bodies (collectively the antennal system) appear to identify odors autonomously from the contralateral antennal system (Masuhr and Menzel, 1972; Fig. 6A,C). Several studies have documented how olfactory memories formed in one antennal system are not accessible to the contralateral hemisphere via the other antenna. If subjects are conditioned with one antenna exposed while the other is covered, the odor–sucrose association is restricted unilaterally to the trained antennal pathway. If the cover is switched to the other antenna after training, the animal fails to respond to the conditioned odor and must be retrained in order to obtain a conditioned response (Masuhr and Menzel, 1972; Macmillan and Mercer, 1987). There are no direct decussations between contralateral antennal sensory cells and the antennal lobes, so this phenomenon has traditionally been interpreted as indicating that odor identity in associative learning is specified in the ipsilateral antennal lobe and/or the associated ipsilateral mushroom body.

There might, however, be some inhibitory interaction between the hemispheres that would give rise to the lower generalization to the mixture (AX) and the subsequent delay in acquisition we observed when the cover was switched between antennae. This delay could be explained by the multiple US presentations without associated stimulation of the covered antenna during pretraining (i.e. a US–pre-exposure effect, as shown for honeybees by Abramson and Bitterman, 1986). Any subsequent associational odor learning in that antennal system would first have to overcome this inhibition. Furthermore, any transfer of inhibition between antennae might be related to the mechanism that gives rise to blocking.

This situation is rather different from that observed in rats, where unilateral odor training results in bilateral memory for the CS odor (Teitelbaum, 1971). This difference is somewhat surprising, given that in both honeybees and rats the laterality of the left and right olfactory pathways is largely maintained until higher-order associative centers are reached. Mammals appear to have no direct decussations between the left and right olfactory lobes, while honeybees have only a small commissure (Mobbs, 1985). In rats, a portion of the anterior commissure of the cerebrum must be lesioned in order to prevent memory sharing (Teitelbaum, 1971; Kucharski and Hall, 1988). Thus, mature rats appear to be capable of identifying odors unilaterally, but the information is typically quickly shared with the contralateral hemisphere.

A striking parallel with honeybees exists in the early postnatal development of rats, where prior to day 12 the commissures between the olfactory association areas are not yet organized. Younger animals trained with one naris occluded could only remember the training odor association through the naris that was open at the time of training (Kucharski et al. 1986). This odor memory could be accessed by the contralateral olfactory bulb after the commissure had developed, indicating that both sides of the olfactory bulb were monitored for memories in the mature animal (Kucharski and Hall, 1988). This delayed bilateralization may have some important consequences for neonatal rats, since unilateral odor learning in bees has been shown to be important for navigating along an odor gradient (Martin, 1964; Kramer, 1976). Given that similar odor navigation is found in some reptiles (Schwenk, 1994) and may be important in neonatal marsupials (Gemmell and Rose, 1989), it is conceivable that it could also be used by blind neonatal rats.

The results of this paper strongly suggest that input from both antennae is crucial for initiating blocking in honeybees. While odor identity may be a product of each autonomous hemisphere (Masuhr and Menzel, 1972; Fig. 6), it is apparent from our results that inputs from both antennae are integrated when utilizing odor information in higher-order decisions. Anatomically, the connections between the brain hemispheres that help mediate blocking might pass through the mushroom bodies, since that is one of the points at which olfactory information decussates. Mushroom bodies clearly have a critical role in associative odor learning in Drosophila (de Belle and Heisenberg, 1994). In addition, there are interneurons that span both pairs of mushroom bodies and antennal lobes (Hammer, 1993; Hammer and Menzel, 1995), so it is certainly possible that the interaction we describe may not involve intrinsic mushroom body neurons. Such modulation by central associative areas also has a correlate in rats, where hippocampal lesions can interfere with an odor learning task similar to blocking (Schmajuk et al. 1983) as well as blocking in non-odor learning paradigms (Solomon, 1977; Rickert et al. 1978).

Note that our hypotheses do not eliminate a role for the antennal lobes in odor blocking. The commissure that has been described as passing between the two antennal lobes (Mobbs, 1985) affords one avenue for direct cross-talk and it appears to contain neuromodulatory processes. It is also possible that bilateral inputs are weighed in the mushroom bodies, and that this information is then centrifugally relayed back to the antennal lobes where it aids in setting up blocking conditions. Interneurons that connect the mushroom bodies and each antennal lobe are present in the honeybee (Hammer, 1993). Thus, information could flow between the antennal lobes or from the mushroom body to the antennal lobes via this route. Similar centrifugal projections to the olfactory lobes in rats have important effects upon odor learning and memory in neonates (Wilson and Sullivan, 1994) and adults (Brennan et al. 1990).

Fig. 8 summarizes how disrupting bilateral inputs attenuates blocking and reveals the potential complexity of the underlying mechanism. Disruption throughout all three phases interferes with blocking. This result would be consistent with a model that incorporates transmission of inhibitory information between the
antennal lobes. That is, when activity in one hemisphere is eliminated, then inhibition of the contralateral hemisphere would be diminished and would be incapable of giving rise to blocking. Furthermore, blocking was attenuated by disruption during the initial acquisition phase, by disruption during the testing phase and by disruption during both the mixture and the testing phases. Such attenuation did not occur when bilateral input was disrupted only during mixture training. These results are also consistent with a bilateral inhibition model and would seem to indicate that bilateral inhibitory transmission is most important during the acquisition and testing phases. However, the experiment that disrupted bilateral input during the acquisition and mixture phases (Fig. 7E) revealed that the mechanism must be more complex than the one just implied. That is, blocking remained intact in that experiment (Fig. 7E) even though disruption of bilateral inputs only during acquisition was sufficient to attenuate it (Fig. 7B).

Linster and Smith (1997) have developed a computational model of antennal lobe glomeruli to examine how the antennal lobes could be organized to produce odor identification and odor blocking. The primary features of this model are (1) odor-stimulated sensory axons are active in a discrete subset of antennal lobe glomeruli; (2) that local inhibitory interneurons activated by these glomeruli act to suppress activity in other, less-active glomeruli in the lobe; and (3) that a neuromodulatory neuron active only during US presentation acts to stabilize this pattern of heightened synaptic activity for the learned odors and depressed synaptic activity for all other odors. This mechanism can sharpen the antennal lobe representation of an odor by reducing anomalous background activity (Linster and Masson, 1995). Furthermore, if reinforcement enhances the spread of inhibition, it could also act to temporarily disable the representation of another odor (e.g. X) presented with a learned odor (Linster and Smith, 1997), which produces blocking.

**Bilateral sensory inputs and odor blocking**

This model assumes unilateral odor processing, but several different components of it can be made ‘bilateral’ to test whether the model can accommodate our results: (1) the excitatory input could have bilateral effects; (2) the inhibitory interneurons could have bilateral effects; or (3) the US-correlating interneuron(s) could have bilateral effects. It remains to be determined how or whether these modifications to the model can accommodate our results, particularly those presented in Fig. 7E. The answer to this question remains to be elucidated by future behavioral and physiological analyses.

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