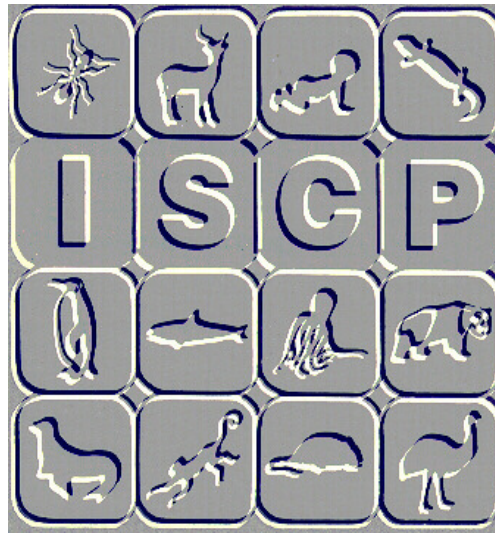


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2008, Volume 21, Number 2

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- 59 Relative Pattern Preferences by Bumblebees
Catherine M. S. Plowright, Mathieu Lebeau, and Martine J. Perreault
- 70 Differing Pattern of the Development of Mother–Infant Interactions in
Cynomolgus Monkeys Due to Exposure of an Environmental Chemical,
Bisphenol A
*Akiko Nakagami, Takamasa Koyama, Katsuyoshi Kawasaki, Takayuki
Negishi, Toshio Ihara, Yoichiro Kuroda, Yasuhiro Yoshikawa*
- 84 Recognition and Discrimination of Human Actions Across the Senses of
Echolocation and Vision in the Bottlenose Dolphin: Evidence for Dolphin
Cross-modal Integration of Dynamic Information
Stan Kuczaj, Moby Solangi, Tim Hoffland, and Marci Romagnoli
-

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Relative Pattern Preferences by Bumblebees

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University of Ottawa, Canada

Bumblebees were trained to discriminate between two patterns, one rewarding (S+) and another unrewarding (S-) consisting of four orthogonal bars. Training and testing conditions were manipulated in a 2 X 2 between groups design. The training patterns differed only in the positioning of the bars in the inferior or the superior portion. The same was true of the testing patterns, both of which were unrewarding. A significant interaction between training and testing conditions was obtained on preference for one pattern, the diamond, which was present at testing for all four conditions. For the groups that were trained with the patterns that differed only in the inferior portion, when tested with patterns that differed in (1) the inferior portion: the diamond, for which the inferior portion matched that of the S+, was chosen at a level significantly above chance (2) the superior portion: the preference for the diamond disappeared--no discrimination was found, even though the alternative to the diamond was the same as the S+. For the groups that were trained with the patterns that differed only in the superior portion, the opposite effect of testing conditions was found: when tested with patterns that differed in (1) the inferior portion: the bees avoided the very same diamond that was preferred by the bees trained differently, and favoured the alternative, which was the same as the S+ (2) the superior portion: no pattern discrimination was found (i.e. the avoidance of the diamond disappeared). Two predictions were disconfirmed: that during testing bees would only (1) approach the pattern that was the same as the S+, or (2) discriminate between patterns that differed in the same area (inferior or superior) as did the training patterns. The data were in line with the interpretation that during differential conditioning the visual field used in future pattern discriminations is expanded to include not only the inferior portion of the pattern but more of the superior portion as well.

This paper addresses a general question in animal cognition as it applies to learning in bumblebees: once a discrimination has been learned, what is it that is remembered and used in future discriminations? In other words, what are the contents of memory? This question has been comparatively neglected in the literature (Dyer, 1998). We have recently addressed this question in the context of spatial learning (Church & Plowright, 2006). Here we consider the case of visual pattern discriminations, which are particularly relevant to the broader question of how invertebrates categorize visual stimuli (Benard, Stach, & Giurfa, 2006).

Three different research strategies in pattern learning can be identified in the literature. (1) The first is to train bees to discriminate between patterns by rewarding approach to one (the positive stimulus, or S+) and not rewarding approach to the other (the negative stimulus, or S-). A learning curve can be traced, and final discrimination performance measured. A rich catalogue has now been assembled of patterns that can and can not be distinguished by honeybees following training (see reviews by Horridge, 1997, 2005, 2007). (2) The second strategy builds on the first: training experience is manipulated and then a common test is given. This method truly assesses learning because group differences on a common test can not be attributed to the current conditions but are traceable to the memories of prior experience (Shettleworth, 1998). As an example, we have recently used this method to show that patterns seem, by and large, undiscriminable on their own (e.g., a square vs. a square

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rotated by 45° when presented at a visual angle of 50° degrees) can be discriminated by bumblebees, but only if prior training with different patterns (e.g., a square and a chevron) is given (Perreault & Plowright, in press). (3) Finally, the third is another way of building on the first: after training on a pattern discrimination (S+ vs. S-), bees are given more than one test. This method has been applied successfully to show, for example, that for honeybees (Gould, 1988) and for bumblebees (Korneluk & Plowright, 1995) there is a “facultative mirror image ambiguity”, at least for some floral patterns: while a previously rewarded pattern and its mirror image are not confused, in the absence of the S+ the mirror image is chosen over a new stimulus, which shows that it is accepted as a substitute for the S+. The same is true for choice of a left-right transposition of a floral pattern by bumblebees (Plowright, 1997) and honeybees (Stach & Giurfa, 2001). The strategy of manipulating testing conditions following S+ vs. S- learning was also used to address the question of recognition of rotated patterns (Plowright et al., 2001). In this paper, we combine all three strategies. Four groups of bees were trained on a pattern discrimination (S+ vs. S-), the training experience was manipulated, and the testing conditions were manipulated as well. The use of a design that manipulates training and testing conditions factorially preserves all the strengths of the three approaches delineated above, with the added advantage of being able to detect an interaction between training and testing conditions.

Prior research has pointed to a dorso-ventral asymmetry in pattern perception in honeybees (Wehner, 1972) and bumblebees (Thivierge, Plowright, & Chan, 2002) with stronger weighting of the ventral portion. Prior research has also shown that for honeybees, differential conditioning (S+ vs. S-), as opposed to absolute conditioning (no alternative to the S+), results in an increase in the visual field assigned to the visual recognition task (Giurfa et al., 1999). Here we extend the research by attempting to manipulate the relative weighting of the inferior and superior portions of the patterns within a differential conditioning task. We created two sorts of patterns (shown in Figure 1), both for training and for testing: (1) those that were identical above the midline (so identical superior portions of the S+ and the S-) and could only be distinguished by attention to the inferior portions (2) those that could only be distinguished by attention to the superior portions, the inferior portions being identical. Depending on the contents of learning during training, several possible experimental outcomes can be envisaged, three of which are described below (see Predictions).

Method

Subjects

Three colonies of bumblebees (*Bombus impatiens*) were purchased from Biobest Canada, Ltd (Leamington, Ont.). Bees were individually labeled with coloured tags glued to the thorax. They were fed with sugar solution (2:1 sugar and water by volume) and pollen *ad libitum*. The supply of sugar solution was removed one day before training and testing periods to motivate foraging behaviour.

Apparatus

A 12-arm radial arm maze was used. It was modelled on that of Lehrer, Horridge, Zhang, and Gadagkar (1995), diagrammed in Séguin and Plowright (2008) and described by Simonds and Plowright (2004) and by Plowright, Simonds, and Butler (2006). One advantage of

using this maze over the Y-maze is that it increases the frequency of exposure to the S+ and S- within one visit to the maze (Perreault & Plowright, in press). Each corridor (14 X 15 X 15cm, W X L X H) opened onto a central area (22cm wide). The corridors' entrances from the central area were 6 cm wide. The vertical walls of the maze were made from opaque grey Plexiglas. The maze was connected through one corridor (the entrance corridor) by a wooden walkway covered with glass plates. Once a bee had entered the maze, the entrance corridor was blocked off, as was the corridor facing the entrance corridor, and so the bees made choices among the 10 remaining corridors during the experiment. The maze was positioned on a rotating platform so that with a 180° rotation, which was done four out of five times that a bee was allowed to enter the maze, the entrance corridor could be changed as could be the positions of the patterns relative to the entrance. Lighting was provided by daylight, two 32watt tubular incandescent lights located over the apparatus and fluorescent room lights.

Patterns

The four patterns were first created by Horridge (1996) and also used by Perreault and Plowright (in press): the cross (more specifically, the Saint-Andrew's cross, or the multiplication sign), the diamond (i.e., a square rotated by 45 °) and two chevrons (one pointing upwards and one pointing downwards, heretofore referred to, respectively, as the chevron-point-up and the chevron-point-down). They all consisted of two pairs of black orthogonal bars (4.3cm x 1.3cm)—since the bars were perpendicular, their orientations cancelled out, and so there was no overall orientation to the patterns that could serve to discriminate between them (Horridge, 1997). Patterns were printed on white paper (same dimensions as the end of the corridor) and laminated. A hole of 1.5cm diameter was cut through the center of each pattern for the end of the feeder tube.

Procedure

The experiment consisted of three consecutive phases (shaping, training and testing) described below. The sequence was repeated for new groups of 3-5 bees being tested until ten bees per group had been tested.

Shaping. The purpose of this phase was to allow the bees to explore the apparatus, to motivate them to forage inside, to approach the ends of the corridors and to allow us to select the most active foragers. A white square was positioned vertically on the wall at the end of each of the 10 corridors that were used in the maze. A hole (1.5cm in diameter) was pierced through the centre to allow the end of a feeding tube filled with sugar solution to protrude. Over three days, the colony was unstoppered so as to allow bees unrestricted access for 2hr inside the maze. The identities of the most active individuals were recorded so that they could be selected for training and testing.

Training. Bees were trained individually to discriminate between a rewarding pattern (S+), for which the feeder was filled with sugar solution, and an unrewarding pattern (S-), for which the feeder was filled with water. In a pilot study, presenting 10 patterns simultaneously seemed to create a "noisy" visual environment, and so we began by presenting only one S+ and one S- while blocking the other corridors, with the S+ and S- on opposite sides of the entrance. After each visit to the maze, the positions of the two patterns were changed in a pseudo-random order. A choice was defined as contact between the antennae or the proboscis and the feeder. When a bee had met a criterion of 12 choices of the S+ in 15 consecutive choices, we proceeded to train the bees in an environment similar to the one they would encounter during testing, i.e., patterns were available in all 10 corridors. The same criterion of 80% choices of the S+ was used for 10 consecutive choices, and a bee was tested immediately after training.

Testing. All feeders were filled with water and the training patterns were replaced with new testing patterns. Only one visit to the maze was allowed and the test concluded when the bee had made 15 pattern choices or had stopped foraging for 5 minutes.

Design

Training and testing conditions were manipulated factorially in a 2 X 2 between groups design (Figure 1). During training, the S- was the cross. The S+ was either a chevron-point-down

or the chevron-point-up. In this way, the difference between the S+ and S- was either in the positions of the two lines in the inferior (Inf) or superior (Sup) portion of the pattern (these portions are framed in Figure 1). During testing, the choice was between a diamond and another pattern (again, the chevron-point-down or chevron-point-up). In this way, the difference between the two patterns was again either in the positions of the two lines in the inferior or superior portions of the pattern. The remaining lines, that did not differ between the testing patterns, were oriented in such a way that the test would not consist of repeating the S+ vs. S-discrimination that had just been succeeded, if for no other reason than to avoid a ceiling effect. The four groups will be referred to as Inf-Inf, Inf-Sup, Sup-Inf and Sup-Sup, with the labels specifying first the portions of the pattern that differed in training and then in testing. We examined the proportion of the choices of the diamond as a function of training and testing.

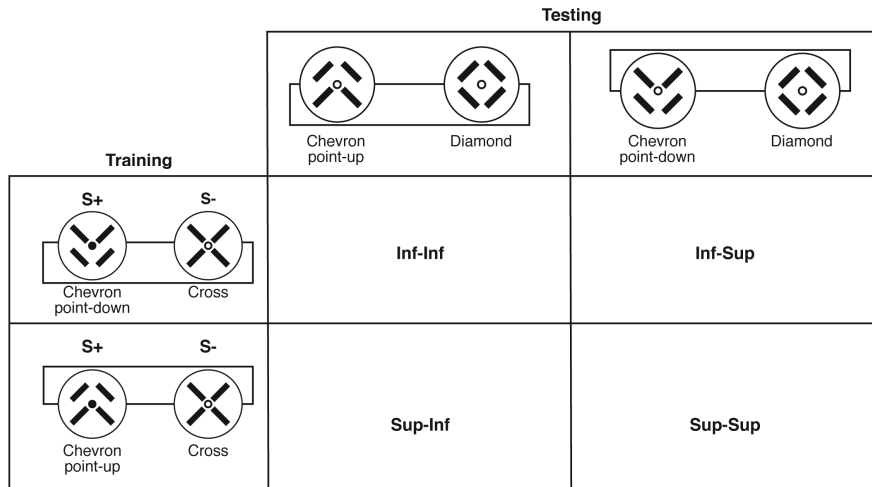


Figure 1. Training and testing stimuli used for each of four groups of bumblebees. The circle at the center of each pattern shows the position of the feeder. An open circle shows that the feeder contained only water and a full circle shows that it contained sugar solution. Either the inferior (Inf) portions or the superior portions (Sup) differed within a pair of patterns. To highlight the difference these portions are framed.

Predictions

Depending on what bees learned during training, at least three experimental outcomes were possible:

(1) The naïve notion that during training the bees would learn a rule “Approach the pattern that was rewarded during training” predicts that during testing, bees would only succeed in discriminations where a copy of the S+ was presented (Groups Inf-Sup and Sup-Inf) but would fail when two new patterns were given (Groups Inf-Inf and Sup-Sup).

(2) If our training manipulation were successful in shifting attention away from the inferior towards the superior portions of the patterns, then testing performance would depend on the match between the training and the testing conditions: the bees in groups Inf-Inf and Sup-Sup should be more successful in the test discriminations. This prediction is the opposite of the first. The prediction is strong in that the dorso-ventral symmetry documented in the literature would have to be so malleable as to be reversed by prior experience.

(3) A third possibility is that training bees to attend to the superior portion of the patterns would not shift the attention away from the inferior portion so much as extend it to encompass more of the visual patterns. This prediction differs from the effect, already documented for honeybees by Giurfa et al. (1999), that pattern encoding differs under conditions of differential conditioning vs. absolute conditioning. Here, our manipulation of training conditions occurs within a differential conditioning task. There still might be a dorso-ventral asymmetry, with the inferior portion of the pattern being weighted relatively more strongly than the superior portion, but nonetheless, the superior portion of the pattern would matter.

Training the bees to discriminate between patterns that differed in their inferior portions would only allow them to distinguish patterns that differed in their inferior portions

(group Inf-Inf) but not their superior portions during testing (group Inf-Sup). Training them to discriminate between patterns that differed in their superior portions would allow the bees to make similarity judgments between the testing stimuli and the training stimuli based on both the inferior and superior portions of the stimuli. If a copy of the S+ were present at testing (the chevron-point-down), then it ought to be preferred over the diamond (group Sup-Inf). The behaviour towards the diamond, however, should be context specific. The diamond would become more attractive in the absence of a copy of the S+ (group Sup-Sup). The diamond would now become the stimulus that most resembled the S+ and, conversely, the chevron-point-down would most resemble the S-.

Statistics

Choice proportion of the diamond vs. the alternative pattern is a binary variable, and so a logistic model, which specifies a binomial error term, was fit to the data using GLIM (Generalized Linear Interactive Modelling; Francis, Green, & Payne, 1993) to determine whether the choice proportion of the diamond depended on training and testing.

To determine whether two patterns at testing could be discriminated, a replicated goodness-of-fit test with the G-statistic was used (Sokal & Rohlf, 1995) because the data set consisted of repeated choices by individual bees. Two G values were obtained: G_H and G_P . The G_H value tests for heterogeneity (i.e., individual differences). The G_P value tests for whether the pooled data (i.e., the group choice proportions) deviated from a theoretical value of chance (50:50). The G values are compared to a χ^2 value in tests of significance.

Results

Figure 2 shows the mean choice proportions for the diamond for each of the four experimental conditions. The main effect of training condition was significant ($X^2_{(1)} = 17.71, p < 0.0001$) while the main effect of testing condition was not ($X^2_{(1)} = 0.13, p = 0.72$). The presence or absence of main effects, however, are comparatively uninformative in view of the significant interaction ($X^2_{(1)} = 34.62, p < 0.0001$). The top two bars show the choices of the two groups that had been trained to discriminate between the chevron-point-down (S+) and the cross (S-). The diamond, though it had not been presented during training, was preferred during testing over the chevron-point-up (group Inf-Inf). This preference was eliminated, however, when the alternative was the chevron-point-down (group Inf-Sup). The bottom two bars show the choices of the two groups that had been trained to discriminate the chevron-point-up (S+) from the cross (S-): the preferences for the diamond “move” in the opposite direction. In marked contrast with the group Inf-Inf, the diamond was distinctly avoided compared to the chevron-point-up (group Sup-Inf). The preference increased (i.e., the avoidance disappeared) when the alternative was the chevron-point-down (group Sup-Sup).

The analysis above compared the four groups to each other. The following analysis compares the choice proportions of each group to a chance value of 50:50. Table 1 shows the results of the G-test for each of the four experimental conditions. For the two groups tested with the chevron-point-up and the diamond, the discrimination was significant. The preferences shown, however, were in opposite directions: Group Inf-Inf favoured the diamond over the chevron-point-up, whereas group Sup-Inf did the reverse. For the two groups tested with the chevron-point-down and the diamond (groups Inf-Sup and Sup-Sup), the choice proportions did not differ significantly from chance. No individual differences were detected in any of the four groups: G_H was non-significant in all cases.

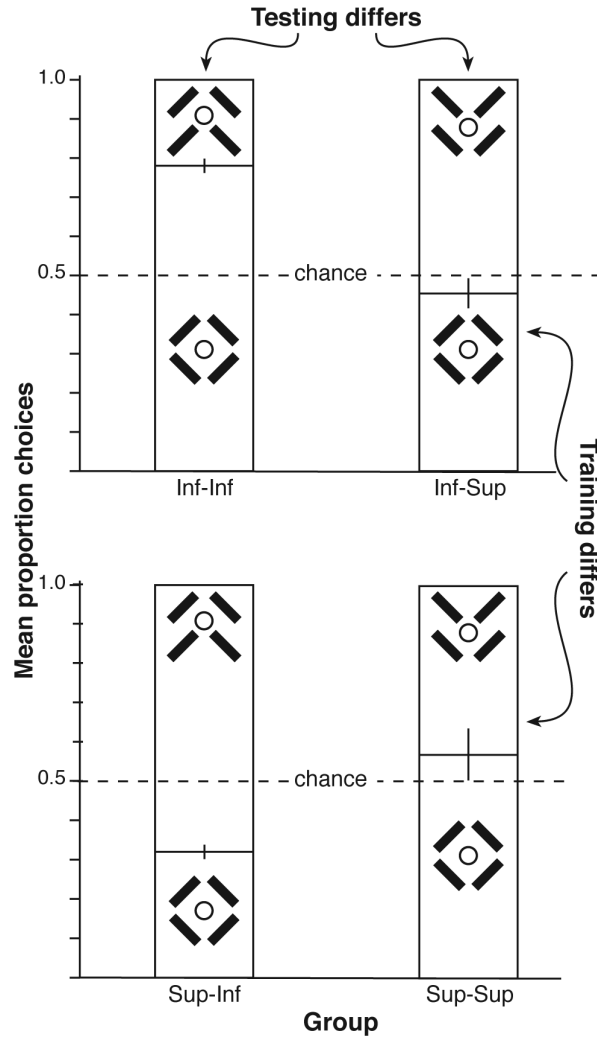


Figure 2. Choice proportions, with standard error bars, for the test stimuli for each of the four groups shown in Figure 1. Table 1 shows which proportions differed from a chance value of 0.5. The interaction between training and testing was significant.

Table 1.

Results of the repeated measures G test for the four experimental conditions. The G_p value tests for a deviation between the observed choice proportion for the diamond shown in Figure 1 and a chance value of 0.5. The G_H value tests for individual differences. In the tests of significance, the G value is compared to a χ^2 value.

Group	G_p (1 df)	p	G_H (9 df)	p
Inf-Inf	32.44	< 0.0001	5.98	0.74
Inf-Sup	0.41	0.52	8.51	0.48
Sup-Inf	18.67	< 0.0001	3.90	0.92
Sup-Sup	1.45	0.23	14.25	0.11

Discussion

For visual pattern discriminations, the effects of training and testing conditions can not be understood independently. The effect of manipulating testing conditions was to reduce a preference for a diamond given one type of training but to increase that preference given another. The same interaction can be described as a significant effect of type of training given one testing context, and an absence of such effect in another. The effects reported above were not attributable to particularities of a few individuals, since individual differences were non-significant in all the analyses, and bees from three different colonies were used. The implications of this interaction in terms of how patterns are encoded are described next with reference to the three predictions outlined above.

The first two of our predictions were disconfirmed. Specifically, a simple rule such as “approach the pattern that was rewarded during training” predicted a discrimination between the two test patterns by the group Inf-Sup because a copy of the S+ was present during the test. No discrimination was obtained. More importantly, the rule predicted a failure in discrimination by the group Inf-Inf because a copy of the S+ was absent during the test. In our experiment a strong preference for the diamond was obtained. The second prediction was the opposite of the first, and it did not fare better. If training with patterns that differed either in the superior or the inferior portions of the patterns served to shift the attentional focus of the bees, then only patterns that differed in the same areas as in training would be differentiated at the time of testing; i.e., successful discrimination would be obtained only for groups Inf-Inf and Sup-Sup. In fact, groups Inf-Inf and Sup-Inf succeeded while Inf-Sup and Sup-Sup failed.

Our data are in line with the third prediction. If bumblebees are indeed disposed to weight the inferior portion of the patterns, then training with patterns that differed only in the inferior portion would lead to a discrimination by the group that was shown patterns that differed in the inferior portion at the time of testing (Group Inf-Inf) but not by the group that was shown patterns that differed in the superior portion (Group Inf-Sup). This is exactly what happened. Training with patterns that differed in the superior portion would not shift attention so much as expand it to include not only the inferior but, to a certain extent, the superior portion as well. This would predict a preference for the chevron-point-up over the diamond by group Sup-Inf, since both inferior and superior portions are the same as the previously rewarded stimulus. This preference was obtained. It would be at the very least reduced if not eliminated or reversed by group Sup-Sup since the superior portion of the diamond corresponds to the superior portion of the S+ and the superior portion of the chevron-point-down corresponds to the superior portion of the S-. This effect too, was obtained.

The fact that the choice proportion by group Sup-Sup was not significantly greater than chance, though it was in the predicted direction, is in no way incompatible with our explanation. It merely shows that the representation of the superior portion of the patterns was still weak relative to the inferior portion. Both testing patterns would have been unattractive by virtue of the dissimilarity between the inferior portions of both patterns to either the S+ or the S-. The possibility that the training with the superior part of

the patterns may have taken longer than the training with the inferior parts in no way detracts from our arguments either. When the extent of training is explicitly manipulated, it does affect pattern encoding (Stach & Giurfa, 2005). In our experiment, training length was not manipulated but if the bees were predisposed to attend to the inferior portions, then the bees may have adjusted learning time to attain a learning criterion themselves: it may well have taken them longer to locate the portions of the training patterns that afforded a key to the discrimination.

Using just the four patterns in this experiment, it would be possible to train the bees on six different S+ vs. S- combinations. If making all these comparisons becomes necessary in the future, then our results point to the importance of manipulating not only the training stimuli but also the test stimuli. With the same six combinations of test stimuli, it would have been possible to obtain 36 choice proportions to compare with each other. Making such comparisons was beyond the scope of this paper, and our conclusions are limited to (1) the hypotheses described above, and (2) the general point that in the presence of a significant interaction between training and testing conditions such as the one documented here, conclusions regarding the effect of training in one particular context, or for that matter the effect of testing context after one particular learning experience, must be carefully circumscribed.

Our results should be interpreted with two cautions, both of which have been made by Giurfa et al. (1999) in their study demonstrating the effect of absolute vs. differential conditioning on visual recognition. The first is that no claim is made as to the nature of coding of the stimuli. The issue of whether visual patterns are represented as a set of unassembled features or an image that preserves the spatial layout of those features has been explored at length elsewhere (e.g., Srinivasan, 1994; Efler & Ronacher, 2000; Horridge, 2000; Stach, Benard, & Giurfa, 2004). The second is that the superior and inferior portions of the patterns do not necessarily correspond to the dorsal and ventral portions of the insect's visual field. Given that the feeders were in the center of vertically positioned patterns, it stands to reason nonetheless that the inferior portion of the pattern was detected in a more ventral portion of the visual field than the superior portion.

In terms of ecological significance, this study provides a laboratory demonstration of relative preferences (for review of factors influencing floral choices, see Goulson, 2003). A floral pattern can not be thought of as attractive to bees in an absolute sense: its drawing power depends on prior experience, present context, and the combination of the two. By showing that bees do find the relevant cues that will allow them to differentiate sources of food from unrewarding patterns, this study also highlights the problem solving nature of learning, and so it is particularly relevant to current efforts to understand bumble bee foraging by integrating the study of cognition and behavioural ecology (e.g., Chittka & Thomson, 2001; Dukas, 2008). It does, however, raise a question that is traditionally the province of psychology: What is the effective stimulus and what are the cognitive processes that give rise to its perception and retention? While these questions belong to mainstream animal learning (Fetterman, 1996), "bee cognition" (e.g., Cheng, 2000; Gould, 1990, 2002) has not always made contact with other-animal-cognition (and more generally, comparative psychology has not always made contact with cognitive psychology (Smith, 2003)). As noted by Bitterman (1996, p. 123) not so long

ago, “zoologists interested in invertebrates have not been much interested in learning” and “psychologists have not been much interested in invertebrates”. Much headway has been made in recent years. For instance, the binding problem in perception has recently been addressed for bees (Fauria, Colborn, & Collett, 2001) as have the topics of working memory (Brown & Demas, 1994), concept formation (Giurfa, Zhang, Jenett, Menzel, & Srinivasan, 2001) and timing (Boisvert & Sherry, 2006). Nonetheless, for the sake of argument, we suggest the following point for debate: that questions in bee cognition are still currently constrained by an inward looking perspective that may well have been born, understandably, from a deliberate attempt to avoid species generalizations and especially generalizations from vertebrates to invertebrates. The benefits of integrating bee cognition with other disciplines such as behavioural ecology (Dukas, 1998; Dukas & Ratcliffe, in press; Chittka & Thomson, 2001), neuroscience (Menzel, 2001) and robotics (Srinivasan & Venkatesh, 1997) are undeniable, but bee cognition is, after all, cognition, and it remains to be fully integrated with its own field.

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Differing Pattern of the Development of Mother–Infant Interactions in Cynomolgus Monkeys Due to Exposure of an Environmental Chemical, Bisphenol A

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Recent studies have focused on the effects of low doses of Bisphenol A (BPA) on the central nervous system, which may prevent sexual dimorphism of the brain in rodents. To assess sensitivity to BPA, mother–infant behaviors in the cynomolgus monkey were studied longitudinally after treating the mothers with low-dose BPA during pregnancy. Mother–infant interaction was observed for 6 months after the birth of the infants. In conclusion, male offspring of BPA-treated females showed female-like behavior patterns. Prenatal BPA exposure altered infant behavior in the early stages of mother–infant interaction, and male infants were affected more seriously than females.

More than a decade has passed since the 1996 publication of *Our Stolen Future* by Colborn, Dumanoski, and Myers. They stated, “For all these systems, normal development depends on getting the right hormone messages in the right amount to the right place at the right time. As this elaborate chemical ballet rushes forward at a dizzying pace, everything hinges on timing and proper cues. If something disrupts the cues during a critical period of development, it can have serious lifelong consequences for the offspring” (p. 46). If traces of environmental endocrine disruptors (EEDs) persist and no decrease in their environmental concentrations have been observed, it is clear that the hormone exposure problem during the fetal period is related to the difference in the mouse’s behavior. Reproductive capacity has addressed this issue (e.g. (Dhar, & vom Saal, 1992; Even, Walker, Keisler, Caldwell, Kier, & vom Saal, 1996; vom Saal & Dhar, 1992). Many studies (Calafat, Ye, Wong, Reidy, Needham, 2008; Cao & Corriveau, 2008; Kang, Kondo & Katayama, 2006; Le, Carlson, Chua, & Belcher, 2008; Mahalingaiah et al., 2008;

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Welshons, Nagel, & vom Saal, 2006; Tsai, 2006;). Bisphenol A (BPA) is an EED that may be released from products such as polycarbonate plastics and epoxy resins which have been used to manufacture food containers. BPA mimics estrogen as well. Recent studies have focused on the effects of low-dose BPA on the central nervous system (CNS), particularly, its effect in preventing the development of sexual dimorphism in the brain of rodents. Kubo et al. (2003) showed the following result in rats: in the control group, females were more active in open-field behavior and had a larger locus coeruleus (LC) volume than males. BPA abolished and inverted the sex differences of both open-field behavior and the LC volume, respectively, without affecting the reproductive system; whereas the rats showed normal phenotype of sexual organs. The behavioral regulatory system in the brain is likely to be more sensitive to EEDs than the systems that regulate reproduction. Moreover, the brain is sensitive to BPA at concentrations lower than the tolerable daily intake (TDI) in humans. Kubo et al. (2003) suggested that the abnormalities in sexual differentiation produced by BPA differ from those caused by resveratrol (RVT) and diethylstilboestrol (DES). Their study showed that DES and RVT affected the LC volume and reproductive system, while BPA abolished and inverted the sex differences of open-field behavior and the LC volume, without affecting the reproductive system. Animal studies indicate that DES acts on parts of the developing fetus other than the reproductive tract, including the brain, the pituitary gland, the mammary glands in the breast, and the immune system, causing permanent changes (Colborn et al., 1996). According to Hines (1996), in animals, exposure to DES or higher levels of estrogen causes “dramatic and permanent changes in brain structure and behavior” (p. 64). The brain and behavior of rats were altered by BPA (Fujimoto, Kubo, & Aou, 2006); that is, BPA affected brain development and altered behavior such as open-field behavior or maternal behavior among rats and mice (Della Seta, Minder, Dessi-Fulgheri, & Farabollini, 2006; Palanza, Howdeshell, Parmigiani, & vom Saal, 2002; Rubin et al., 2006). Moreover, there is clear, although indirect, evidence of long-term alterations in the monoaminergic functions of the brain after perinatal BPA exposure (Adriani, Della Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003; Negishi et al., 2004). Kuroda (2003) suggested a “spatial–temporal complex pollution theory of toxic chemical substances” (p. 23), which emphasized the importance of the duration and site of exposure of the fetal and infant brain to toxic chemicals. These chemicals (EED, BPA, PCB, etc.) are known to affect neurogenesis during this crucial period as well as during the prenatal, perinatal, and infant periods. Various symptoms and abnormal behaviors have been observed because of slightly altered functions or neural impairment in specific cerebral regions. Studies during the prenatal period involving different species and different methods of administration showed that rats excreted BPA immediately after oral and subcutaneous administration; in contrast, monkeys absorbed BPA very rapidly, maintained a high concentration in the blood, and excreted the compound at a slower rate (Negishi et al., 2004; Tominaga et al., 2006). Chemicals in the blood easily pass into the brain because the blood–brain barrier is not fully functional during infancy. Therefore, the brain of the fetus and neonate is intrinsically sensitive to environmental changes. Environmental factors affecting the brain during this period may cause irreversible and permanent changes (Aou, 2004).

Monkeys exhibit sexual differences in behavior in early life. In normal primate mother-infant relations, it is generally recognized that male rhesus monkey infants are more active and more playful than their female counterparts (Hansen, 1966). Male infants in the wild have been reported to leave their mothers sooner than female infants (Itani, 1959). The female rhesus monkeys (*Macaca mulatta*) remain closely attached to the mothers; the males interact more frequently with other members at the periphery of the troop (Mitchell, 1968). Thus male infants are more active than female infants, while female infants are more frequently in contact with the mother. Rodents exposed to BPA showed behavioral changes and the LC volume of male rodents was equal to that of the females'. This indicates that the brain of the male rat was feminized. Is the sexual difference impaired in the early stage of development reversed later in primates? We consider that it is important for us to identify whether these alterations appear in primates and in which stage of development amelioration occurred.

We considered these neurobehavioral effects to be a result of BPA exposure. In the present study, we administered low-dose BPA during the prenatal period to cynomolgus monkeys (*Macaca fascicularis*), a phylogenetically close relative and a useful model for humans. We report that mother-infant interactions were observed longitudinally. We also observed social interactions between mother and infant immediately after birth and evaluated the behaviors in the offspring to assess sensitivity toward BPA.

Method

Subjects

Colony-bred adult female cynomolgus monkeys (*M. fascicularis*) (body weight 3–4kg, generally healthy) were maintained and bred at Shin Nippon Biomedical Laboratories Ltd. in Kagoshima, Japan. The animals were housed in stainless-steel cages, and received approximately 108g (12g × 9 pieces) of solid diet (Harlan Tekland, Harlan Sprague Dawley Inc., Indianapolis, IN, USA), on a daily basis, which was supplied once a day at 15 h; any food remaining by 9 h the next day was removed. The animals had free access to drinking water.

All mothers were primiparous monkeys. They were housed individually during pregnancy in stainless steel cages (69cm × 61cm × 75cm) according to the National Institute of Health guidelines. In addition, they continued to care for their offspring until weaning. The duration of pregnancy and body weight of the offspring at birth were recorded (Table 1).

Table 1

Effect of BPA-exposure on gestation length and body weight at birth in cynomolgus monkeys.

	Control		BPA	
	Male	Female	Male	Female
Number of pregnant animals	19		18	
Number of animals normally delivered	6	10	4	9
Gestational length (days) ^a	161.7 ± 5.0	160.9 ± 6.4	159.3 ± 5.0	159.7 ± 7.0
F1 body weight at birth (g) ^a	369 ± 34	372 ± 40	368 ± 40	350 ± 51
Number of animal subjected to observations	6	9	4	6

^aNormally delivered mothers and infants were used. Values are mean ± SD.

Maternal BPA Exposure

Eighteen pregnant monkeys received BPA (10µg/kg/day) in a mixture of N, N-dimethylacetamide and polyethylene glycol (400) (1:1) through Alzet® osmotic pumps. These pumps were surgically implanted in the dorsal subcutaneous tissue and each pump released a fixed amount of the solution (6µl/day) from 20 days of pregnancy until delivery. The pumps were renewed every 28 days. Oral administration of 1mg/kg is an effective low dose among rats. Species differences of ADME (absorption, distribution, metabolism and excretion) of BPA between rodents and primates are 1:10, and blood concentration of BPA in subcutaneous injection (s.c.) is 10 times higher than per os (p.o.). (Tominaga et al., 2006). Thus, BPA concentrations were calculated as [body weight (kg) × 10 (µg/kg/day)/6 (µl)]. Control pregnant monkeys received only the vehicle solution using the same osmotic pumps.

Observation Procedure

Each mother and infant lived together in the same cage (69cm × 61cm × 75cm). We observed mother–infant behavior twice a week for a period of 1 week during the first 90 days after birth (the infant has greater developmental changes in the early stage of development) and once a week during the period from 90 to 180 days after birth. The observation started in the morning before food was given. Interactions between the mother and infant in their home cage were recorded for 20min using a digital video camera. The front mesh of the cage was replaced with an acrylic sheet for the observation period.

Table 2
Behavioral Categories of Infant in the Mother–Infant Interactions

Item (Abbreviation)	Explanation
Social behaviors	
Approach (Appr)	Approach mother within infant's reach.
Contact cling (Cntc)	Active body contact, excluding ventral contact.
Lip smacking (Lips)	Rhythmic movements of the lips, sometimes with an occasional click noise.
Nipple contact (Nipc)	Sucking mother's nipple.
Orient (Ornt)	Paying attention to animals and humans outside the cage. Reaction to mainly voice and noise.
Proximity (Prox)	Sitting or standing within mother's reach.
Reject/withdraw (Rjwz)	Rejection of mother's approach or withdrawing to the corners of cage, sometimes shaking its head.
Social exploration (Soce)	Nonaggressive interactions with mother such as licking and visual exploratory action toward her.
Ventral contact (Vntc)	Abdominal clinging to mother.
Nonsocial behaviors	
Auto grooming (Autg)	Grooming oneself.
Environmental exploration (Enve)	Exploratory actions toward surroundings with forelimbs or mouth (licked the glass).
Locomotion (Loco)	Moving on foot or brachiating by clinging to tall limbs and branches.
Outward interest (Outw)	Looking out of the cage from the gap between the acrylic front board and the wall, observing the wire on the floor.
Self-directed behavior (Slfd)	Stimulating oneself with its mouth, hand, or foot, and biting or licking for a long time.
Stereotypy (Styp)	Repetition of the same pattern behaviors in the monotonous rhythm.

We did not use video for five minutes at the beginning of the observation. We used the later 10 minutes for observation to prevent bias, because the part immediately after the beginning of the recording was not suitable for observation. We omitted the first and last 5 minutes of a 20

minute recording period to avoid human influence caused by the technician who switched on and off the video camera. Video recordings of the mother and infant were analyzed separately by the one-zero sampling method every 5s. Infants were observed exhibiting 15 behaviors (Table 2) that were defined beforehand. Each observer was blind to all information about the subjects (BPA group or control group, sex). We used The Observer 5.0 (Noldus Information Technology, Netherlands) to analyze the video observations. The total inter-observer agreement (Cohen's kappa) was 0.88.

We obtained approval for the study and conducted the experiments according to a guideline by IACUC Institutional Animal Care and Use Committee, Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Statistical Analysis

For each behavioral item, we calculated the percentage of total observation time. Each behavioral change was expressed graphically as an average for each month. Infant behaviors were first analyzed by canonical discriminant analyses. Each behavioral category was then analyzed by a three-way analysis of variance (ANOVA). All statistical analyses were performed using SPSS (SPSS Inc.) and StatView (Hulinks Inc.). Significance was set at $p < .05$.

Results

General Observations

During experimental exposure to BPA, three fetal and two neonatal deaths occurred among 18 pregnant animals in the experimental group. Of the 19 animals in the control group, three fetal deaths and one neonatal death were observed. Gestation period and body weights at birth until weaning of the surviving animals are summarized in Table 2. BPA exposure had no significant effect on sex ratio, gestation length, or body weight at birth. In addition, no external abnormalities were observed in either group.

Effect of BPA Exposure on Four Classes of Infant Behaviors

Infant behaviors were analyzed for nine social behaviors and six nonsocial behaviors. The four groups (males and females of the BPA group and males and females of the control group) were discriminated by canonical discriminant analysis based on age-pooled data sets. Discriminant scores of all subjects in the four groups were plotted separately in two dimensions (Figure 1). The result showed that male infants of the control group were distinguishable from the remaining three groups. Control females, BPA females, and BPA males showed similar behavioral tendencies; that is, BPA males showed behavior similar to the female groups (BPA and control female infants). The cumulative contribution to the two functions in the canonical discriminant analysis was 97.1% (Function 1, 89.3%; Function 2, 7.8%). The behaviors contributing to Function 1 were outward interest, i.e. locomotion, orientation, ventral contact, and social exploration. Function 1 represents a measure of static or dynamic mother–infant relationships.

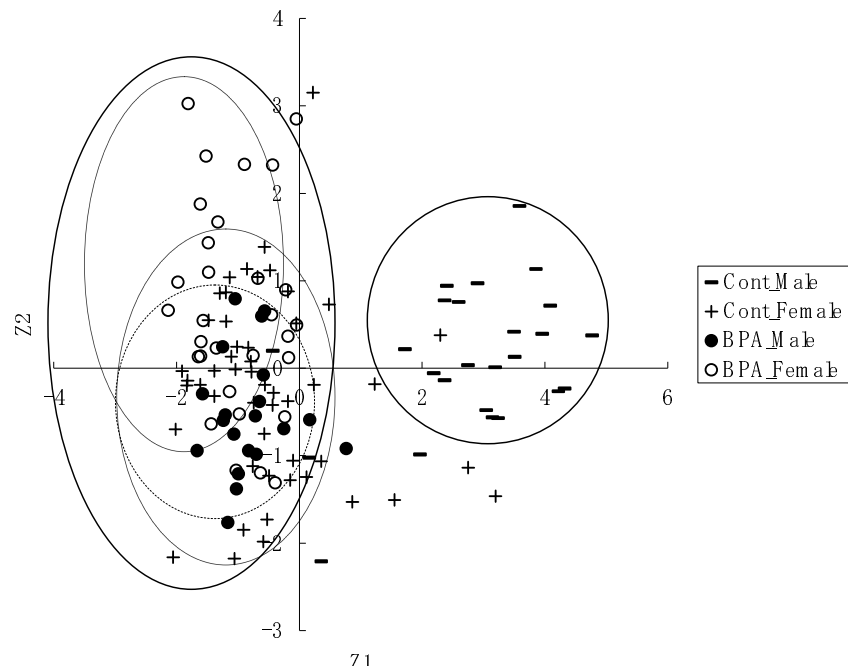


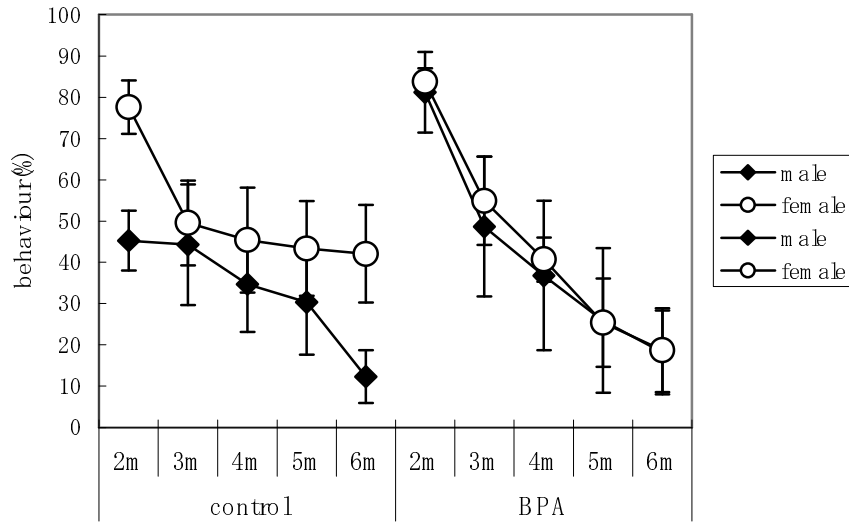
Figure 1. Canonical discriminant analysis (four groups). Subjects were classified into four groups (males & females of the BPA group and males & females of the control group, i.e., cont_male...) in consideration of the sexual differences in behaviors. Z1 represents the measure of static or dynamic mother–infant relationship.

Developmental Changes

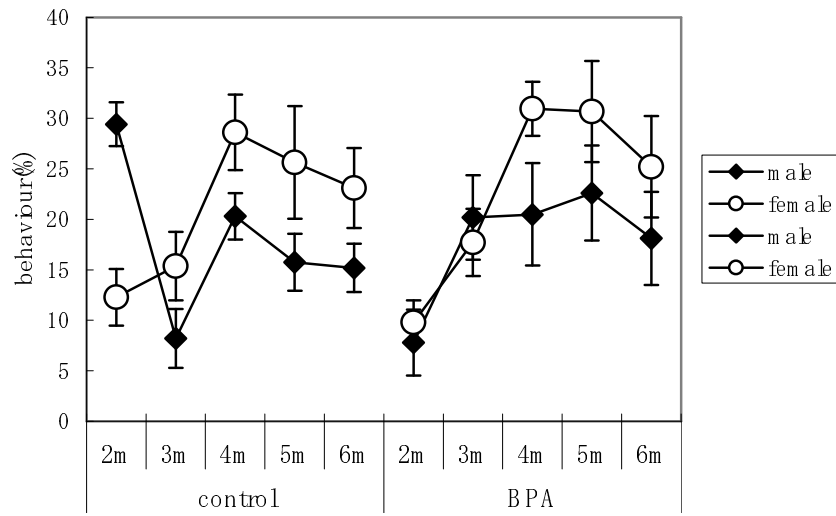
The developmental changes associated with behaviors that contributed to function 1 of the canonical discriminant analysis are indicated in Figure 2. As for these behaviors, analyses of variance (ANOVA) were performed in order to explain the developmental profile. A three-way ANOVA was performed across categories (treatment, sex, and age) to explain which factors caused significant changes. A significant interaction between age and treatment was shown in locomotion ($F_{4,84} = 2.69, p < 0.04$), ventral contact ($F_{4,84} = 2.66, p < .04$), social exploration ($F_{4,84} = 5.03, p < .001$), and outward interest ($F_{4,84} = 4.98, p < .001$) among the behaviors contributing to function 1 in the canonical discriminant analysis.

In addition, a two-way ANOVA (one factor about age) was performed to examine the developmental profile on the behavior categories related to the significant interaction of age. These analyses included: (1) two-way ANOVA between the BPA and control groups with two factors: sex \times age, and (2) two-way ANOVA (treatment \times age) factored by sex.

Ventral contact



Social exploration



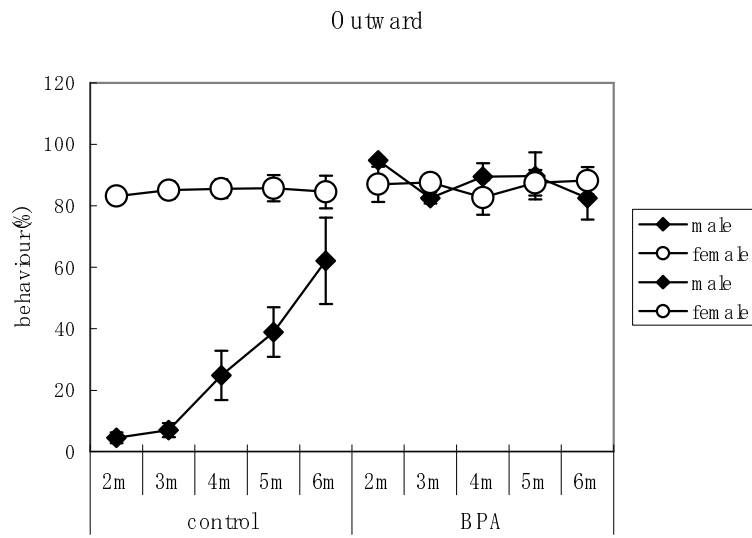
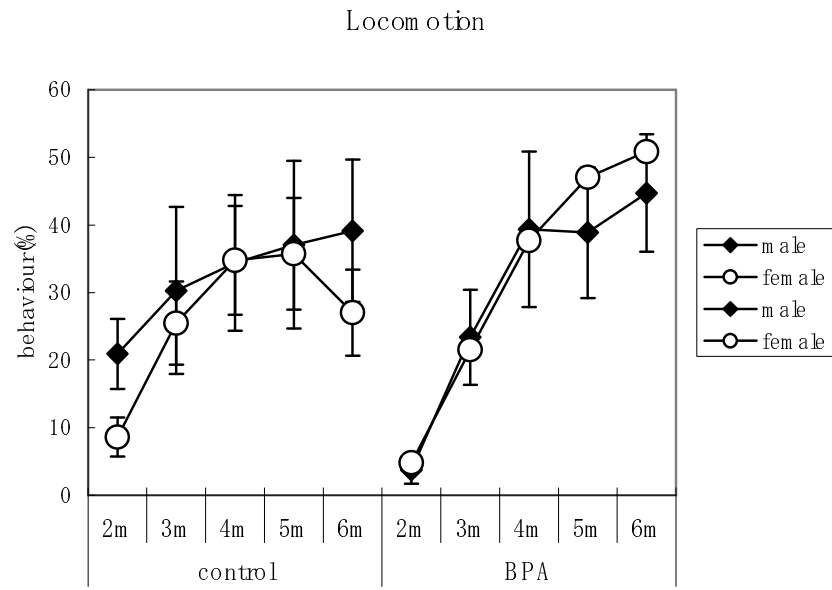


Figure 2. Developmental changes of infant behaviors.

Outward Interest. The three-way ANOVA showed a significant main effect of treatment ($F_{1, 21} = 31.3, p < 0.01$) as well as an age/treatment interaction. A significant main effect was also shown for sex ($F_{1, 21} = 16.63, p < .01$) and age ($F_{4, 84} = 3.70, p < 0.01$). The two-way ANOVA (sex \times age) by treatment, showed a significant main effect of sex ($F_{1, 13} = 27.46, p < 0.01$) and age ($F_{4, 52} = 8.07, p < 0.02$) in the control group. There was also a significant interaction between sex and age ($F_{1, 13} = 5.76, p < 0.01$). This result reflects a difference in Outward Interest: males slowly increased, whereas females remained flat from the early stage of development. On the other hand, the main effect and the interaction were not significant in the BPA group. Males and females in the BPA group, as well as females of the control group remained constant in the frequency of Outward Interest from the early stage of development.

Locomotion. The two-way ANOVA (sex \times age) showed a main effect of age to be significant in both the BPA group ($F_{4, 32} = 16.04, p < 0.01$) and control group ($F_{4, 52} = 3.62, p < 0.01$). Both groups showed increase of the behavior with age. Subsequently, another two-way ANOVA (treatment \times age) showed a significant main effect ($F_{4, 56} = 15.0, p < 0.01$) in females and a significant interaction (treatment \times age) ($F_{4, 28} = 2.69, p < 0.05$) in males. Locomotion of females increased in both the control group and the BPA group. However, males showed development in the BPA group but not in the control groups.

Ventral Contact. A two-way ANOVA (sex \times age) showed that only the main effect of age was significant in both the BPA group and control group: ($F_{4, 32} = 18.22, p < 0.01$), ($F_{4, 52} = 5.11, p < 0.01$). This indicates that Ventral Contact decreased with development in both groups. Another two-way ANOVA (treatment \times age) was performed with respect to both sexes and the results showed the main effect of age to be significant in males ($F_{4, 28} = 6.22, p < 0.01$) and females ($F_{4, 56} = 17.76, p < 0.01$). Both males and females exhibited a similar decrease of Ventral Contact as they developed.

Social Exploration. As a result of the two-way ANOVA (sex \times age), the control group showed a significant main effect of age ($F_{4, 52} = 3.85, p < 0.01$) and a significant interaction of age and sex ($F_{4, 52} = 3.91, p < 0.01$), while a main effect of age was significant ($F_{4, 32} = 7.83, p < 0.01$) in the BPA group. This is an indication that the developmental pattern of Social Exploration is different in males and females of the BPA group but similar in males and females of the control group. According to the two-way ANOVA (treatment \times age), male infants showed a significant interaction ($F_{4, 28} = 7.34, p < 0.01$) and female infants showed a significant main effect of age ($F_{4, 28} = 9.6, p < 0.01$). This indicates that female infants in both the control group and the BPA group presented similar developmental changes, but in contrast male infants showed different developmental changes of Social Exploration in both groups.

Discussion

Previous rodent studies have shown that low-dose BPA exposure affected sexual behaviors and brain dimorphism in offspring (Della Seta et al., 2006; Fujimoto et al., 2006; Fujimoto, Gioiosa, Fissore, Ghirardelli, Parmigiani, & Palanza, 2007; Kawai et al., 2003; Kubo, & Aou, 2007; Kubo et al., 2003; Kubo et al., 2001; Porrini et al., 2005; Rubin et al., 2006; Tando et al.,

2007). Only two studies have reported the effect of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin exposure on nonhuman primates (Negishi et al., 2006; Schantz & Bowman, 1989), but no studies have addressed the effect of BPA exposure on primate offspring. Findings from rodent studies would be applicable to other species, including humans. Therefore, the present study is an important step in demonstrating the effects of BPA in primates.

The evidence that BPA exposure affects behavior of offspring has statistical significance. Overt behavior is considered the final output of CNS activities, and therefore, behavioral changes suggest the possibility of neurological and neuroendocrinological alteration in the brain.

The present study revealed that prenatal exposure to BPA prevented the sexual differentiation (dimorphism of the brain gender) of infant behaviors, such as Outward Interest and social exploration, and the development of ventral contact and locomotion behaviors in male infants. These observations led to two conspicuous features of prenatal exposure to BPA in cynomolgus monkeys. First, BPA exposure altered infant behaviors in the early developmental stages of the mother-infant interaction. Some of the behaviors in the BPA-exposed group were different from those in the control group, and male behaviors in the BPA-exposed group were feminized. A developmental difference was seen between the BPA-exposed and control groups with regard to locomotion and ventral contact. The control group showed sex differences at an early developmental stage, but the BPA-exposed group did not. Second, male infants were more susceptible to a low dose of BPA. Behaviors in the BPA-exposed males were similar to female behaviors. This shows that prenatal exposure to BPA influenced male infant behavior immediately after birth. Female infant rhesus monkeys received, as well as reciprocated, more positive physical contact than did male infant rhesus monkeys (Mitchell, 1968). Minami (1997) reported that sexual difference was generally apparent in social contacts. Ventral contact rapidly decreased as the infant developed; however, females often showed ventral contact for a longer period than males. Males exhibited locomotion behavior earlier than females. These behavioral differences in the mother-infant interactions were related not only to the experience of the mother but also to the sex of the infant (Mitchell & Brandt, 1970). This difference between sexes gradually increased and appeared among infants at an early stage of development; it influenced their growth and maturation through mother-infant interactions. Our results showed that the effect of BPA exposure on the sexual differentiation of the brain in monkeys was similarly estrogenic in nature, since it interfered with the sexual differentiation and social behavior from an early developmental stage. Normal brain development requires internal signals, such as environmental sensory stimuli, hormones, and growth factors at appropriate times and in appropriate amounts from the perinatal period to the lactation period. Sex hormones play an important role in the sexual differentiation of the brain. When cerebral neurons were exposed to testosterone during the third and fourth fetal months in humans, the brain showed masculine characteristics (Arai, 2004). Such differentiation was evident in a wide range of behaviors, from learning to emotional regulation, and in the autonomic nervous system. It also influenced higher brain functions such as homeostasis, stress responses, emotion, and memory (Aou, 2004). Tominaga et al. (2006) examined how BPA passed through the monkey's placenta on fetal days 50, 80, and 120. BPA was almost

completely excreted in urine within 24hr on fetal day 50, depending on maternal metabolism. However, on fetal day 80, increased blood concentration of BPA in the fetus is higher than that of mother, and was found to be circulating in the CNS of the growing brain. Yoshikawa (2005) considered that nearly the entire amount of BPA can pass through the placenta and affect the spatial-temporal development of the fetal brain.

In the present study, prenatal BPA exposure influenced the development of the brain gender in the CNS. Low-dose exposure to BPA resulted in feminization of masculine behavior. This low-dose exposure during the fetal period functioned as an estrogenic agent and prevented the sexual differentiation of male infants. It is believed that BPA can penetrate the CNS; thus, BPA passes through the placenta and possibly impairs the sexual differentiation of the behavior in the brain. It has been said that transgenderism is physiological in origin, probably because of neurological accidents during the prenatal period (Hood, 2005). Although our results cannot be applied directly to humans, they do support the concept of endocrine-disrupting chemicals destabilizing the sexual differentiation process in the brains of nonhuman primates.

As seen in this study, behavioral feminization of male infants during the early neonatal period diminished in the later stages suggesting that the effects of BPA exposure did not necessarily continue after birth. Further observations are needed to examine whether the effects last until the later developmental stages.

Our study focused on behaviors in the early period of development. Future research into what kinds of sexual behavior would be affected and at which reproductive age the effects are exhibited would be of value. Recently, it is suspected that BPA may be affecting the nervous systems of people in Japan. Therefore researchers should begin to reassess the effects of BPA, not only in rodents and non-human primates, but in humans as well.

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Recognition and Discrimination of Human Actions Across the Senses of Echolocation and Vision in the Bottlenose Dolphin: Evidence for Dolphin Cross-modal Integration of Dynamic Information

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The ability of cetaceans to explore and interpret their world via echolocation has received considerable attention during recent years, and the resulting body of work has revealed a sophisticated cetacean echolocation system. In addition, a number of recent studies suggest that dolphins can relate information that they receive from vision with information that they obtain from echolocation when this information concerns stationary objects. However, the present study is the first test of the cetacean ability to integrate dynamic information about movement across the two senses. Three adult female bottlenose dolphins that had previously learned to interpret visible movements produced by humans stationed on floating docks were asked to interpret a set of these movements produced by an underwater human located behind a visually opaque screen. Although each dolphin had previously demonstrated its ability to reliably interpret movements produced by a human in the air above the surface of the water, none of the dolphins had any previous experience with underwater humans producing movements that the dolphins could see or with underwater humans producing movements behind an opaque screen that prevented the dolphins from using visual information to interpret these signals. The dolphins quickly learned to correctly respond to signals that they could not see but could observe via echolocation. These results demonstrate that dolphins can relate visual and echoic representations of actions, although the amount of experience necessary for such integration has yet to be determined.

The manner in which cetaceans use echolocation to make sense of their world has received considerable attention since the possibility of a cetacean echolocation system was advanced during the 1950s (Kellogg, 1958; Kellogg & Kohler, 1952; McBride, 1956). Subsequent research has revealed a sophisticated echolocation system that enables dolphins to both locate objects and to discriminate objects based on a number of factors, including size, shape, and material composition (Akamatsu, Nakagawa, & Hatakeyama, 1995; Au, 1993; Au & Benoit-Bird, 2003; Au & Hastings, 2008; Harley & DeLong, 2008; Harley, Putman, & Roitblat, 2003; Helweg, Roitblat, Nachtigall, & Hautus, 1996; Pack, Herman, & Hoffmann-Kuhnt, 2004; Roitblat, 2004). These findings are consistent with the notion that wild cetaceans may spontaneously use echolocation to identify and distinguish obstacles, prey, predators and conspecifics, a possibility also supported by the observations that free-ranging harbor porpoises use echolocation to inspect areas before swimming through them (Akamatsu, Wang, Wang, & Naito, 2005).

In addition to their remarkable ability to make sense of their world using echolocation, dolphins possess impressive visual skills (Dawson, 1980; Herman, Hovancik, Gory, & Bradshaw, 1989; Herman, Peacock, Yunker, & Madsen, 1975; Madsen & Herman, 1980). The combination of a sophisticated

echolocation system and a sophisticated visual system resulted in a number of researchers investigating the possibility that dolphins might be able to integrate information across the senses of vision and echolocation. The extent to which information from multiple perceptual systems is integrated by organisms has intrigued scholars for centuries (see Marks, 1978; Stein & Meredith, 1993), and previous research has found that an ability to relate information from different modalities exists in a variety of species, including bushbabies (Ward, Yehle, & Doerflein, 1970), chimpanzees (Hashiya & Kojima, 2001; Izumi & Kojima, 2004; Parr, 2004; Savage-Rumbaugh, Sevcik, & Hopkins, 1988), humans (Arabzadeh, Clifford, & Harris, 2008; Ernst & Banks, 2002; Giard & Peronnet, 1999; Meltzoff & Borton, 1979; Rose & Ruff, 1987), pigtailed macaque monkeys (Gunderson, Rose, & Grant-Webster, 1990), rats (Delay, 1986), rabbits (Yehle & Ward, 1969), and rhesus monkeys (Cowey & Weiskrantz, 1975).

To date, research with bottlenose dolphins has revealed that dolphins are capable of integrating information from vision and echolocation when the information involves stationary physical objects (Harley & DeLong, 2008; Harley et al., 2003; Harley, Roitblat, & Nachtigall, 1996; Pack & Herman, 1995; Pack, Herman, Hoffmann-Kuhnt, & Branstetter, 2002). Given this ability, one might also expect dolphins to be able to integrate information about actions that they experience visually with information about actions that they obtain via echolocation. However, this ability has yet to be demonstrated empirically. The present experiment tested the ability of one cetacean species, the bottlenose dolphin, to interpret information about the movements of others obtained through echolocation in terms of previous visual experiences with these movements. We did so by taking advantage of the dolphins' previous experience with human movements in the context of human-dolphin training.

Dolphins housed in oceanariums are often trained to respond to movements produced by humans. These movements range from small subtle behaviors (such as a human lightly tapping her foot or slightly nodding her head) to large vigorous behaviors (such as a human quickly moving both arms in a windmill fashion), and are used to request specific behaviors from dolphins. When interpreting these movements, dolphins are typically asked to produce behaviors that serve a variety of functions, including animal husbandry, public education, public entertainment, and human-dolphin interaction (Kuczaj & Xitco, 2002).

The dolphins used in the present study had considerable experience with humans producing movements that the dolphins had learned to discriminate in order to produce the correct responses. However, the dolphins' experience with interpreting human movements was limited to the visual domain. Movements were always produced in the air by a human while the dolphins had their heads above the surface of the water and oriented toward the human. Consequently, the dolphins learned to discriminate these movements visually. Prior to this study, the dolphins had no experience with humans producing movements underwater. This provided a unique opportunity to assess the dolphins' ability to relate information they had learned in the visual modality with information they subsequently obtained from echolocation.

Method

Subjects

Three adult female bottlenose dolphins participated in the present study. These dolphins were housed at MarineLife Oceanarium in Gulfport, Mississippi. Although each dolphin had previously learned to discriminate and correctly respond to many human movements that they perceived visually, none of the dolphins had been asked to respond to human movements produced underwater prior to their participation in this study. Nor had any of the dolphins participated in any research involving echolocation prior to this study.

Apparatus

A portable underwater screen was used to test the dolphins' ability to discriminate movements using echolocation. The screen consisted of an aluminum frame and a lexan panel approximately 6' high, 4' wide, and ½" thick. The screen was positioned below the surface of the water so that a human diver could be stationed on one side of the screen while a dolphin oriented towards the diver from the other side of the screen.

Target movements

Five target movements were selected because they involved relatively large movements, which we believed would increase the possibility that the dolphins would be able to process the movement information using echolocation. Each of the five movements that were selected asked the dolphins to perform distinct behaviors. The *bow* movement informed the dolphin to leap above the water in a particular manner. The *hula* movement informed the dolphin to position itself vertically in the water and spin in a circle. The *bubbles* movement informed the dolphin to produce underwater bubbles by exhaling through its blowhole. The *yes* movement informed the dolphin to nod its head as if it were answering yes to a question. Finally, the *wave* movement informed the dolphin to orient itself vertically in the water and move its pectoral fins back and forth as if it were waving goodbye. Each of these movements was used in dolphin shows, and each dolphin had considerable experience with each of these movements in the above water context.

Procedure

The first step involved desensitizing the dolphins to the portable underwater screen. Desensitization was important because dolphins sometimes avoid novel objects, and we wanted the animals to become comfortable with this aspect of the testing situation. Desensitization was accomplished by placing the screen in the animals' pool for brief periods of time (approximately 5-10 minutes per desensitization session). Ten desensitization sessions proved sufficient to familiarize the dolphins with the screen. By the end of these sessions, the dolphins neither approached nor avoided the screen more often than other changeable aspects of their environment, suggesting that it was no longer a novel stimulus.

After the dolphins had been desensitized to the screen's presence, human divers began to present underwater movements to the dolphins. These movements were ones that the dolphins had already demonstrated that they were able to interpret when the movements were seen in the air. The presentation of underwater movements involved placing the screen in the pool, positioning a diver on one side of the screen, and then sending a dolphin to look at the diver through the screen. In this phase and all subsequent phases, dolphins were tested individually. Initially, the diver produced movements for the dolphin to interpret while the diver was located behind a visually transparent screen. This aspect of the training procedure allowed the dolphins to use the same modality (vision) to interpret movements that they had learned to use in a different context (in air rather than under water). Each dolphin quickly learned to respond correctly to the human's underwater movements, all three dolphins achieving 90 – 100 % correct performance within four sessions. The *bow*, *hula*, and *bubbles* movements were used during this aspect of the training procedure.

Once a dolphin had demonstrated that she could reliably interpret underwater movements visually, the concept of using echolocation in this context was introduced to the dolphin. In order to familiarize the dolphins with the novel notion of interpreting human movements using echolocation rather than vision, the transparent screen was gradually made

darker until it was completely visually opaque. This transitional phase provided the dolphins with the knowledge that the diver was producing movements behind the screen even when the dolphin could not see the diver, and resulted in the dolphins learning to use echolocation rather than vision during this task, as evidenced by the dolphins' increased production of clicks during the transition phase. The *bow*, *hula*, and *bubbles* movements were used to help the dolphins learn to use echolocation during this process.

The testing phase began once the dolphins were reliably producing echolocation clicks. The opaque screen was used exclusively during testing sessions. Each animal was tested with the five individual human movements described above, each of which the dolphin had consistently responded to correctly when the movements were produced above water and visible. Comparing how the dolphins reacted to individual movements produced in two different domains allowed us to better determine each dolphin's ability to relate information that it had obtained through vision with that it had obtained via echolocation. More specifically, we were able to compare a dolphin's ability to interpret movements it had learned by watching humans above the surface of the water and in air (which required processing visual information) with its interpretation of movements that were produced underwater and behind an opaque barrier (which required processing information obtained via echolocation). In addition, we were able to compare the dolphins' interpretation of movements that it had never before had to interpret underwater (*yes* and *wave*) with those with which it had previous underwater visual experience and underwater echolocation experience (even though both types of experiences were limited, particularly those involving echolocation in a non-visible condition).

Target movements were presented in a random order constrained only by the fact that each dolphin was to receive a total of fifty target movements (each of the five target movements on ten separate occasions). Each test trial began with the dolphin positioning itself on the side of the screen opposite the human diver. Once the dolphin was orienting on the screen, the diver on the opposite side produced a target movement. The dolphin's response was ascertained by observers, one of who blew a whistle and rewarded the dolphin with a fish if it had correctly interpreted the target movement.

There were approximately six test trials per session, and a maximum of one test session per day. We opted for short sessions for three reasons. First, it proved easier to maintain each dolphin's interest if individual sessions were relatively brief. Second, shorter sessions made it easier to conduct the sessions. Third, the shorter sessions more closely approximated other training and relationship sessions that occurred at MarineLife Oceanarium.

Results

The results were very straightforward. Given their previous experience with the target movements as visual cues provided by humans in the air, we expected the dolphins to perform well when they were asked to respond to target movements that they could see while watching a human stand on a floating dock. This prediction was confirmed. In fact, two of the dolphins were perfect, responding correctly each of the ten times they were asked to do so for each target movement that was presented in the above water and visible condition. The remaining dolphin made two mistakes, one each for the *yes* movement and the *wave* movement. These results are summarized in Figure 1.

But how did the dolphins perform when asked to respond to the same movements produced by an underwater human hiding behind an opaque screen? As shown in Figure 2, this depended on their previous experience with humans producing the movements underwater. For the three movements that had been used in the training phases (*bow*, *bubbles*, and *hula*), two of the three dolphins were perfect when asked to interpret these movements in the non-visible underwater condition, and the other dolphin made one mistake. Correct responses were less common for the two movements (*wave* and *yes*) that the dolphins had never observed humans performing underwater prior to the underwater non-visible test situation than was the case for the three movements that had been used in the training phase, although this difference was not statistically significant ($t(2) = 2.29$, ns).

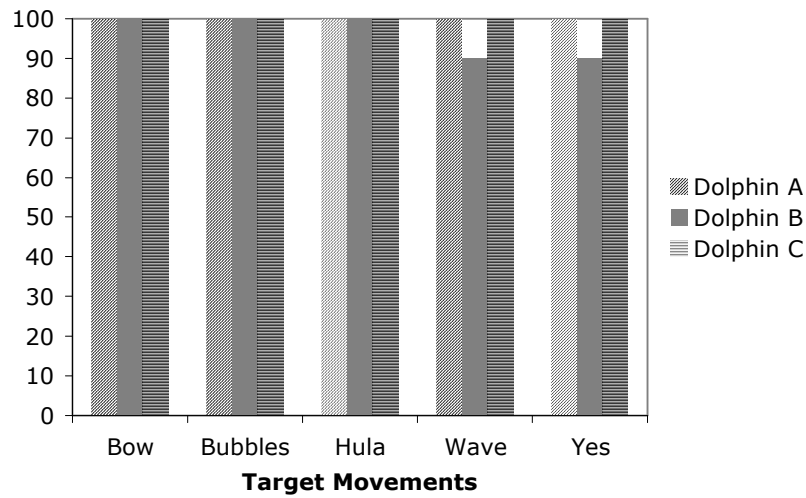


Figure 1. Correct responses to target movements in visible above water condition for each dolphin.

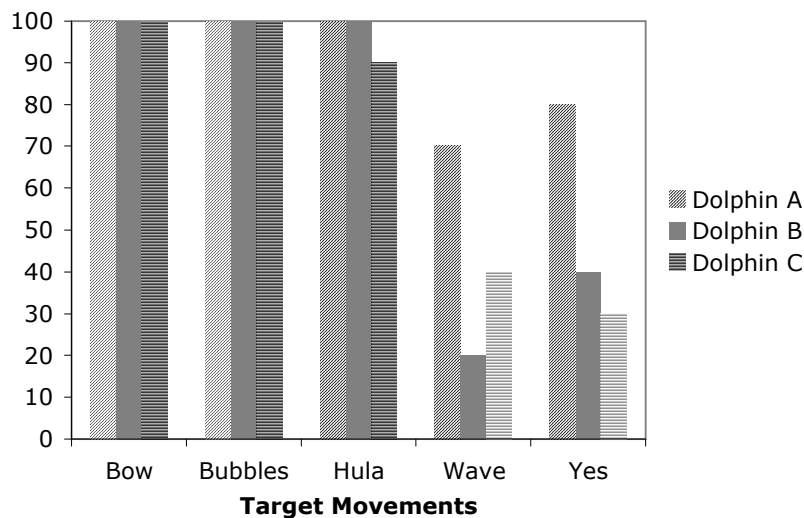


Figure 2. Correct responses to target movements in non-visible underwater condition for each dolphin.

Given the near perfect performances for all target movements in the visible condition, it is not surprising that statistical comparisons yielded no significant differences for either dolphins or target movements in this condition. Similarly, the dolphins' correct responses in the non-visible underwater condition for the three target movements (*bow*, *bubbles*, and *hula*) that were used in training were also near perfect, and consequently there were no significant differences in the dolphins' correct responses to these three target movements in the visual condition compared to the non-visible condition. Moreover, binomial tests demonstrated that the dolphins performed well above chance for each of these three movements in each condition.

Specifically, each dolphin responded correctly to the *bow* movement significantly more often than she responded incorrectly, both in the visual condition ($p < 0.001$ for each dolphin) and in the echolocation condition ($p < 0.001$ for each dolphin). The same pattern held for the *bubbles* movement. Each dolphin responded correctly to the *bubbles* movement significantly more often than she responded incorrectly, both in the visual condition ($p < 0.001$ for each dolphin) and the echolocation condition ($p < 0.001$ for each dolphin). The *hula* movement yielded similar results. Each dolphin responded correctly to the *hula* movement significantly more often than she responded incorrectly, both in the visual condition ($p < 0.001$ for each dolphin) and the echolocation condition ($p < 0.001$ for dolphins A and B, and $p < 0.01$ for dolphin C).

The dolphins did not fare so well with target movements in the non-visible underwater condition if they had never witnessed a human producing the movements in an underwater environment prior to the test condition. The dolphins were less likely to produce correct responses for the *wave* movement in the non-visible underwater condition than in the visible above-water condition ($t(2) = 3.26, p < 0.05$). They were also less likely to produce correct responses for the *yes* movement in the non-visible underwater condition than in the visible above-water condition, although this difference did not achieve statistical significance ($t(2) = 1.85, ns$). Moreover, only dolphin A achieved a success rate of over 50% with either of these movements (and she did so for both target movements). Although binomial tests demonstrated that the dolphins performed well above chance for each of these two movements in the visible condition, such was not the case for the non-visible underwater condition. Specifically, each dolphin responded correctly to the *wave* movement significantly more often than she responded incorrectly in the visual condition ($p < 0.001$ for two dolphins and $p < 0.01$ for the other dolphin). The same pattern held for the *yes* movement. However, none of the dolphins responded correctly significantly more often than they responded incorrectly to these target movements in the non-visible underwater condition (dolphin A did respond to the *yes* movement correctly eight out of ten times, but this performance did not reach statistical significance, $p < 0.055$).

Discussion

Our results demonstrate that dolphins can respond to underwater movements that they cannot see, at least in the case of movements that they have previously experienced visually. This suggests that dolphins are able to relate dynamic information obtained through echolocation with similar information obtained visually. This ability has been demonstrated for static objects (Harley, 2004; Harley & DeLong, 2008; Harley et al., 2003; Harley et al., 1996; Herman & Pack, 1992; Herman, Pack, & Hoffman-Kuhnt, 1998; Pack & Herman, 1995; Pack et al., 2002), and the present results suggest that the ability to relate visual information and echolocation information exists for dynamic events as well. The ability to relate actions and objects observed visually with those sensed via echolocation suggests that dolphins represent their world in a way that makes cross-modal comparisons possible and meaningful. For example, echolocation and vision are both thought to be important for finding and capturing prey (Au, 1993; Barrett-Lennard, Ford, & Heise, 1996; Purves & Pilleri, 1983; Rossbach & Herzing, 1997), and the

ability to integrate information from the two senses most likely maximizes dolphins' predatory efficiency and success. The ability to discriminate and recognize objects echoically and visually facilitates prey detection and selection, and the ability to discriminate, recognize, and anticipate actions in both modalities is a crucial component of successful prey capture and predator avoidance.

Research in recent years has resulted in significant increases in the understanding of the acoustic characteristics of both echolocation clicks and the subsequent returning echoes (Au, 1993; Au & Hastings, 2008; Au et al., 2008; Houser, Helweg & Moore, 1999; Houser et al., 2005; Moore, 1997; Moore, Dankiewicz, & Houser, 2008), as well as an appreciation of the dolphin ability to eavesdrop on the echoes that result from the clicks produced by another dolphin (Götz, Verfaß, & Schnitzler 2005; Gregg, Dudzinski, & Smith, 2007; Xitco & Roitblat, 1996). However, at the present time we know little about the manner in which information is represented and used by dolphins (Harley, Xitco, & Roitblat, 1995; Popper, Hawkins, & Gisiner, 1997; Roitblat, Helweg, & Harley, 1995; Roitblat, 2004). The best available evidence suggests that dolphins recognize equivalences between their visual representations and acoustic representations of objects, although the exact nature of the equivalences is a matter of some dispute (Au & Hastings, 2008; Harley, 2004; Harley & DeLong, 2004; Harley et al., 1996; Herman et al., 1998; Pack & Herman, 1995; Roitblat et al., 1995).

Similarly, the dolphin ability to integrate dynamic information across the two senses of vision and echolocation likely results from the dolphin's recognition of dynamic equivalences between its visual representations and acoustic representations of actions. In their discussion of the role of imagery in dolphin echolocation, Roitblat et al. (1995) suggested that the object-centered representations that result from dolphin echolocation preserve the structural properties of the object, but noted that these representational images need not be vision-like. We also suspect that the representations of actions that result from dolphin echolocation are not visual in nature.

In his theory of perceptual modalities, Gibson (1966) emphasized that the perceptual systems of organisms have evolved to take advantage of the information that exists in the physical world, supposedly because that information is important for the survival of the species. He also suggested that the identification of perceptual stimuli precedes decisions about actions to be taken. Or, in Gibson's words, "what is it?" comes before "what to do about it?" (p. 148). This idea is relevant to our discussion in that dolphins most likely decide what something is before they decide what actions to pursue, regardless of whether the information is obtained via vision or echolocation. Previous experimental work has demonstrated that dolphins can readily identify target objects (Au, 1993; Harley et al., 2003; Houser et al. 2005; Moore et al., 2008), sometimes by eavesdropping on the returning echoes produced by another dolphin (Xitco & Roitblat, 1996), and the present results suggest that dolphins may be able to incorporate an object's movements into decisions about object identity and appropriate reactions to the object, regardless of whether this information comes from information obtained from vision or information obtained from echolocation.

Although our results suggest that dolphins can relate dynamic information obtained via echolocation with that obtained from vision, we

recognize that the results are limited. The dolphins in the present study were only tested with human movements that they could readily interpret visually. Consequently, it is possible that the dolphins' ability to relate dynamic information from echolocation and vision depended on prior visual experience, and that dolphins might prove incapable of integrating echoic information about movements with visual information about similar movements if the echoic experiences preceded the visual ones. Although this possibility needs to be tested by training animals to respond to human movements that they perceive echoically before the dolphins are exposed to the movements in the visual domain, we suspect that dolphins will be able to integrate information from the two modalities regardless of which modality provides the initial experience. This has proven to be the case for static object identification (Harley et al., 1996; Harley & DeLong, 2008; Pack & Herman, 1995; Pack et al., 2002), and there is no reason to believe that cross-modal integration of dynamic information should not occur in both directions as well. Of course, data from future studies will determine if our speculations are correct.

The amount of experience that dolphins need to correctly identify dynamic equivalences across the two modalities is also unclear. Dolphins that have visual experience with stationary objects can sometimes recognize one of the objects the first time they are asked to do so using echolocation, and dolphins that have initial echolocation experience with stationary objects can also sometimes recognize one of the objects the first time they are asked to do so visually (Herman et al., 1998; Pack & Herman, 1995). Nonetheless, experience with objects does facilitate dolphins' ability to match the objects across the two modalities. Harley et al. (1996) reported that "dolphins can cross-modally match familiar objects, and when the objects are easily identifiable, the dolphins can cross-modally match in both directions" (p. 172). They suggested that dolphins may find it difficult to cross-modally match unfamiliar objects because their limited experience with the objects has not resulted in the dolphins' encoding sufficient information about the objects to allow cross-modal comparisons.

The results of the present study suggest that dolphins' ability to integrate information about actions across the modalities of vision and echolocation may also depend on the dolphins' experience with the actions. Although each dolphin had considerable visual experience with all five of the target movements, the dolphins had limited experience with three of the movements in an underwater context prior to testing and no experience with the remaining two movements in an underwater context prior to testing. The previous underwater experiences with the *bow*, *bubbles*, and *hula* movements were sufficient to result in near perfect performances when the dolphins were asked to respond to the movements in the test condition. But the lack of underwater experience with the *wave* and *yes* movements resulted in dramatic decreases in correct responses to these movements for two of the dolphins when they were asked to respond in the test condition. These two dolphins, although near perfect when asked to respond to these movements produced by humans in the visual condition, fared much more poorly in the underwater test condition. Neither of these dolphins produced a correct response the first three times they were exposed to *wave* and the first three times they were exposed to *yes* in the underwater test condition. Evidently, despite their visual experience with these movements, these two dolphins needed echolocation experience

with the movements before they were able to relate the information about these movements that they were obtaining via echolocation with the information about the movements that they had previously obtained from their visual experiences. The remaining dolphin proved much better at interpreting the *wave* and *yes* movements in her initial encounters with the movements in the underwater test condition. She responded correctly the first time that she echolocated on the *yes* movement in the test condition. She also responded correctly the second time that she echolocated on the *wave* movement in the test condition. The ability of this dolphin to relate echoic information about movements with previously acquired visual information about these same movements suggests that dolphins can relate dynamic information from the two modalities even with limited experience in one modality. Future studies that systematically examine the role of experience in each modality with dolphins' ability to relate cross-modal information about actions will increase our understanding of the manner in which dolphins represent and make sense of their world.

The finding that dolphins can integrate information about dynamic events obtained via echolocation with that previously obtained from visual experience is not surprising if we assume that dolphins' sensory and perceptual capacities have evolved in order to enhance their ability to make sense of their world, a world in which both vision and echolocation help dolphins detect and avoid obstacles, detect and recognize prey, predators and conspecifics, and determine actions that should be taken to capture prey, meet and interact with conspecifics, and deter or avoid predators. When dolphins are near the surface of the water and the water is clear, vision may suffice to make such decisions. But when dolphins are in deeper or murkier water, the use of vision becomes limited and so dolphins must then rely on their echolocation skills to interpret their world. Although it is possible that dolphins could use two independent representational systems, one for information gathered via vision and the other for information gleaned from echolocation, the results of previous studies with stationary objects and the results of the present study with dynamic events suggest that such is not the case. Dolphins seem predisposed to integrate information from the two perceptual systems, a predisposition that likely facilitates their ability to gather meaningful information about their world.

But what happens when information from one sense conflicts with information from another sense? Sometimes the information is integrated into a perception that is different from what would have occurred if only information from one sense had been involved. For example, when a video of human visual lip movements suggests that one sound will be produced but another sound actually occurs, an audio-visual illusion known as the McGurk effect occurs (McGurk & MacDonald, 1976). Thus, if an adult human sees a video in which the speaker mouths the sound *ga*, but the soundtrack actually produces a *ba* sound instead, the adult will hear the sound *da* (a similar effect has been observed in human infants, which suggests that the integration of auditory and visual information in speech perception requires little experience; Rosenblum, Schmuckler, & Johnson, 1997). The McGurk effect demonstrates that conflicting information from different modalities sometimes results in an integrated perception that does not accurately reflect what actually occurred in either modality. However, this is not always the case when an organism experiences conflicting information from multiple senses. When human adults

experience a conflict between visual information and tactile information, the conflict is resolved in favor of the visual information (Rock & Harris, 1967; Power & Graham, 1975). For example, when adults view a square shape through a lens that makes it appear rectangular and also examine the square haptically, subsequent tactual matches are more likely to be based on the visual information (rectangular) than on the haptic information (square). Experiments designed to assess how dolphins respond when visual and acoustic information provide conflicting information about objects and/or actions would be beneficial in terms of increasing our understanding of how dolphins relate information from each sense. Would such information be integrated to create a perception that differs from what would result if the information from either sense was considered independently, as happens in the McGurk effect? Or would one sense “win out” over the other, as happens when vision and touch are put in conflict for human perceivers? Clearly, much remains to be done.

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