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WITHIN-SUBJECTS EXPERIMENTS ON BLOCKING AND FACILITATION IN HONEYBEES (*Apis mellifera*)

R. E. Blaser, P. A. Couvillon, and M. E. Bitterman

Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii

Abstract

It has long been suspected in the vertebrate literature, but demonstrated only recently in work with honeybees (*Apis mellifera*), that the different treatments of non-target stimuli in conventional between-groups blocking experiments may give the appearance of blocking independently of experience with the target stimulus. The same difficulty does not arise in within-subjects experiments, and in a series of such experiments with odors and colors free-flying honeybees gave no evidence of blocking; separate reinforced presentations of one element of a reinforced compound failed to reduce responding to the second. There was, however, clear evidence of facilitation; separate nonreinforced presentations of one element of a reinforced compound increased responding to the second. The implications of the results for further work on compound conditioning in honeybees and other animals are considered.

Keywords

blocking; compound conditioning; cue validity; facilitation; honeybees

The parsimonious assumption of early learning theorists (Hull, 1929; Spence, 1936) that the components of a compound stimulus gain and lose associative strength independently with reinforcement and nonreinforcement has generally been regarded as untenable in the light of experiments on “blocking” in rats and a variety of other animals, but those experiments have been clouded from the outset by a variety of control problems. In the prototypical case (Kamin, 1969), a blocking group has reinforced trials with stimulus *A* (*A*+ trials) that are followed by reinforced trials with a compound of stimuli *A* and *B* (*AB*+ trials), while a control group has reinforced trials with the compound alone. In a subsequent test, the blocking group responds less to *B* than does the control group, although the contiguity of *B* with reinforcement has been the same for both. In his work on “cue validity,” Wagner (1969) soon found that subsequent responding to *B* is less also when the *A*+ training trials do not precede the *AB*+ trials but are interspersed among them, an effect that has been described as “concurrent” blocking to distinguish it from the prototypical “forward” blocking.

Kamin’s control procedure – the elimination of *A*+ trials – leaves us uncertain as to whether it is the conditioning of *A* that is responsible for the difference, or only the additional experience with reinforcement apart from *A*, or the additional experience with *A* apart from reinforcement.

Correspondence concerning this article should be addressed to R. E. Blaser, now at the Department of Psychology, University of San Diego, 5998 Alcala Park, San Diego, CA, 92110, Telephone: (619) 260-4511, Fax: (619) 260-6867, E-mail: rblaser@sandiego.edu.

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Wagner (1969) substituted unsignalled reinforcements for the $A+$ trials, which equated experience with reinforcement, but not with A . In later experiments, A was backwardly paired or explicitly unpaired with reinforcement, thus equating experience both with A and with reinforcement, or reinforced trials with a different stimulus ($C+$ trials) were substituted for the $A+$ trials; in their work with honeybees, Smith and Cobey (1994) notably used all three of these control procedures. Although none of the control procedures, either alone or in combination, can provide unequivocal evidence of blocking, their various shortcomings have commonly been lost sight of because the same outcome -- less responding by the blocking group than by the control group in the test with B -- has usually (but not always) been reported for all of them.

The traditional explanation of blocking is that the conditioning of B on $AB+$ trials is impaired by the conditioning of A , with which B must compete for associative strength (Rescorla & Wagner, 1972) or for the attention assumed to support the acquisition of associative strength (Sutherland & Mackintosh, 1971). A more recent suggestion (Miller & Matzel, 1988) is that the competition is not for associative strength (the old independence assumption is here resurrected) but for control of performance. The pairing of A and B is held to establish a so-called "comparator" relation between them which determines that responding to each alone will be an increasing function of its own associative strength relative to that of the other.

A limitation of all conventional between-groups designs, as Rescorla (1981) noted to little effect some decades ago, is that the two treatments compared may differentially affect responding to B quite apart from experience with it. As the most obvious example, the enhancement of contextual conditioning by unsignalled reinforcement in the training of a control group like Wagner's might be expected to produce greater responding in the test with B even where the associative strength of B is the same in both groups. Just such an experiment, and several others that had given the appearance of blocking in honeybees (Blaser, Couvillon, & Bitterman, 2004), was recently repeated (Blaser, Couvillon, & Bitterman, 2006) with only one difference in procedure: B was absent in training and encountered for the first time in the test. In every case, even so, there was less responding to B by the group whose treatment was otherwise the same as that of the blocking group than by the group whose treatment was otherwise the same as that of the control group, an effect we have termed "pseudoblocking." These results demonstrate that different treatments of non-target stimuli may in fact influence responding to B in the test quite apart from experience with B in the training. They suggest that a demonstration of blocking in any conventional between-groups experiment can carry little conviction unless accompanied by null results for parallel B -absent groups. Fortunately, this difficulty can be avoided by comparing the treatments within subjects, a much more efficient strategy as well, since a single group takes the place of four.

In a within-subjects experiment with pigeons by Rescorla (1981), differentially reinforced trials with stimuli A and C ($A+$ and $C-$ trials) were followed by consistently reinforced trials with compounds of A and B ($AB+$ trials) and of C and D ($CD+$ trials); in a subsequent test, the animals responded less to B than to D . This design confounds the possibility of a facilitating effect of the $C-$ training on responding to D (examined early by Rescorla, 1971) with the possibility of a blocking effect of the $A+$ training on responding to B , both of which are anticipated by current theories of blocking. Confounded designs have since been used also in conventional between-groups experiments to study the tenability of the independence assumption for honeybees (Couvillon, Arakaki, & Bitterman, 1997; Couvillon, Campos, Bass, & Bitterman, 2001). In within-subjects experiments, if not in between-groups experiments, blocking and facilitation effects can readily be separated, the $C-$ trials eliminated to look for blocking alone and the $A+$ trials eliminated to look for facilitation alone. What may be the first unambiguous evidence of blocking in any animal has, in fact, recently been provided by pure within-subjects blocking experiments ($A+$ trials, followed by $AB+$ and $CD+$ trials, and then by

a test with *B* and *D*) on conditioned fear in rats (McNally & Cole, 2006; Cole & McNally, 2007).

Reported here are some within-subjects experiments with free-flying honeybees that were designed to look for blocking and facilitation unquestionably dependent on experience with the putative blockers and facilitators. For honeybees, it should be noted, even conventional between-groups experiments have as yet produced no indisputable indications of blocking (Gerber & Ulrich, 1999; Guerrieri, Lachnit, Gerber, & Giurfa, 2005). The concurrent procedure, expected by contemporary theories to produce both blocking and facilitation, was used in these initial within-subjects experiments because of its operational convenience. The strategy for each set of stimuli – odors and colors -- was to begin with a confounded design and then, if a confounded effect appeared, to analyze it in pure blocking and facilitation experiments.

Method

The subjects were foraging honeybees (*Apis mellifera*), all experimentally naive, from our own hives situated near the laboratory. They were trained individually, each in a single session lasting several hours. In the pretraining, a forager was selected at random from a group of foragers at a feeding station providing 10-12% sucrose solution, picked up in a match box, carried to a laboratory window, and set down at a 100- μ l drop of 50% sucrose solution on a gray pretraining target centered on the deep sill of the window. The animal was marked with a spot of colored lacquer as it fed to repletion, after which it left for the hive to deposit the sucrose. Typically, the animal would return after a few minutes, continuing to fly back and forth between the hive and the window as long as sucrose was available there. If the marked animal did not return after its first placement, it was taken again from the feeding station (where it usually could be found) to the pretraining target and permitted again to feed to repletion. More than two placements rarely were required. The pretraining ended with the first return of the animal to the window of its own accord, when discriminative training began. The location of the window was such that intruders were uncommon, but any follower that did arrive was caught in a small net and removed.

The targets were plastic petri dishes, 5.5 cm in diameter, with opaque covers varying in color. Drilled in each cover, 6 mm from its outer circumference, was a circle of eight equally-spaced holes, 5 mm in diameter, and the dishes contained cotton batting that could be impregnated as required with distinctive odors. The covers of the dishes were painted grey for the odor experiments, or faced with yellow, orange, green, and blue plastics for the color experiments. A target of a single color was labeled with that color alone, and a compound of two colors was labeled with 180 degrees of each color, the line of their separation on the target placed perpendicular to the outer edge of the window sill. The batting within odor targets was labeled with 0.5 mL diluted peppermint, geraniol, hexanol, or jasmine odorants. The targets used on each trial were drawn at random from sets of identical targets, to which they were returned after washing of their covers at the end of the trial. The purpose of this procedure was to randomize any irrelevant stimuli. All of the colors and odors had previously been found to be readily discriminable in simple choice experiments.

The training trials (one on each visit) were of two kinds, each ending with reinforcement. On trials of one kind, the animal found a single target containing a 100- μ l drop of 50% sucrose solution from which it fed to repletion; on an A+ trial, for example, the target was labeled with stimulus A. On trials of the second kind, two targets, sequentially presented, were differentially reinforced in a procedure that provides for prolonged exposure to the negative stimulus -- a delay-to-choice procedure. A differentially reinforced trial with A+ and C-, for example, began with presentation of the negative target, C, containing a 100- μ l drop of water (unacceptable

to the animal and indistinguishable from the sucrose except by taste). All contacts with the target in a period of one minute were recorded, after which *C* was replaced with a pair of fresh *A* and *C* targets set 10-cm apart and parallel to the edge of the windowsill, their lateral arrangement varying quasi-randomly from one such trial to the next. If the animal went first to *C*, it was free to correct its choice, and the trial ended with feeding to repletion on *A*. The various trials that composed the training portion of each experiment also were scheduled quasi-randomly. On the visit following the last training trial, there was a nonreinforced 10-min preference test with *B* and *D* (both now containing drops of water), during which all contacts with each target were recorded. Finding water on one of the targets, the animal typically would fly up from the target, then return to it or go to the other target, and so forth, the interval between successive responses increasing progressively in the course of the test period.

Experiments with Compounds of Two Odors

Evidence of blocking-facilitation in free-flying honeybees, now suspect because of the lack of *B*-absent treatments, has appeared in confounded between-groups experiments with odor-odor compounds (Couvillon, Arakaki, & Bitterman, 1997). In the present Experiments 1-3, odor-odor compounds were studied in within-subjects designs. The first produced a credible confounded effect, which then was analyzed in pure facilitation and pure blocking designs.

Experiment 1 (Odor-Odor Confounded)

Eight foragers were trained with gray targets labeled with the odors of peppermint, geraniol, hexanol, and jasmine -- the pretraining target with all four. The assignment of the odors to the roles of *A*, *B*, *C*, and *D* was counterbalanced over subjects in latin square fashion (the practice throughout). In quasi-random sequence, there were four trials with *AB*+, four with *CD*+, and eight delay-to-choice trials with *A*+ and *C*-. Then there was a preference test with odors *B* and *D*.

Results—In the left portion of Figure 1, performance in the training with *A*+ and *C*- is plotted in terms of the proportion of animals choosing correctly on each presentation of the pair. It is clear from the plot that the two odors were sharply discriminated, $t(7) = 9.7$, $p < .05$ (the level of confidence employed throughout). In the right portion of Figure 1, performance in the test with odors *B* and *D* is plotted in terms of the mean cumulative number of responses to each of the stimuli in successive 30-s intervals of the 10-min test period. There was less responding to odor *B* than to odor *D*. ANOVA (based as always on the uncumulated data) yields a significant Stimulus effect, $F(1, 7) = 11.3$ ($\eta_p^2 = 0.62$), as well as significant change in responding over 2.5-min Blocks, $F(3, 21) = 7.4$ ($\eta_p^2 = 0.51$), without a significant Stimulus X Block interaction, $F(3, 21) = 1.8$. Here, then, is unambiguous evidence of a blocking-facilitation effect, the basis of which was examined in Experiments 2 and 3.

Experiment 2 (Odor-Odor Facilitation)

In an experiment designed to look for facilitation apart from blocking, 12 foragers had concurrent experience with *C*-, *AB*+, and *CD*+. There were eight delay-to-choice trials, four with *C*- and *AB*+, and four with *C*- and *CD*+, in quasi-random sequence, followed by a terminal preference test with odors *B* and *D*.

Results—In the left portion of Figure 2, performance in the training is plotted in terms of the proportion of animals choosing correctly on each presentation of each pair. It is clear from the plot that odor *C* was readily discriminated from each of the odor-odor compounds, for *AB*+, $t(11) = 7.1$, and for *CD*+, $t(11) = 5.9$. In the test with odors *B* and *D*, as shown in the right portion of Figure 2, the animals responded less to odor *B* than to odor *D*. ANOVA yields a significant Stimulus effect, $F(1, 11) = 6.0$ ($\eta_p^2 = 0.35$), as well as significant change in responding over

2.5-min Blocks, $F(3, 33) = 22.8$ ($\eta_p^2 = 0.68$), without a significant Stimulus X Block interaction, $F(3, 33) = 2.6$. Here, then, is clear evidence of a facilitation effect.

Experiment 3 (Odor-Odor Blocking)

In an experiment designed to look for blocking apart from facilitation, 10 foragers had concurrent experience with $A+$, $AB+$, and $CD+$, as well as unreinforced experience with a fifth odor, E . The added odor was lemon, and the roles of A , B , C , D , and E were balanced over the five odors in latin-square fashion, the pretraining target labeled with all five. Of 16 training trials scheduled in quasi-random order, eight were $A+$ trials and eight were delay-to-choice trials, four with $AB+$ and $E-$, and four with $CD+$ and $E-$. The $E-$ trials were introduced in an effort to ensure attention to the odors and to provide a measure of the effectiveness of the training procedure. On the final visit, there was the standard preference test with odors B and D .

Results—In the left portion of Figure 3, performance in the training is plotted in terms of the proportion of animals choosing correctly on each presentation of each pair. It is clear from the plot that odor E was readily discriminated from each of the odor-odor compounds -- on trials with $AB+$, $t(9) = 11.1$, and on trials with $CD+$, $t(9) = 13.5$. In the test with odors B and D , as shown in the right portion of Figure 3, response to odor B was not less than response to odor D . There was no evidence of blocking, but the reverse if anything -- a statistically unreliable preference for odor B . ANOVA yields a significant change in responding over 2.5-min Blocks, $F(3, 27) = 11.5$ ($\eta_p^2 = 0.56$), but neither a significant Stimulus effect, $F(1, 9) = 3.3$, nor a significant Stimulus X Block interaction, $F(3, 27) = 2.9$. These negative blocking results together with the positive facilitation results of Experiment 2 suggest that the confounded effect with compounds of two odors found in Experiment 1 was due to facilitation alone.

Experiments with Compounds of Two Colors

Evidence of blocking-facilitation in free-flying honeybees has appeared also in a confounded between-groups experiment with compounds of two colors (Couvillon, Arakaki, & Bitterman, 1997). The present within-subjects Experiments 4-6 were like Experiments 1-3, but with color-color rather than odor-odor compounds.

Experiment 4 (Color-Color Confounded)

Twelve foragers were studied in a confounded experiment like Experiment 1, except that the colors of the petri dishes serving as targets were substituted for odors emanating from cotton batting in their interiors. As in the confounded experiment with odors, there were, in quasi-random sequence, four trials with $AB+$, four with $CD+$, and eight delay-to-choice trials with $A+$ and $C-$. Then there was a preference test with colors B and D .

Results—In the left portion of Figure 4, performance in the training with $A+$ and $C-$ is plotted in terms of the proportion of animals choosing correctly on each presentation of the pair. It is clear from the plot that the two colors were discriminated, $t(11) = 4.3$. The right portion of Figure 4 shows less responding to B than to D in the test with the two colors. ANOVA yields a significant Stimulus effect, $F(1, 11) = 5.0$ ($\eta_p^2 = 0.31$), as well as significant change in responding over 2.5-min Blocks, $F(3, 33) = 20.7$ ($\eta_p^2 = 0.65$), without a significant Stimulus X Block interaction, $F(3, 33) = 2.4$. Here again for colors -- as there was for odors in Experiment 1 -- is unambiguous evidence of a blocking-facilitation effect, which was analyzed in Experiments 5 and 6.

Experiment 5 (Color-Color Facilitation)

Twelve foragers were studied in a pure facilitation experiment like Experiment 2, except that the targets were labeled with colors rather than odors. There were eight delay-to-choice trials in quasi-random sequence, four with *C*- and *AB*+, and four with *C*- and *CD*+, in quasi-random sequence, followed by a terminal preference test with colors *B* and *D*.

Results—In the left portion of Figure 5, performance in training is plotted in terms of the proportion of animals choosing correctly on each presentation of each pair. The discrimination was not very good, but each of the color-color compounds was preferred to color *C*; for *AB*+, $t(11) = 2.2$, and for *CD*+, $t(11) = 4.0$. In the test with colors *B* and *D*, as shown in the right portion of Figure 5, the animals responded less to *B* than to *D*. ANOVA yields a significant Stimulus effect, $F(1, 11) = 5.1$ ($\eta_p^2 = 0.32$), as well as significant change in responding over 2.5-min Blocks, $F(3, 33) = 11.0$ ($\eta_p^2 = 0.50$), without a significant Stimulus X Block interaction, $F(3, 33) < 1$. Here again for colors -- as there was for odors in Experiment 2 -- is evidence of a pure facilitation effect.

Experiment 6 (Color-Color Blocking)

Twelve foragers were studied in a pure blocking experiment like Experiment 3, except that the targets were labeled with colors rather than odors. The fifth target, *E*, was gray. For this experiment, all training trials were delay-to-choice, and of the 16 trials, scheduled in quasi-random order, eight were with *E*- and *A*+, four with *E*- and *AB*+, and four with *E*- and *CD*+. On the final visit, there was the standard preference test with *B* and *D*.

Results—In the left portion of Figure 6, performance in the training is plotted in terms of the proportion of animals choosing correctly on each presentation of each pair. It is clear from the results that the colors were discriminated. On trials with *A*+, $t(11) = 4.5$, on trials with *CD*+, $t(11) = 4.5$, and on trials with *AB*+, $t(11) = 1.5$, the latter not significant although the terminal performance was much the same as for the other pairs. In the test with colors *B* and *D*, as shown in the right portion of Figure 6, there was again little sign of a pure blocking effect. ANOVA yields a significant change in responding over 2.5-min Blocks, $F(3, 33) = 18.6$ ($\eta_p^2 = 0.16$), but neither a significant Stimulus effect, $F(1, 11) = 2.0$, nor a significant Stimulus X Block interaction, $F(3, 33) = 1.4$. The failure to find blocking, together with the positive facilitation results of Experiment 5, suggests that the confounded effect with compounds of two colors found in Experiment 4 was due to facilitation alone. The pattern of results for compounds of two colors is the same as the pattern of results for compounds of two odors (Experiments 1-3).

Discussion

The essential features of the 6 experiments are summarized in Table 1, which shows the design of each experiment (confounded, facilitation, or blocking), the stimuli employed as *A*, *B*, *C* and *D*, and the outcome (response to target stimulus *B* relative to *D*).

A striking feature of the results is that they provide evidence of facilitation but none whatever of blocking, although popular explanations of blocking lead to the expectation of both effects. After confounded training (*A*+/*C*-/*AB*+/*CD*+) with compounds of two colors or of two odors, there was less responding to *B* than to *D*, which may be understood as a facilitation effect, since responding to *B* was less than to *D* after training designed to look for facilitation alone (*C*-/*AB*+/*CD*+) but not after training designed to look for blocking alone (*A*+/*AB*+/*CD*+/E-). It is possible, of course, that the pure blocking design was not sensitive enough -- that the *E*- trials incorporated in the training were not sufficient (as they were intended to be) to promote the scrutiny of the compounds that presumably would be necessary for differential conditioning of their components. The *C*-/*CD* training might, of course, have done just that. Nevertheless,

given the shortcomings of between-groups experiments, which even so have produced no clear results, we are still without a convincing demonstration of blocking in honeybees and therefore without empirical support for any theory of facilitation that contemplates both.

The implications of this work with honeybees, which are only distantly related to the more familiar vertebrate subjects of conditioning experiments but whose performance in a wide range of such experiments has shown many detailed similarities to the performance of vertebrates (Bitterman, 1988, 1996), seem perfectly general. On the intuitively reasonable assumption reflected in the traditional theories that much the same mechanism underlies both blocking and facilitation, the indications of facilitation in the present work with honeybees should intensify the search for clean evidence of blocking – either in within-subjects experiments or in between-subjects experiments with B-absent controls. By the same token, the indications of blocking in recent within-subjects experiments with rats (Cole & McNally, 2007; McNally & Cole, 2006) should prompt further work on facilitation. Both phenomena can be expected to be parametrically sensitive, and the search for them would involve systematic variation in the stimuli employed, in the number and order of training trials, in training techniques, and in measures of performance. Conventional between-groups experiments with honeybees already have shown wide variation in outcome with the stimuli employed (Couvillon et al., 1997, 2001; Guerrieri et al., 2005; Hosler & Smith, 2000). Study of the conditions under which each of the phenomena occurs or fails to occur certainly can be expected to provide valuable clues to its nature. What must be settled on first of all, however, is a meaningful and efficient methodology, which the within-subjects logic seems to provide.

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Experiment 1 (Odor-Odor Confounded)

Figure 1. Results of Experiment 1

Left panel: Performance in the A+/C- discrimination is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the odors, B and D, is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Experiment 2 (Odor-Odor Facilitation)

Figure 2. Results of Experiment 2

Left and center panels: Performance in the *AB+/C-* and *CD+/C-* discriminations is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the odors, *B* and *D*, is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Experiment 3 (Odor-Odor Blocking)

Figure 3. Results of Experiment 3

Left and center panels: Performance in the *AB+/E-* and *CD+/E-* odor discriminations is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the odors, *B* and *D*, is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Experiment 4 (Color-Color Confounded)

Figure 4. Results of Experiment 4

Left panel: Performance in the A+/C- discrimination is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the colors, B and D, is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Experiment 5 (Color-Color Facilitation)

Figure 5. Results of Experiment 5

Left and center panels: Performance in the $AB+/C-$ and $CD+/C-$ discriminations is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the colors, B and D , is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Experiment 6 (Color-Color Blocking)

Figure 6. Results of Experiment 6

Left and center panels: Performance in the $A+/E-$, $AB+/E-$, and $CD+/E-$ discriminations is plotted in terms of the proportion of animals choosing correctly on each trial. Right panel: Performance in the nonreinforced preference test with the colors, B and D , is plotted in terms of the mean cumulative number of responses ($\pm$ CI) in successive 30-s intervals.

Table 1

Summary of Experiments

Experiment	Design	Stimuli	Preference test
1	Confounded	Odors	$B < D$
2	Facilitation	Odors	$B < D$
3	Blocking	Odors	$B = D$
4	Confounded	Colors	$B < D$
5	Facilitation	Colors	$B < D$
6	Blocking	Colors	$B = D$

Note. Confounded, $A+/C-/AB+/CD+$; facilitation, $C-/AB+/CD+$; blocking, $A+/AB+/CD+$; B and D , target stimuli.