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Olfactory cues and *Vespula* wasp recognition by honey bee guards

Matthew J. Wood* and **Francis L. W. Ratnieks**

Laboratory of Apiculture and Social Insects,
Evolution and Behaviour Group,
Department of Animal and Plant Sciences,
The University of Sheffield,
Sheffield S10 2TN,
England.

* Corresponding author:

Email: m.j.wood@sheffield.ac.uk

Telephone: +44 114 2220064

Fax: +44 114 2220002

Keywords: honey bee *Apis mellifera*, guard behaviour, olfactory cue,
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Running head: Olfactory cues and honey bee predator recognition

1 **General Summary**

2

3 As honey bees live in large colonies with central food reserves and developing brood,
4 these crucial resources are protected by guards at the colony entrance. Guards can
5 discriminate between nestmate and non-nestmate honey bees, but little is known about
6 the mechanisms underlying defence against predators. In this study, we examined the
7 role of guards in defending the colony against a commonly observed predator of honey
8 and brood: the common wasp *Vespula vulgaris*. In late summer, we transferred colony-
9 specific olfactory cues between nestmate honey bees and wasps, and vice versa (Bee
10 carrying bee odour, bee carrying wasp odour, wasp carrying bee odour and wasp
11 carrying wasp odour). By observing the response of guard honey bees when these
12 insects were introduced to the colony entrance, we aimed to determine whether predator
13 olfactory cues influence honey bee guarding behaviour. Odour cues were transferred by
14 taking chilled, inactive insects and shaking them together gently in a glass tube. After
15 separating the insects, they were kept chilled before being revived and introduced to the
16 colony entrance. Guarding behaviour was recorded on video and observed for
17 aggressive attacking behaviour, eviction from the hive and antennation. The insect
18 carrying the odour was important in predicting guarding behaviour: nestmate honey
19 bees were evicted less than wasps, attacked less and antennated more frequently.
20 Honey bees or wasps carrying wasp odour were also evicted more frequently and
21 treated more aggressively than those receiving bee odour. Furthermore, introduced
22 insects carrying an incongruous, allospecific odour (a conflicting cue) were antennated
23 more than those carrying a conspecific odour. The magnitude of some of these
24 behavioural responses varied between colonies and trial days. We clearly show that
25 olfactory cues were transferred between honey bees and *Vespula* with marked

- 1 consequence for guarding behaviour and discuss the potential importance of olfactory
- 2 cues in predator recognition.

1 **Abstract**

2

3 Guard honey bees patrol the entrance to the nest and are thought to recognise nestmates

4 by cuticular hydrocarbons. We aimed to determine whether honey bee guards can

5 recognise predatory common wasps *Vespula vulgaris* and nestmates by olfactory cues.

6 Odours were transferred between both honey bees and wasps and the responses of

7 guards to controlled introductions monitored. When controlling for the species of

8 introduced insect, the transferred odour was a predictor of aggressive attacks on both

9 bees and wasps. Carriers of incongruous, allospecific odours were antennated by more

10 guards than conspecific odours. Olfactory cues were, therefore, transferred and guards

11 responded not only to odour *per se* but also odour incongruity. Olfactory cues may

12 therefore be important in predator recognition by honey bee guards.

13

1 **Introduction**

2

3 Guard honey bees *Apis mellifera* patrol the nest entrance and prevent entry by intruders
4 (Butler and Free, 1952; Free, 1954). Conspecific intruders - bees from other colonies -
5 steal honey and this robbing can result in the total loss of honey stores and the death of
6 the plundered colony (Winston, 1987). Many other species also attack honey bee
7 colonies. Hornets *Vespa* spp. are large enough to kill adult honeybees and may enter
8 the colony to carry off developing larvae and pupae (Futuyama, 1986; de Jong, 1990).
9 Similarly, yellow-jacket wasps *Vespula* spp. frequently kill adult honey bees at the
10 entrance and also steal honey from the colony, a particular problem in late summer and
11 early autumn when the annual wasp colony reaches its peak population (Spradbery,
12 1973; de Jong, 1990). In Sheffield, common wasps *Vespula vulgaris* can become
13 abundant and have been seen to enter hives to steal both brood and honey (N.S.
14 Badcock, pers. comm.).

15 Allospecific intruders could be recognised by a variety of potential cues. Honey
16 bees have well developed vision (Giurfa *et al.*, 1995; Lunau and Maier, 1995) and can
17 discriminate between complex textures and patterns (Maddess *et al.*, 1999). Honey bees
18 also have excellent olfaction (von Frisch 1967), which is important in nestmate
19 discrimination based on genotype-specific cues (Getz and Smith, 1983; but see Downs
20 and Ratnieks, 1999), comb wax hydrocarbons (Breed *et al.*, 1988a; Breed *et al.*, 1995)
21 and floral oil odours (Bowden *et al.*, 1998; but see Downs *et al.*, 2000; Downs *et al.*,
22 2001).

23 Common wasps and honey bees are sympatric in Europe (Spradbery, 1973;
24 Winston, 1987). There exists, therefore, the potential for coevolution between predator
25 and prey (Futuyama, 1986). In Japan, for example, the Asian honey bee *Apis cerana*

1 *japonica* recognises the aggregation pheremone of a sympatric predator, the giant hornet
2 *Vespa mandarinia*, forming a ball around the intruder until the temperature becomes
3 high enough to kill the hornet, but not the bees. By contrast, European honeybees *A.*
4 *mellifera* introduced to Japan do not respond (Matsuura and Sakagami, 1973; Ono *et*
5 *al.*, 1995).

6 In view of the sympatry of honey bees and common wasps and the potential
7 ubiquity of cuticular hydrocarbons in insect recognition, the aim of this study was to
8 examine the importance of olfactory cues in the recognition of *Vespula vulgaris* by
9 honey bee guards, a sympatric wasp predator. We transferred odours between honey
10 bees and wasps and observed their treatment by honey bee guards at the colony
11 entrance. While guards always behaved more aggressively to wasps than bees, they
12 were more aggressive to bees with wasp versus nestmate bee odour, and less aggressive
13 to wasps with nestmate bee versus wasp odour. Incongruous, allospecific odours (i.e.
14 bee with wasp odour or wasp with bee odour) resulted in increased guard antennation.

15

16

17 **Methods**

18

19 *Study species*

20 Three discriminator honey bee *Apis. m. mellifera* colonies were studied at the apiary of
21 the Laboratory of Apiculture and Social Insects. Colonies were situated 2m apart to
22 minimise drifting between colonies, were queenright with approximately 20,000
23 workers and brood and were housed in standard two-deep Langstroth hive bodies. Each
24 colony had a 3.5cm diameter entrance hole in the lower box immediately above a 15cm
25 by 20cm wooden platform to facilitate both introductions and observations. Introduced

1 bees or wasps were placed on the centre of this platform to be contacted by guards
2 patrolling the platform and entrance.

3 Nests of the common wasp *Vespula vulgaris* were collected during pest control
4 visits to houses in Sheffield, and relocated to the laboratory where they were housed in
5 30×30×30cm polystyrene boxes with one 5cm diameter entrance hole.

6

7 *Odour transfer*

8 The following procedure was used to transfer odour within and among groups of honey
9 bees and wasps. Returning honey bee foragers from each of the three discriminator
10 colonies, and foraging wasps returning to laboratory study nests were collected.

11 Captured insects were separated into odour recipients and odour donors. Recipients
12 were chilled in a refrigerator at 5°C for 20 minutes to anaesthetise them and prevent
13 fighting. Donors were killed by placing them in a freezer at -19°C for 20 minutes, and
14 then kept at 5°C until used for odour transfers within one hour. For each odour
15 treatment group, three recipients were placed in a sealed, sterile 20ml plastic Universal
16 tube containing four donors and lightly shaken for five minutes. The recipients were
17 then separated, placed in individual 2ml Eppendorf tubes, and labelled such that
18 introductions were blind with respect to odour treatment. Recipients were kept in an
19 ice-box to await introduction to a discriminator colony within 30 minutes.

20 All honey bees were introduced to their own colonies and, where appropriate,
21 received nestmate honey bee odour. This avoided the complicating factor of non-
22 nestmate honey bee olfactory cues. Similarly, introductions to a discriminator colony
23 involving wasps or wasp odour transfer were conducted using wasps from the same
24 nest. The four treatment groups were arranged thus, shown as 'recipient insect (odour
25 transferred)': bee (bee), bee (wasp), wasp (bee), wasp (wasp).

1

2 *Guarding assay*

3 The behaviour of guard honey bees was observed using a standard bioassay adapted
4 from Breed (1983) (see Downs and Ratnieks, 1999; Downs and Ratnieks, 2000; Downs
5 *et al.*, 2000; Downs *et al.*, 2001) in which the cooled introduced bees and wasps were
6 allowed to warm up enough to walk but not to fly away. Using forceps, the introduced
7 insect was placed on the centre of the entrance platform, taking care not to disturb the
8 guard bees. To minimise the observer's disturbance of the colony, each introduction
9 was filmed (Sony Hi8 digital camcorder).

10 From video footage, each introduced insect was classified as rejected (when it
11 was either bitten, held, carried away or stung by guards) or accepted (when no such
12 aggressive approach was made for two minutes following introduction). In addition, we
13 also noted evictions – when the introduced insect was physically removed from the
14 colony entrance by guards and thrown from the platform. Eviction, therefore, is a more
15 violent and aggressive sub-category of rejection. At least five minutes was allowed
16 between introductions, for the number of guards to return to normal. We also
17 determined from the video the number of approaches by guards to each introduced
18 insect. These were classified as either aggressive or non-aggressive (guard merely
19 antennated the introduced insect before moving away). Guard approach behaviour was
20 not considered if the insect was evicted or entered the colony in less than 10 seconds,
21 due to the difficulties in observing guard behaviour in such a short time. Thereafter, the
22 behavioural observations over a maximum of two minutes were corrected to the mean
23 number of approaches made per 10 seconds.

24 Twelve insects were introduced into each discriminator colony on each trial day
25 of the experiment – three insects from each of the four treatment groups: bee (bee), bee

1 (wasp), wasp (bee), wasp (wasp). These twelve insects were introduced in a random
2 order to each discriminator colony. Introductions took place on three separate days in
3 September 2001, giving a total of 108 introductions.

4

5 *Statistical Analysis*

6 The response variables considered were the rejection/acceptance and
7 eviction/non-eviction of the introduced insect, and the numbers of aggressive and non-
8 aggressive guard approaches. Guard approach data were normally distributed
9 following $\sqrt{(x+1/2)}$ transformation prior to analysis (Finney, 1973) (Kolmogorov-
10 Smirnov test, $P>0.05$).

11 Two predictor factors described the transferred odour: origin of the odour (bee
12 or wasp) and its comparison with the recipient (conspecific or allospecific). This allows
13 a distinction to be made between the odour *per se*, and an introduced insect with an
14 incongruous odour (i.e. bee with wasp odour and vice versa). The sequence of
15 introduction was included as a covariate (0-12) to control for the potential effect of an
16 increased guard response as the introductions continue, for example in response to
17 alarm pheromone (Morse, 1966; Morse *et al.*, 1967). The potential random effects of
18 discriminator colony (1-3) and the trial day (1-3) were included as categorical variables.

19 The response variables, rejection (0/1) and eviction (0/1) were analysed using a
20 binomial logistic regression, with a logit link and the backward stepwise elimination of
21 non-significant variables. Guard approach behaviour was analysed using a backward
22 stepwise general linear model. In the starting model, all predictor variables and their
23 two-way interactions were entered. At each step in model optimisation one variable
24 was eliminated, being the variable making the least significant change in the variance
25 explained by the model (if $P>0.05$), until arriving at the final model (all predictors

1 $P < 0.05$) (Crawley, 1993). All statistical tests were two-tailed and means are displayed .
2 ± 1 s.e. Analyses were conducted using S.P.S.S. version 11.0.1.

3

4 **Results**

5

6 *Guard rejection and eviction*

7 All wasps were rejected by guards (Figure 1) as opposed to 35.2% of bees, so the
8 statistical analysis of guard rejection was restricted to bees. The transfer of wasp odour
9 was a highly significant predictor of rejection (Table 1a); 59.3% of bees with wasp
10 odour were rejected by guards compared to just 11.1% of bees with bee odour. In this
11 analysis, which is restricted to bee rejection, the origin of the odour (bee or wasp) and
12 the incongruity of the odour (conspecific or allospecific) are equivalent. No other
13 predictors or interactions were retained in the final model (Table 1a).

14 In the case of the eviction of introduced insects, involving sustained aggressive
15 attacks by guards, the species of the introduced insect could be entered as a predictor,
16 and was retained in the model (Table 1b). Furthermore, the species of the transferred
17 odour (bee/wasp) was retained , as eviction rate was greater in bee (wasp) relative to bee
18 (bee) and smaller in wasp (bee) relative to wasp (wasp), (Figure 1). Eviction rate also
19 varied between trial days and no further predictors or interactions were retained in the
20 final model (Table 1b).

21

22 *Guard approaches: aggression and antennation*

23 The number of aggressive approaches by guards to introduced insects was significantly
24 affected by the species of introduced insect and trial day (Table 2a). In addition, the
25 species of transferred odour was also retained in the final model. As in the case of

1 eviction, aggression towards introductions was lower with transferred bee odour rather
2 than wasp odour, whether the introduced insect was a bee or a wasp (Figure 2).

3 By contrast, the odour classification ‘conspecific/allospecific’ was retained as a
4 significant predictor of non-aggressive guard approaches to introductions - those that
5 involved antennation (Table 2b). As Figure 2 shows, bees or wasps receiving an
6 allospecific odour treatment were antennated more by guards than bees or wasps
7 receiving a conspecific odour. The recipient insect was a significant predictor of guard
8 antennation, and guard antennation also varied between trial days and discriminator
9 colony (Table 2b).

12 **Discussion**

13
14 Our guarding bioassay clearly showed that odours were transferred between bees and
15 wasps, as demonstrated by their marked effects on guard behaviour. The species of the
16 transferred odour (i.e. bee or wasp) had a significant effect on eviction and aggression
17 by guards. Transferred wasp odour increased eviction rate and aggressive approaches,
18 relative to the transfer of bee odour, whether or not the introduced insect was a bee or a
19 wasp. The species of introduced insect, bee or wasp, was always a highly significant
20 predictor of eviction by guards, and both aggressive and non-aggressive approaches by
21 guards. This experiment clearly shows that honey bee guards can recognise an
22 introduced insect using transferred olfactory cues, independent of the insect carrying the
23 odour, and modify their behaviour accordingly. Olfactory cues may therefore be of
24 considerable importance in predator recognition by honey bee guards.

25 The incongruity of the transferred odour (i.e. conspecific or allospecific)

1 remained as a significant predictor of non-aggressive approaches by guards: the
2 presence of an incongruous odour on the recipient insect increased guard antennation.
3 Downs *et al.* (2000) found that nestmate bees introduced to their natal colony carrying
4 incongruous floral oil odours were examined for longer by guards than honey bees
5 carrying only the colony floral odour; an increased guard decision effort with no effect
6 on the eventual decision.

7 The olfactory cues involved in honey bee nestmate recognition by guards may
8 be derived from several sources. No evidence for the use of floral oils has been found
9 in field conditions (Bowden *et al.*, 1998; Downs *et al.*, 2000; Downs *et al.*, 2001) and
10 queen-derived chemicals (Moritz and Crewe, 1988; Breed and Stiller, 1992) are not
11 present in detectable amounts on workers or the honeycomb (Breed *et al.*, 1998).
12 Endogenous heritable cues have been shown to be important in within-colony
13 recognition, such as the aggression between sisters and half-sisters (Getz and Smith,
14 1983). Comb-derived hydrocarbons are rapidly acquired by workers (Breed *et al.*,
15 1988a) and appear to override endogenous heritable cues in nestmate recognition (Breed
16 *et al.*, 1988b). Honey bee cuticles are also rich in hydrocarbons (Francis *et al.*, 1989),
17 which accounts for the rapid cue transfer from comb to honey bee, so cuticular
18 hydrocarbons are a likely candidate for the olfactory cues transferred from honey bee to
19 wasp in this experiment. The paper nests of some social wasps have been found to
20 contain hydrocarbons, which are crucial for nestmate recognition (Singer and Espelie,
21 1992; Butts and Espelie, 1995; Singer and Espelie, 1996). Remarkably, *Polistes* social
22 parasites avoid detection by host workers by mimicking the host's colony-specific
23 cuticular hydrocarbon profile (Bagnères *et al.*, 1996; Sledge *et al.*, 2001). With the
24 widespread occurrence of cuticular hydrocarbons in insects and their involvement in
25 nestmate and kin recognition by Hymenoptera (Singer, 1998), common wasp olfactory

1 cues are surely available for recognition by honey bee guards, and may have been
2 transferred from wasp to honey bee in our experiment.

3 Should olfaction be revealed to be an important factor in predator recognition by
4 guards, a number of questions arise. Has coevolution between predator and prey
5 resulted in European honey bees being particularly sensitive to the odours of common
6 wasps, as Japanese honey bees are to giant hornets (Ono *et al.*, 1995), or is the degree of
7 unfamiliarity of the olfactory cue the crucial factor? The introduction of an unfamiliar
8 wasp to guards, such as sand wasps *Bembix* spp., may be informative in the latter
9 context. The reaction of guards to such a novel cue may reveal whether responses to
10 olfactory cues are learned or innate. As honey bees can rapidly learn rewarding flower
11 colorations and odours (Menzel *et al.*, 1973) and also have innate flower preferences
12 (Giurfa *et al.*, 1995; Lunau and Maier, 1995), both mechanisms are plausible.

13

14 **Acknowledgements**

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16 Apis Biodiversity in Europe". David Hewett assisted with data collection. We thank
17 Adam Hart and Stephen Martin for their valuable comments on an earlier draft of the
18 manuscript.

Figure 1. Rejection and eviction by honey bee guards of nestmate bees and common wasps introduced to colony entrances

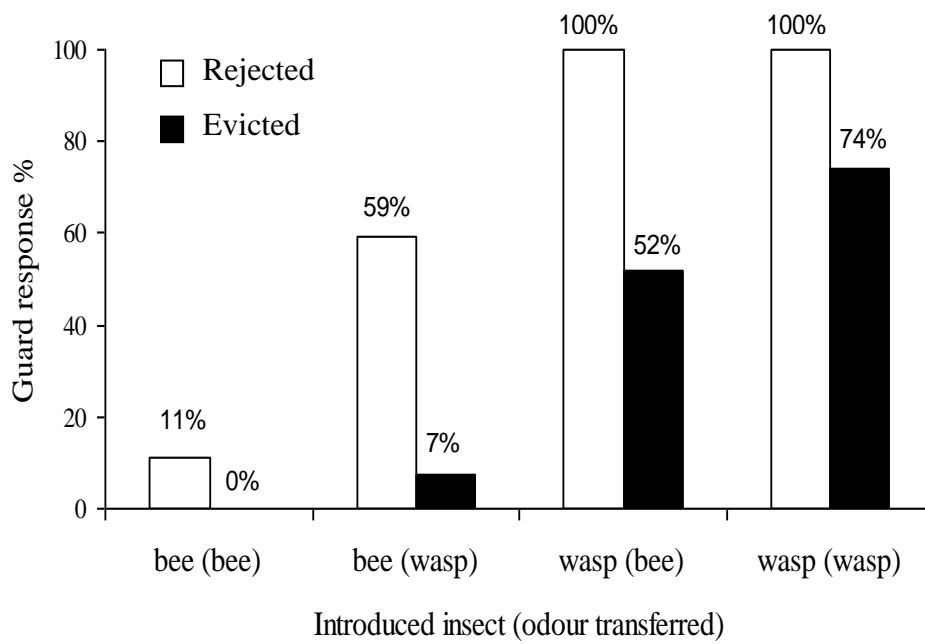


Figure 2. Approaches by honey bee guards to nestmate bees and common wasps introduced to colony entrances

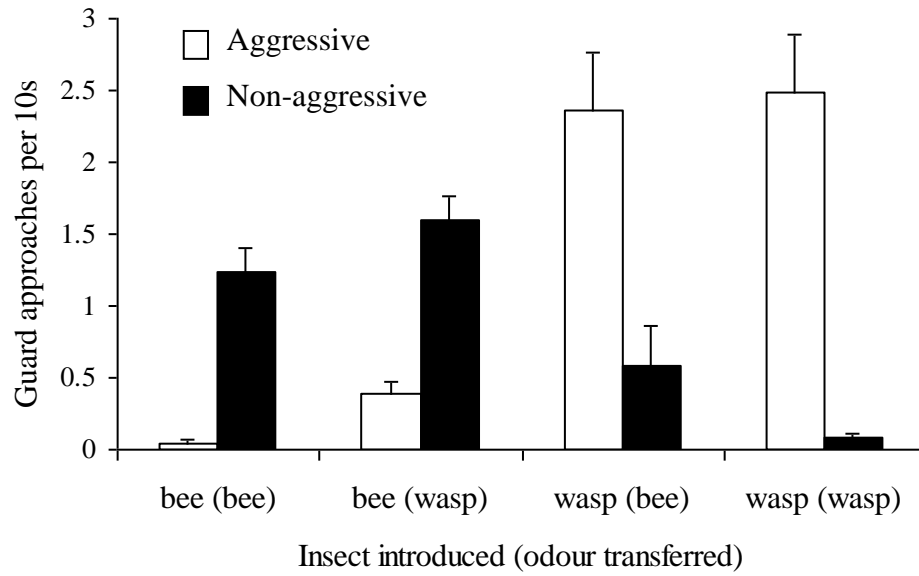


Table 1

Results of a binary logistic regression, showing variables retained in the final model as significant predictors of rejection or eviction, following the stepwise backward elimination of non-significant variables. The change in model deviance (G) and associated P -value caused by the removal of each variable retained in the final model are shown; G and P -values are also shown for the step prior to the exclusion of variables not included in the final model. The analysis of rejection rate (Table 1a) is restricted to introduced nestmate bees, as 100% of wasps were rejected. In this case, transferred odour classifications ‘species’ and ‘conspecific/allospecific’ are equivalent.

a. Rejection of introduced nestmate honey bees by guards

Variable	G	df	P
RETAINED:			
Transferred odour: bee/wasp (conspecific/allospecific)	15.7	1	<0.001
EXCLUDED:			
Introduction sequence	1.50	1	0.22
Trial day	1.61	2	0.45
Discriminator colony	1.67	2	0.44
FINAL MODEL: $G_1=55.3, P<0.001$			

**b. Eviction of introduced nestmate bees and common wasps
by honey bee guards**

Variable	<i>G</i>	df	<i>P</i>
RETAINED:			
Introduced insect: species	65.3	1	<0.001
Transferred odour: species	4.97	1	0.026
Trial day	5.70	2	0.017
EXCLUDED:			
Introduction sequence	3.25	1	0.072
Transferred odour: conspecific/allospecific	1.40	1	0.24
Discriminator colony	2.05	2	0.36
FINAL MODEL: $G_4=78.2$, $P<0.001$			

Table 2. Results of a general linear model, showing the variables retained in the final model as significant predictors of the number of approaches by guards that were aggressive and non-aggressive (antennation), following the stepwise backward elimination of non-significant variables. *F*-ratios and associated *P*-values are shown for the variables retained in the final model, and also for the contribution of each excluded non-significant variable in the step prior to its elimination. Count data were $\sqrt{(x+1/2)}$ transformed prior to analysis.

a. Aggression by honey bee guards toward introduced nestmate bees and common wasps

Variable	<i>F</i>	df	<i>P</i>
RETAINED:			
Introduced insect: species	132	1	<0.001
Trial day	6.02	2	0.0040
Transferred odour: species	4.67	1	0.033
EXCLUDED:			
Discriminator colony	1.85	2	0.16
Introduction order	0.58	1	0.45
Transferred odour: conspecific/allospecific	0.29	1	0.59
FINAL MODEL: $F_{4,89}=201.3, P=0.001$			

b. Antennation of introduced nestmate bees and common wasps by honey bee guards.

Variable	<i>F</i>	df	<i>P</i>
RETAINED:			
Introduced insect: species	82.4	1	<0.001
Transferred odour: conspecific/allospecific	6.70	1	0.011
Trial day	4.31	2	0.016
Discriminator colony	3.25	2	0.043
EXCLUDED:			
Introduction order	0.91	1	0.35
Transferred odour: species	0.083	1	0.78
FINAL MODEL: $F_{6,91}=224.4, P=0.001$			

1 **Legends:**

2

3 *Figure 1:*

4 The percentage of introduced insects in each category rejected or evicted by honey bee
5 guards is presented. Each category, n=27.

6

7 *Figure 2:*

8 Approaches over the observation period were classified as either aggressive or non-
9 aggressive and standardized as the number of approaches per 10 seconds. Each
10 category, n=27. Means displayed \pm 1 s.e.

11

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