

ISSN 0889-3667  
IJCP 20(4)317-373 (2007)

# International Journal of Comparative Psychology



Published by the  

---

International Society for Comparative Psychology

Stan Kuczaj, *Editor*

---

2007, Volume 20, Number 4

***Editor:***

Stan Kuczaj  
*Department of Psychology*  
*University of Southern Mississippi*  
*118 College Drive #5025*  
*Hattiesburg, MS 39406, U.S.A.*  
*ijcp.editor@gmail.com*

***Editorial Advisory Board:***

Michael J. Beran, *Georgia State University, U.S.A.*  
Aaron P. Blaisdell, *University of California at Los Angeles, U.S.A.*  
Daniela Brunner, *PsychoGenics and Columbia University, U.S.A.*  
Jonathon D. Crystal, *University of Georgia, U.S.A.*  
Nancy K. Dess, *Occidental College, U.S.A.*  
Fabiana Kubke, *University of Auckland, New Zealand*  
Suzanne E. MacDonald, *York University, Canada*  
Lori Marino, *Emory University, U.S.A.*  
Helena Matute, *Deusto University, Spain*  
Roger Mellgren, *University of Texas at Arlington, U.S.A.*  
Alba E. Mustaca, *University of Buenos Aires, Argentina*  
Sadahiko Nakajima, *Kwansei Gakuin University, Japan*  
Sergio M. Pellis, *University of Lethbridge, Canada*  
Irene Pepperberg, *Harvard University, U.S.A.*  
Steve Reilly, *University of Illinois at Chicago, U.S.A.*  
Todd R. Schachtman, *University of Missouri, U.S.A.*  
Roger K. R. Thompson, *Franklin and Marshall College, U.S.A.*  
Masaki Tomonaga, *Kyoto University, Japan*

***Editorial Assistants:***

Kelly Caffery and Lauren Miller

# International Journal of Comparative Psychology

2007, Volume 20, Number 4

---

Copyright © 2007 by the International Society for Comparative Psychology

## Research Articles

- 317 Temporal Organization of Eating in Low- and High-Saccharin-Consuming Rats  
*Nancy K. Dess, Jocelyn M. Richard, Susan Fletcher Severe, and Clinton D. Chapman*
- 341 The Role of Visual Cues in the Comprehension of the Human Pointing Signals in Dogs  
*Gabriella Lakatos, Antal Dóka, and Ádám Miklósi*
- 351 A Comparative Analysis of the Preference for Dark Environments in Five Teleosts  
*Caio Maximino, Thiago Marques de Brito, Fernanda Dias de Moraes, and Flávia Volta Cortes de Oliveira, Iza Batista Taccolini, Patrícia Mendes Pereira, Rafael Colmanetti, Rafael Lozano, Rangel Antônio Gazolla, Raquel Tenório, Renata Inah Tavares de Lacerda, Silvana Terume Koshikene Rodrigues, Soraia Valéria de Oliveira Coelho Lameirão, Alvaro Antonio Assis Pontes, Cynthia Ferreira Romão, Vitor Miranda Prado, and Amauri Gouveia Jr.*

## Brief Report

- 368 A Brief Report: The Use of Experimenter-Given Cues by South American Sea Lions  
*Lauren E. Highfill, Harald Schwammer, and Stan A. Kuczaj*
-

***The International Journal of Comparative Psychology*** is sponsored by the International Society for Comparative Psychology, an affiliate of the International Union of Psychological Sciences. In consonance with the goals and activities of the Society, it publishes studies on the evolution and development of behavior, broadly defined, and in all animal species; on the use of the comparative method for the understanding of behavioral processes; and the examination of the adequacy of psychological and evolutionary theories. It accepts for review articles that deal with historical and contemporary relationships between humans and other animals that elucidate problems of ecological and behavioral processes in resource management that explicate fundamental concepts about human evolution, and that present research results and develop theories about the development and evolution of behavior. The Editor is elected by the Operations Committee of the Society. The Editorial Advisory Board is appointed by the Editor and the Operations Committee.

***Manuscripts*** can only be submitted electronically (MS Word, preferred). The *IJCP* adheres to APA format (see inside back cover for further details).

***Copyright*** © 2007 by the International Society for Comparative Psychology.

***Published quarterly.***  
ISSN 0889-3667

IJCPE820(4) 317-373(2007)

## **Temporal Organization of Eating in Low- and High-Saccharin-Consuming Rats**

**Nancy K. Dess, Jocelyn M. Richard,  
Susan Fletcher Severe, and Clinton D. Chapman**  
*Occidental College, U.S.A.*

When, where, and how much animals eat are influenced by food scarcity and risk of predation. The present study concerned the mediation of risk-related feeding patterns by emotion. Occidental Low-saccharin-consuming (LoS) and High-saccharin-consuming (HiS) rats, which differ in both ingestion and emotionality, were studied in three steady-state paradigms: an “open economy” procedure (discrete session cyclic-ratio operant schedule) and two “closed economy” procedures (meal patterning, free feeding with running wheel access). Cyclic-ratio performance showed better defense of stable food intake against variable cost among LoS rats. In closed economies, LoS rats consumed a larger number of smaller meals and showed a more pronounced circadian rhythm in meal initiation and running than HiS rats. Taste finickiness appears to serve as a marker for heightened cross-modal risk reactivity, the expressions of which include tighter behavioral regulation of eating in conditions of scarcity and exaggerated nocturnality.

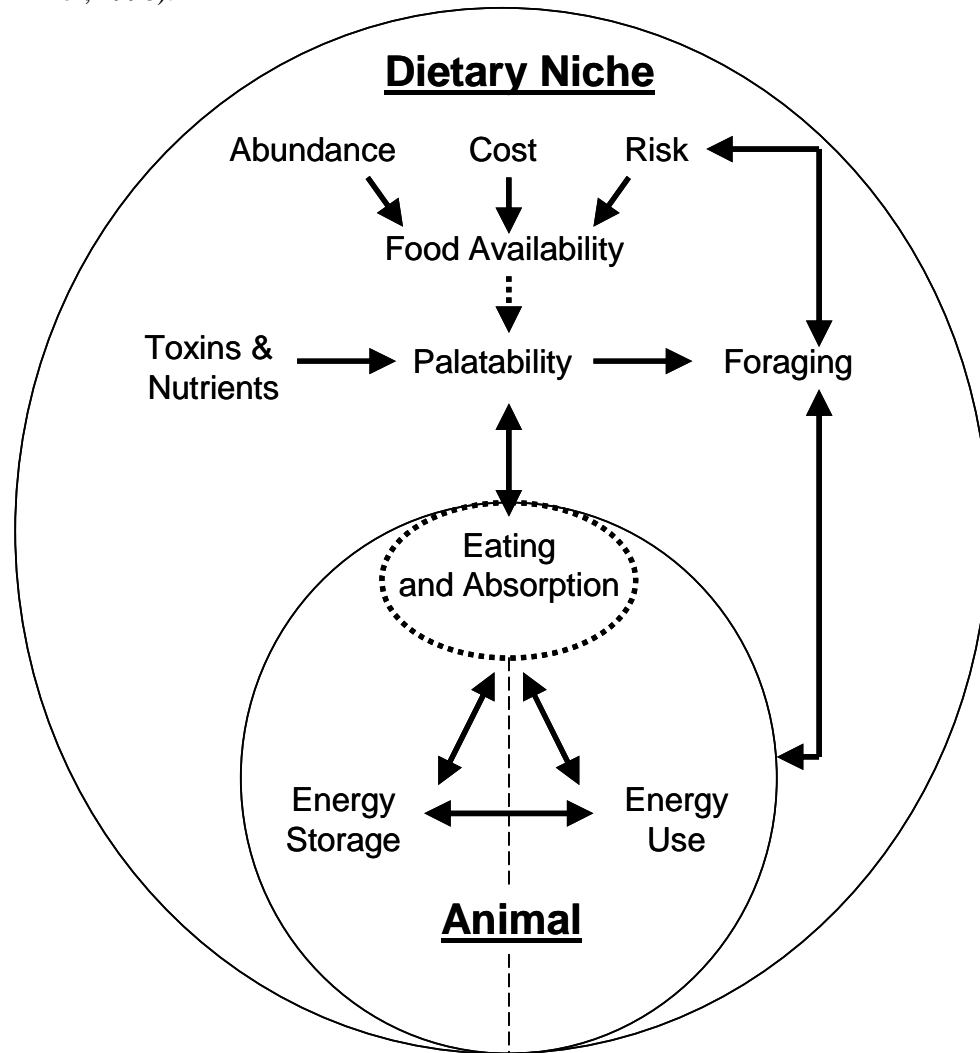
### **Temporal Organization of Eating in Low- and High-Saccharin-Consuming Rats**

For many species, eating poses a high-stakes dilemma. On one hand, animals must eat or they die. On the other hand, eating can be risky because food can be toxic and because procuring or consuming food exposes the animal to environmental dangers. This dilemma is particularly acute for opportunistic omnivores who are both predator and prey, such as rats and humans (Agrawal, 2003). For them, food selection and the organization of eating in time and space are shaped by myriad mutually constraining internal and external variables.

Figure 1 schematically represents such an animal in an ecological context (from Dess, 1991). The figure depicts two interfaces between internal processes comprising energy balance and external variables comprising the dietary niche. “Eating and absorption” mark, respectively, the mouth and gut, each of which is a semi-permeable barrier between the animal and its environment. “Foraging” marks the interface between the niche and the animal’s molar behavior within it. Processes operating at these interfaces influence, and are influenced by, variables on both sides of the interface. Rather than emphasizing the distinctiveness of inner and outer spaces (e.g. *gut defense* versus *skin defense*, Garcia, 1989), this model integrates them. Evidence concerning many of the relationships is presented in Dess (1991), as is the argument that negative affect can be usefully conceptualized in terms of shifts in energy regulation. For instance, one adaptive response to a fresh whiff of lurking predator is to hide and meet metabolic needs by utilizing calories stored as glycogen or fat rather than venturing out to forage. From this

This work was supported by a Howard Hughes Biomedical Institute grant to the Occidental College Undergraduate Research Center and by the Dean of the College. Their support is gratefully acknowledged, as is the invaluable assistance of Roberto Pelayo and Jenny Alonzo with data collection. Correspondence concerning this article should be addressed to Nancy K. Dess, Department of Psychology, Occidental College, 1600 Campus Road, Los Angeles, CA, 90041, U.S.A. (dessnk@oxy.edu).

perspective, short-term weight loss after exposure to a stressor reflects an orderly shift in energy regulation rather than dysregulation (Dess, 1997; Dess, Choe, & Minor, 1998).



**Figure 1.** Schematic representation of an animal in its dietary niche, depicting energy balance, ecological constraints on eating, and some interrelationships. Originally published in Dess (1991, with permission).

Our strategy for examining relationships depicted in Figure 1 centers on rats selectively bred for relatively low versus high saccharin consumption. The phenotypic trait used for selection is intake of 0.1% saccharin solution in a 24-hour two-bottle test as change from a water-only baseline, expressed relative to body weight ( $\Delta\%$ ). As expected (Nachman, 1959), our line difference in saccharin intake emerged by Generation 3 (Dess & Minor, 1996) and stabilized quickly. In samples from Generations 3 through 30 in two laboratories, phenotypes average about  $6\Delta\%$

for LoS rats and 32Δ% for HiS rats (Carroll, Morgan, Anker, Perry, & Dess, 2008). LoS rats also consume less starch, salt, and sugar solution than do HiS rats; they also reject sucrose solution adulterated with quinine, but not with citric acid, at lower adulterant concentrations than do HiS rats (Dess, 2000). Body weight and baseline food and water intake do not consistently distinguish the lines.

The line differences go beyond gustation *per se*. Relative to HiS rats, LoS rats display more aversive taste reactivity to saccharin only after some experience with it (Thiele, Badia Elder, Kiefer, & Dess, 1997), are less prone to intravenous cocaine self-administration (Carroll, Morgan, Lynch, Campbell, & Dess, 2002), and eat less in response to rapid-onset hypoglycemia (VanderWeele, Dess, & Castonguay, 2002). Phenotypic correlates also include behaviors bearing no obvious relationship to appetitive motivation. Relative to HiS rats, LoS rats score higher on several measures of emotional reactivity including defecation in a novel open field (Dess & Minor, 1996), acoustic startle amplitude (Dess et al., 2000), ethanol withdrawal (Dess, O'Neill, & Chapman, 2005), and stress-induced hypoalgesia (Dess et al., 2000).

Line differences observed to date demonstrate a link between the saccharin phenotype and affective processes that are not unique to taste. What sort of linkage might it be? Possibilities include:

- genetic linkage, i.e. multiple genes in close chromosomal proximity are expressed as the selection phenotype and as its correlates;
- pleiotropism, i.e. one gene has several seemingly unrelated phenotypic expressions; and
- functional linkage, i.e. two or more phenotypic correlates play related roles in a *behavioral system* (or systems) comprised of spatiotemporally coordinated sensory, affective, and action-generating processes, such as eating and defense.

The present study focuses on functional linkage. Functionally connecting the saccharin selection phenotype to startle, defecation, withdrawal, and hypoalgesia presents a challenge: The stimuli, contexts and behaviors involved apparently have little in common, differing in sensory modality and locus (external, interoceptive), time scale (milliseconds to hours), and type of motor response (reflexive, consummatory, smooth versus striated muscle). However, the stimuli and situations do all have an aversive component, and the responses are all defensive. Thus, the diverse stimuli and responses could be inputs to or outputs from a *risk reactivity* mechanism sensitive to qualitatively different kinds of risk – risk associated with toxins, metabolic imbalance, dominant conspecifics, and predators. This pattern of phenotypic covariation in LoS and HiS rats may derive from differential risk reactivity, with LoS rats generally reacting as if risks are greater.

Risk reactivity as we conceive of it has counterparts in the human literature, such as *neuroticism*, *negative emotionality*, *trait pleasure*, and *negative affectivity* (Eysenck, 1979; Patrick, Curtin, & Tellegen, 2002; Russell & Mehrabian, 1977; Watson & Clark, 1984). The closest parallel may be with negative affectivity (NA), the disposition to experience aversive emotional states. Individuals high on NA show heightened, persistent reactivity to stressors; thus,

they experience more negative affect than do low NA individuals even under steady state conditions due to the lingering effects of past upsets.

Global affective constructs gave some ground over the last 20 years to more specific emotional modules (e.g. fear versus disgust, Lawrence, Murphy, & Calder, 2004). However, global constructs – e.g. *undifferentiated negative affect* and *core affect* – are again proving valuable in the study of temperament, emotion, and stress vulnerability (Clark, 2005; Nemanick & Munz, 1997; Reich & Zautra, 2002; Russell & Barrett, 1999). In models that integrate an affective valence factor (positive/negative) with specific emotion circuits, the former is more basic, generic, and evolutionarily prior. Generalizing across species with respect to emotion is tricky (Davidson, 2002). Given that it can be valid, risk reactivity is the sort of construct likely to apply across species who either are closely related or have confronted similar ecological problems, and comparative evidence supports this view (Gosling, 2001). We prefer the term risk reactivity to similar terms because *risk* is referential and ecologically grounded.

A domain in which risk reactivity clearly should be expressed is foraging. To the extent that LoS and HiS rats respond differently to external or internal signals for risk, they should forage differently. Pathways to and from “Foraging” in Figure 1 represent functional relationships that should distinguish the two lines. Two experiments to date bear on that prediction. In an early one (Dess & Minor 1996), LoS rats showed greater suppression of homecage food intake by a stressor than did HiS rats, an effect consistent with a regulatory shift toward utilization of stored nutrients. Later, we reported in this journal (Dess et al., 2000) that deprivation-induced hyperactivity (DIH) is greater among LoS rats. When fed one hour per day for two days, LoS rats run more than twice as much in a wheel as HiS rats. DIH arguably is an experimental foraging paradigm (Epling & Pierce, 1996): Severely limited access to food models famine, and the excessive activity it generates models migration in search of a richer food supply (Fessler, 2002; Guisinger, 2003). In terms of Figure 1, depletion of energy stores and increased energy utilization stimulate greater activation of general search behavior (Timberlake, 1984) in LoS rats. In both of those experiments, however, inferences about foraging are necessarily weak because eating was not measured with any precision. The purpose of the present study was to look more directly at eating regulation in LoS and HiS rats with food-rewarded operant procedures.

Food-rewarded operant procedures may be grouped into two general categories. In discrete session (or *open economy*) procedures, rats earn some of a daily food ratio in relatively short (<1 hr) daily sessions. In meal patterning (or *closed economy*) procedures, rats live and earn all of their food in the operant chamber. As noted by Staddon (1979), many of the quantitative relationships between procurement and food intake are the same in discrete and 24-hr sessions. Similarities should be expected given that both procedures require animals to regulate food intake as its cost varies. And, after all, session length and proportion of daily food earned in it are continuous experimental variables; they hardly could be behaviorally discontinuous.

From a behavioral systems perspective, though, the two types of procedure as usually implemented differ in important ways (Collier & Johnson, 1997;

Timberlake & Silva, 1995). For present purposes, the critical difference is overall food availability. In a typical discrete session procedure, rats are chronically food deprived. This experimental practice is ecologically valid: Wild cousins of laboratory animals do encounter and adapt to food scarcity (Holeckova & Chytil, 1963; Poling, Mickel, & Alling, 1990). However, eating by very hungry animals in a short discrete operant session arguably constitutes one meal, with the experimenter controlling when the meal starts and, by selecting conditions to avoid satiation, when it ends (Collier & Johnson, 1997). This type of procedure therefore can reveal little about how rats organize their eating into meal-eating bouts on a daily basis. (See Houston & McNamara, 1989, for a defense of open economies as foraging models.)

In a typical meal-patterning procedure, on the other hand, rats can earn all the food they want and maintain body weight except when adverse procurement schedules are used. They regulate meal initiation and termination and allocate time to other activities over the dark/light cycle, within constraints imposed by the experimenter. As a result, closed economies usually do not generate “the increase in response rate, ingestion rate, or general activity that is associated with increasing intermeal intervals in deprived or depleted animals” (Collier & Johnson, 1997, p. 168). A typical closed economy procedure has its limits. When standard operant chambers are used, alternative activities are few, and some components of the behavioral system (general search, stalking, food handling) are either not engaged or not measured. Compared to a typical discrete-session procedure, however, the organization of eating in time can be expressed more fully.

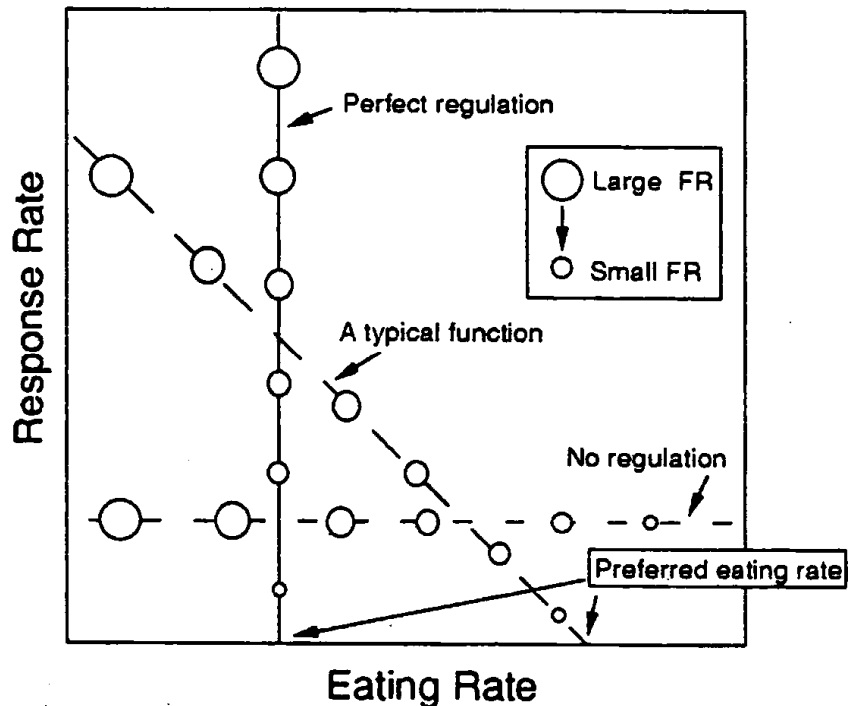
The studies of eating regulation described here employed both types of operant procedure. In Experiment 1, a powerful discrete session operant schedule was used – the cyclic ratio schedule. Meal patterning (Experiment 2) was examined in 23.5-hr sessions of continuous reinforcement. Results from the Experiment 2 led us to revisit activity and eating in running wheels as a foraging paradigm (Experiment 3). Differences between LoS and HiS rats in steady-state performance in these paradigms were assessed to further explore some of the hypothetical relationships depicted in Figure 1.

### **Experiment 1**

The cyclic ratio schedule of reinforcement provides a means of distinguishing the roles in eating of energy balance and the incentive value of the reinforcer (Ettinger & Staddon, 1983; Staddon, 1979). In this schedule, several fixed ratio (FR) schedules occur in ascending and descending orders in strict alternation. An ascending and descending series comprise a cycle, and several cycles comprise a session. When average response rate on each schedule is plotted against the corresponding eating rate (pellets earned per minute on that schedule), the parameters of the best-fit function correspond to different aspects of meal eating (see Figure 2, from Dess, 1997). The slope indexes the degree to which eating is defended against variation in the work requirement, or *behavioral regulation*. The *x*-intercept – the point corresponding to an instrumental response rate of 0 – is the hypothetical eating rate for free food, or the *preferred eating rate*.

The elevation of the function (y-intercept) indexes *reinforcer quality*, with a higher value indicating higher quality.

Several idealized patterns of cyclic ratio performance are shown in Figure 2. A hypothetical rat generating a vertical best-fit function earns pellets at the same rate whatever the schedule of reinforcement, i.e. perfectly regulates eating rate. Shallower functions indicate more variation in eating rate, with maximum variation occurring when the response rate is constant.



**Figure 2.** Idealized best-fit functions for cyclic ratio performance when response rate (bar presses/min) on fixed ratio schedules is plotted against eating rate (pellets/min). *Perfect behavioral regulation* refers to a constant eating rate (vertical function). *Preferred eating rate* refers to the hypothetical rate of consumption of free pellets (response rate equals 0). Originally published in Dess (1997, with permission).

Exposure to a long series of unpredictable, uncontrollable tailshocks reduces food intake in the homecage (Dess, Minor, & Brewer, 1989). We used the cyclic ratio schedule to assess the effect of that stressor on specific eating parameters (Dess, 1997). Consistent with reduced intake of free food in the homecage, preferred feeding rate ( $x$ -intercept) decreased from baseline; schedule control ( $r$ ) was the same as during training, and behavioral regulation *improved* (steeper slope). In the aftermath of an encounter with a stressor, then, rats were not “dysregulated”: They regulated pellet consumption better than before stress, albeit around a lower preferred rate.

Steady state cyclic ratio performance of LoS and HiS rats was examined in Experiment 1. LoS rats are more reactive to metabolic challenges including

hypoglycemia (VanderWeele et al., 2002) and restricted access to food (Dess et al., 2000). Thus, the chronic food deprivation required for stable performance on this schedule should constitute a greater stressor for LoS rats than for HiS rats. In the cyclic ratio paradigm, LoS rats' greater risk reactivity should be expressed as tighter behavioral regulation of eating (steeper slope) and a lower preferred eating rate (lower  $x$ -intercept) relative to HiS rats.

## Method

**Animals.** The rats were 48 experimentally naïve adult female and male LoS and HiS rats from, respectively, six and seven litters of Generation 22 ( $n_s = 12$ ) (see Carroll et al., 2008, for selective breeding information). Initial body weights differed between females and males,  $F(1, 38) = 255.33$ , but not between lines (see Table 1). They were maintained at 85% of their pre-experimental weight with earned pellets and supplemental feeding of Purina 5001 rodent chow. They were housed individually on a 12:12 hr dark/light cycle, with light onset at 7:00 a.m. Care and use of the rats in this and the following experiments complied with institutional policies.

**Apparatus.** Sessions occurred in six computer-controlled standard operant chambers equipped with a lever and pellet dispenser. Each chamber was housed in a sound-attenuating box illuminated by a 7-w houselight. A ventilation fan provided some masking noise. The reinforcer was a nutritionally complete 45 mg pellet (#0021, Bioserv Inc., Frenchtown NJ).

**Table 1**

*Initial Body Weight and Parameters of Best-Fit Lines for Cyclic-Ratio Performance Among Female and Male Rats in the LoS and HiS Lines (Mean  $\pm$  SEM).*

	<u>LoS Females</u> ( $n = 11$ )	<u>LoS Males</u> ( $n = 11$ )	<u>HiS Females</u> ( $n = 9$ )	<u>HiS Males</u> ( $n = 11$ )
Initial body weight (g)	278 $\pm$ 12	475 $\pm$ 10	281 $\pm$ 9	488 $\pm$ 16
Slope ( $\Delta$ resp/pellet)**	-3.1 $\pm$ 0.5	-2.5 $\pm$ 0.6	-1.0 $\pm$ 0.3	-1.4 $\pm$ 0.3
$x$ intercept (pellets/min)*	45 $\pm$ 9	66 $\pm$ 21	120 $\pm$ 60	146 $\pm$ 30
$y$ intercept (resp/min)	102 $\pm$ 7	110 $\pm$ 10	85 $\pm$ 11	116 $\pm$ 11
$r$ (schedule control)	0.76 $\pm$ 0.05	0.71 $\pm$ 0.06	0.63 $\pm$ 0.06	0.64 $\pm$ 0.06

\* LoS  $\neq$  HiS,  $p < .05$

\*\* LoS  $\neq$  HiS,  $p < .01$

**Procedure.** Each rat received one operant session per day between 1:00 and 4:00 p.m. Supplemental feeding occurred 1 hr later. Rats received shaping followed by several sessions which progressed from FR1 to FR10 before cyclic ratio training began. Initial components of the cyclic ratio schedule were FR2, 4, 8, 16, 32, and 64. After a week on this schedule, four rats showed ratio strain at FR64 (two HiS males, two LoS males) and were shifted to a schedule comprised of FR1, 2, 4, 8, 16, and 32, which all completed. (Barring strain on high ratios, cyclic ratio performance parameters should be insensitive to the ratio values comprising the schedule.) After an additional week of training, six rats failed to meet a modest criterion for schedule control ( $r \geq .30$ ; three HiS females and one rat in each of the other three groups) and were eliminated from the study. Final group size was  $n = 9$  for HiS females and  $n = 11$  for the other groups.

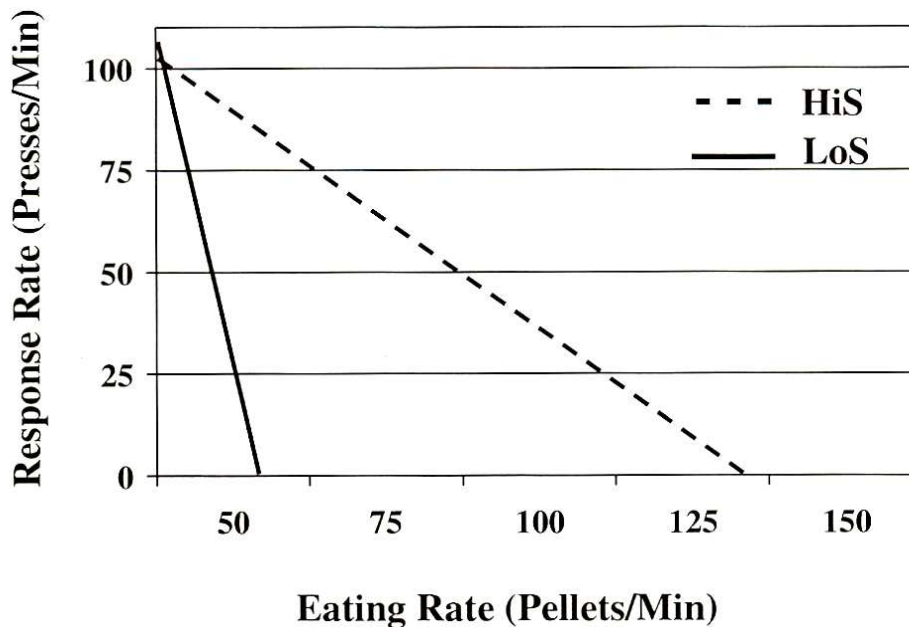
Rats earned 72 pellets (3.24 g of food) in each session. All 42 rats who met the schedule control criterion completed the last three training sessions quickly (less than 30 min) and consumed all of the pellets earned.

**Statistical analyses.** Mean response rate on each of the six FR schedules for each rat was

averaged over the last three training sessions. Dividing the mean response rate (bar presses/min) on each schedule by the schedule's value yielded the corresponding eating rate (pellets/min). Linear regression was performed on each rat's data ( $x$  = eating rates,  $y$  = response rates) to obtain four best-fit function parameters: slope (eating rate stability),  $x$ -intercept (preferred eating rate),  $y$ -intercept (reinforcer quality), and  $r$  (schedule control). Each parameter was subjected to a two-way analysis of variance (ANOVA) with line (LoS, HiS) and sex (female, male) as variables. Test statistics significant at  $\alpha = .05$  are reported.

## Results

Means and standard errors for each of the four meal parameters are shown by line and sex in Table 1. Best-fit functions were steeper and had lower  $x$ -intercepts for LoS rats than for HiS rats,  $F_s(1, 38) = 11.79$  and  $5.72$ , respectively. Thus, LoS rats' performance more closely approximates an idealized, vertical function depicting an eating rate that is constant regardless of work requirement. LoS rats' preferred eating rate, however, is lower than HiS rats'. No sex differences or line  $\times$  sex interactions were significant. The line differences in slope and  $x$ -intercept are depicted graphically in Figure 3.



**Figure 3.** Data-based best-fit functions for the cyclic ratio performance of LoS ( $n = 22$ ) and HiS ( $n = 20$ ) rats. Average slope and  $x$ - and  $y$ -intercepts were used to plot the function for each line.

Skew, kurtosis, and nonhomogeneity of variance were present for  $x$ -intercepts. Though ANOVA is robust against these characteristics,  $x$ -intercepts were reanalyzed nonparametrically. A Mann-Whitney test comparing confirmed the lower preferred eating rate among LoS rats,  $U(20, 22) = 115.00$ .

## Discussion

LoS rats more strongly defended eating rate across work requirements and had a lower preferred eating rate than did HiS rats. Thus, as predicted, chronically food deprived LoS rats behaved in the cyclic ratio paradigm as if they were responding to a greater stressor than HiS rats (Dess, 1997). In the previous study, tailshocks did not affect  $y$ -intercept, indicating no effect on reinforcer quality; similarly, in Experiment 1, LoS and HiS rats had comparable  $y$ -intercepts. Also, in the previous study, the slope of the best-fit function was only steeper than baseline after 100 tailshocks; neither restraint nor 20 tailshocks produced that effect. Thus, the present results suggest that LoS rats' heightened risk reactivity makes chronic deprivation functionally comparable to a major, rather than a mild or moderate, stressor.

LoS and HiS rats responded at equally high rates on the leanest schedule components, with LoS rats showing graded depression of response rates on richer schedule components. In addition, schedule control was equally high among LoS and HiS rats. LoS rats' instrumental response to a metabolic shortfall, then, is not to press the bar faster overall (to earn food faster) or slower overall (to conserve effort) or to behave erratically. Nor are they simply less hungry than HiS rats, as less severe food deprivation makes the best-fit function shallower (Staddon, 1979). Rather, compared to HiS rats, LoS rats more precisely titrate their effort to the work requirement to maintain a more stable, but lower, eating rate.

If discrete session procedures model single meals (Collier & Johnson, 1998), then it follows from the present results that the pace of eating within a meal should be lower among very hungry LoS rats than HiS rats when food is free, as would be the case if foraging led from a depleted patch to a rich one. This prediction is counterintuitive in the sense that one might expect rats who are more reactive to metabolic emergency to eat faster, not slower. It is, however, reminiscent of prior observations that LoS rats eat less than do HiS rats in response to hypoglycemia produced by fast-acting insulin or 2-deoxy-D-glucose (VanderWeele et al., 2002) and respond to food deprivation with a staggering *increase* in energy expenditure by running (Dess et al., 2000). Also, in the delay-discounting procedure, hungry LoS rats are more willing than HiS rats to tolerate a delay to earn a larger food reward (Perry, Nelson, Anderson, Morgan, & Carroll, 2007); the lines did not differ when cocaine was the reward, implicating energy regulation rather than generic incentive value in the effect. A more definitive answer to the question of whether very hungry foraging LoS rats who encounter free food actually will eat slower than HiS rats awaits further study.

In Experiment 1, females and males did not differ on any cyclic ratio performance parameter. More importantly, line differences in the parameters were statistically the same in females and in males. The line difference also has been essentially the same in females and in males in other studies with both sexes: Differences between LoS and HiS rats in open-field emergence (Dess & Minor, 1996), acoustic startle (Dess et al., 2000), and intake of flavored solutions and ethanol (Dess et al., 1998; Dess, 2000) were not sex specific. While the magnitude of the line difference sometimes differed between sexes, the direction of the

difference and its sensitivity to parametric manipulation were comparable in females and in males. Similarly, in the one study we have published on the taste/emotionality relationship in women and men, no sex differences in that relationship were observed (Dess & Edelhait, 1998). Thus, our studies to date of the relationship of saccharin phenotypes to somatic function provide no *a priori* reason to expect line differences to be unique to females or unique to males.

## Experiment 2

The closed economy meal patterning paradigm has been used to study steady state organization, and reorganization, of eating (Clifton, 1999). In this paradigm, rats live in operant chambers and earn all of their food instrumentally. The distribution of meals in time as well as characteristics of individual meals, including meal size, duration, and pace of eating, are measured. When initiating a meal is made energetically costly with a high meal procurement response requirement, meal frequency decreases and meal size increases (Collier & Johnson, 1998). Another kind of cost to meal initiation is exposure to risk of predation (Lima & Dill, 1990), which has been modeled experimentally with intermittent, unpredictable electric shocks. Like high instrumental procurement cost, exposure to shocks in either another apparatus (Dess & VanderWeele, 1994) or the same apparatus (Fanselow, Lester, & Helmstetter, 1988; Helmstetter & Fanselow, 1993) reduces meal frequency and increases meal size and/or eating rate in a closed economy. In the latter two studies, total food decreased at the highest threat levels. Interestingly, the rats did not lose weight, suggesting that adaptation to an adverse foraging environment included increased energy efficiency.

If rats cope with a risky world by eating fewer meals, LoS rats might be expected to eat fewer, larger meals than HiS rats. Confidence about that outcome was compromised by the features of the closed economy necessary to getting a good look at steady state meal patterning. Put simply, the simulated foraging niche we created in Experiment 2 was not very risky. Food was cheap, unlimited, high quality, and controllable, and by the time steady state data were collected, the situation was familiar and predictable. Perturbations were minimal, more so than in regular vivarium life: Rats lived in sound-attenuating chambers without interruption except for a brief daily maintenance routine that did not include handling. Shock has never been administered to rats in the operant chambers, so stress odors would have been minimal.

This relatively safe world did have night and day. Rats are nocturnal, a lifestyle which reduces risk of predation. Endogenous and light-entrained processes mediate various aspects of nocturnality, including more frequent meal initiation in the dark (Strubbe & Woods, 2004). Light avoidance does not account entirely for reduced meal initiation. However, rats do prefer to forage in darker rather than lighter areas (Arcis & Desor, 2002; Whishaw, Dringenbeg, & Comery, 1992). More generally, risk reactivity is greater in the light than in the dark. In rats, darkness onset reduces several measures of emotionality (Nasello, Machado, Bastos, & Felicio, 1998), and vigilance and acoustic startle are greater in the light than the dark (Bertoglio & Carobrez, 2002; de Jongh, Groenink, van der Gugten, &

Olivier, 2003; Godsil & Fanselow, 2004; Whishaw, Dringenberg, & Comery, 1992). Light enhancement of startle is not due to sensory stimulation *per se* because among humans, who are diurnal, acoustic startle is greater in the dark (Grillon, Pellowski, Merikangas, & Davis, 1997). Moreover, light enhancement and light/dark area choice in rats are attenuated by anxiolytic drugs (Chaouloff, Durand, & Mormede, 1997; Walker & Davis, 2002), implicating anxiety and not general arousal (Frankland & Ralph, 1995) in the effects. The organization of rats' behavior over the circadian cycle is best understood from an ethological perspective, as derived from an affect-modulated system for managing risk associated with time of day.

Because the one unambiguous risk in our meal-patterning protocol was simulated daytime, one prediction follows from the hypothesis that risk reactivity is higher among LoS rats: Relative to HiS rats, LoS rats should show a more exaggerated circadian rhythm in meal patterning. Our hypothesis is not helpful in guessing about the particular aspects of meal eating in which exaggeration should be most apparent. It is helpful to note that the biggest difference between dark and light feeding in rats is in meal frequency. Meal initiation is controlled by satiety signals (Strubbe & Woods, 2004; Zanutto & Staddon, 2007), and reduced satiety appears to be a proximate mechanism for more frequent meal initiation in the dark (Kraly, Cushin, & Smith, 1980). Therefore, LoS rats might be expected to show a larger difference between dark and light in meal frequency and in satiety than HiS rats.

If meal frequency does differ between the lines, meal size also should differ. Homecage food intake does not consistently differ between LoS and HiS rats, and the protocol in Experiment 2 was designed to model unconstrained eating as much as possible given a minimal operant requirement. Thus, if meal frequency differs between lines either overall or in the dark, compensatory differences in meal size should be observed. That is, if LoS rats eat more often, the meals should be smaller, such that total consumption does not differ between lines.

## Method

**Animals.** The rats were 36 experimentally naïve adult male LoS and HiS rats ( $n_s = 18$ ) from, respectively, 11 and 15 litters of Generations 29 and 30. LoS rats were slightly heavier on average than HiS rats initially and when weighed before the saccharin phenotype test a few days post-experimentally, but neither body weight nor weight gain differed significantly between lines (see Table 2). Rats lived in operant chambers on a 12:12 light:dark cycle (lights on at 7:00 a.m.) with access to tap water throughout the study. Rat chow was freely available until the experiment began, after which rats ate pellets earned in the chambers.

**Apparatus.** The same operant apparatus and pellets as in Experiment 1 were used.

**Procedure.** Rats were preexposed to the 45-mg pellets in their homecages on the two days preceding placement in the operant chambers. Rats then were weighed and placed in the operant chambers where they could earn food pellets on a continuous reinforcement schedule (1 pellet per lever press). They remained in the chambers until training was complete. Any rat who did not learn the task the first night was given a supplemental chow feeding and another night to learn the task, which all did.

Daily at approximately 4:30 p.m., pellets and water were replenished, dropping pans were changed as needed, data were collected, and the program was restarted; the maintenance routine took approximately 30 minutes, so rats were free to earn meals for 23.5 hr daily. Meal initiation was defined as at least 10 pellets earned within 10 min, and meal termination was defined as 10 minutes

without a response (Dess & VanderWeele, 1994; Kraly et al., 1980). Military time at each meal initiation, intervals between the end of one meal and initiation of the next meal, meal duration, and number of pellets earned within the meal were recorded. Training continued until meal eating stabilized, defined as the number of meals varying by no more than two meals in three successive 23.5 hr periods. Stabilization occurred in about a week in both lines.

**Statistical analyses.** Meal parameters were averaged over the last three days of training. Parameters analyzed included number of meals and intermeal interval (minutes), meal size (pellets/meal), meal duration (minutes), within meal pace (pellets/min), and total pellets earned. Additional derived measures were analyzed. The first was *satiety ratio*, an index of how long a meal is delayed per pellet consumed in the preceding meal (intermeal interval/meal size; Kraly et al., 1980); a smaller value indicates more transient satiety.

Second, a measure of circadian rhythm was calculated for each meal parameter. To obtain a *dark ratio*, the average value for meals initiated in the dark phase was calculated and divided by the average for 23.5 hr periods. This transformation was used instead of direct comparison of dark phase to light phase values because the latter often would be based on few meals and thus would be unstable. Presence of a circadian rhythm is indicated by a dark ratio different from 0.5 for number of meals and total pellets earned and different from 1.0 for other parameters.

A series of three statistical analyses was performed. First, LoS and HiS groups were compared on the daily average for each meal parameter using independent *t*-tests. The other two analyses concerned circadian rhythm. To determine which of the parameters were, in an absolute sense, robustly sensitive to simulated daylight, the overall dark ratio for each meal parameter was assessed with a one-sample *t*-test (grand mean dark ratio collapsed across lines versus 0.5 or 1.0 as appropriate). Finally, LoS and HiS groups were compared on the dark ratio for each meal parameter using independent *t*-tests. All *ts* significant at  $\alpha = .05$  are reported.

**Table 2**

*Body Weight and Meal Parameters for LoS and HiS Rats (ns = 18, Mean  $\pm$  SEM. Parameters are Shown as 23.5 hr Average (First Line) and Circadian Dark/Light Shift (Second Line).*

	LoS	HiS
Initial body weight (g)	443 $\pm$ 8	428 $\pm$ 6
Post-experimental body weight (g)	483 $\pm$ 7	460 $\pm$ 7
Number of meals *	11.9 $\pm$ 0.5	9.6 $\pm$ 0.7
Dark ratio	0.83 $\pm$ 0.02	0.84 $\pm$ 0.02
Meal size (pellets/meal) *	52.6 $\pm$ 2.4	61.8 $\pm$ 3.5
Dark ratio	1.06 $\pm$ 0.02	1.07 $\pm$ 0.04
Intermeal interval (min)	86.7 $\pm$ 3.9	98.7 $\pm$ 8.2
Dark ratio **	0.78 $\pm$ 0.02	0.97 $\pm$ 0.06
Satiety ratio (IMI/Meal size)	1.67 $\pm$ 0.07	1.62 $\pm$ 0.12
Dark ratio*	0.74 $\pm$ 0.03	0.92 $\pm$ 0.06
Meal duration (minutes)	6.2 $\pm$ 0.6	6.7 $\pm$ 0.4
Dark ratio	1.11 $\pm$ 0.04	1.09 $\pm$ 0.06
Within meal pace (pellets/min)	9.2 $\pm$ 0.6	9.5 $\pm$ 0.5
Dark ratio	0.98 $\pm$ 0.04	1.00 $\pm$ 0.03
Total pellets earned	605.3 $\pm$ 16.0	559.2 $\pm$ 18.9
Dark ratio	0.80 $\pm$ 0.02	0.78 $\pm$ 0.03

\* LoS  $\neq$  HiS,  $p < .05$

\*\* LoS  $\neq$  HiS,  $p < .01$

## Results

### *Daily meal parameter values*

Means and standard errors for each meal parameter in an average 23.5 hr period are shown in Table 2. Compared to HiS rats, LoS rats ate more meals, independent  $t(34) = 2.54$ , and the meals were smaller, independent  $t(34) = 2.15$ . No other meal parameters differed between lines.

### *Parameters showing circadian rhythm*

Dark ratios for each meal parameter also are shown in Table 2. Overall, a circadian rhythm occurred for every meal parameter except pace of eating within a meal. Number of meals, meal duration, meal size, and total pellets earned were higher in the dark phase, one-sample  $ts(35) = 23.84, 2.90, 2.76,$  and  $16.85,$  respectively. Intermeal interval and satiety ratio were smaller in the dark phase, one-sample  $ts(35) = 3.46$  and  $7.78,$  respectively.

### *Line differences in circadian rhythm*

Circadian rhythm was exaggerated among LoS rats by two measures. Dark ratios were smaller for LoS rats than for HiS rats for intermeal interval and satiety ratio, independent  $ts(34) = 2.83$  and  $2.64,$  respectively. Among HiS rats, the dark ratio for those parameters was close to 1.0, indicating that the time between meals was nearly the same in the dark as in the light, in terms of both absolute length and length relative to meal size. LoS rats, on the other hand, initiated successive meals sooner in the dark than in the light, in both absolute and relative senses. The dark/light phase difference was comparable in the two groups for all other meal parameters.

### *Body weight considerations*

Supplemental analyses were conducted to determine whether the nonsignificant body weight difference between lines could account for significant line differences in meal parameters. Correlations of initial and post-experimental body weight with each meal parameter were examined, and meal parameters that distinguished the lines were reexamined with initial or post-experimental body weight as a covariate. Neither body weight measurement correlated with any meal parameter, and line differences in overall number of meals and meal size and dark ratios for intermeal interval and satiety ratio all remained significant when either body weight measurement was used as a covariate. Body weight does not explain the observed line differences in meal patterning.

## Discussion

On average, LoS and HiS rats weigh about the same amount and eat about the same amount of food daily. Given the opportunity, however, they go about

provisioning themselves differently. LoS rats eat more frequently and show a stronger nocturnal pattern than do HiS rats. Specifically, LoS rats initiate meals in faster succession in the dark. Smaller meals do not explain this line difference because the satiety ratio takes meal size into account. LoS rats' relatively shorter intermeal intervals in the dark imply reduced generation of or sensitivity to satiety signals (Kraly et al., 1980). Candidate brain sites include the hippocampus and nucleus accumbens, lesions of which result in more frequent, smaller meals (Clifton & Somerville, 1994; Clifton, Vickers, & Somerville, 1998); potential neurochemical mediators include bombesin, gastrin-releasing peptide, insulin, and neuropeptide Y (Lynch, Hart, & Babcock, 1994; McGowan, Andrew, Kelly, & Grossman, 1990; Strubbe & Woods, 2004). Satiety ratio did not distinguish the lines overall (i.e. 23.5 hr averages), indicating that the mechanisms of the LoS rats' reduced satiety in the dark are synchronized to the circadian cycle. Hypothalamic nuclei such as the suprachiasmatic and paraventricular nuclei may modulate the central or peripheral mediators of the line difference in the temporal distribution of meals (Strubbe & Woods, 2004).

We are disinclined to link LoS rats' higher meal frequency over 23.5 hr periods directly to risk reactivity. The kind of risk to which it might be a reaction is unclear. Their exaggerated circadian rhythm, on the other hand, clearly follows from heightened risk reactivity. This interpretation also is consistent with higher meal frequency in the dark among RLA/Verh rats (Rossi, Driscoll, & Langhans, 1997). Selection on the basis of two apparently dissimilar phenotypes – low saccharin intake and low active avoidance – seems to have yielded rats who score higher on diverse measures of risk reactivity (see also Brush et al., 1988).

Over 23.5 hr periods, LoS rats' meals were smaller than HiS rats' meals. Circadian variation in meal size did not differ between lines. Taken together, these results suggest that the robust difference between lines is the temporal distribution of meals, and that meal size is secondary to meal frequency. That is, regulatory signals such as cholecystokinin or insulin likely reduce LoS rats' meal size to compensate for high meal frequency, such that body weight and total food intake do not differ between lines either in the homecage or in an operant closed economy.

Relatively higher meal frequency in the dark among LoS rats is consistent with results from Roman Low Avoidance (RLA/Verh) rats who, relative to their High Avoidance counterparts (RHA/Verh), are more anxious (Steimer & Driscoll, 2005). Compared to RHA/Verh rats, RLA/Verh rats eat more frequently and have lower satiety ratios (intermeal interval relative to meal size) in the dark phase. That two pairs of independently selected lines of rats distinguished on measures of anxiety differ in these ways strongly implicates anxiety in these aspects of eating regulation.

In contrast to meal frequency and size, within meal pace of eating did not distinguish LoS and HiS rats. Moreover, pace of eating showed no circadian variation. These results concur with Collier & Johnson's (1998) observation that ingestion rate is independent of intermeal interval in closed economies. The results also suggest that the LoS rats' lower preferred eating rate in Experiment 1 was indeed contingent on chronic deprivation.

Other variables also must influence within meal pace of eating. Whereas freely feeding RLA/Verh rats eat more slowly than their RHA/Verh counterparts (Steimer & Driscoll, 2005), LoS rats earn “cheap” food more slowly than HiS rats only when very hungry. Similarities between LoS and RLA/Verh rats probably only go so far, and procedural differences such as level of dietary fat and meal definitions might contribute to the discrepancy (Castonguay, Kaiser, & Stern, 1986). Even if so, puzzles remain. For instance, Whishaw, Dringenberg, & Comery (1992) reported that rats ate faster at night in the colony than during the day in the test apparatus but, within the apparatus, ate *slower* in relatively safe dark areas than in riskier light areas. They attributed the reversal to “time sharing” between surveillance and eating in uncovered areas of the apparatus. For present purposes, the important point is that compared to pace of eating, circadian variation in meal initiation more robustly distinguishes the LoS and HiS lines and their RLA/RHA counterparts.

### Experiment 3

In Experiment 2, LoS showed an exaggerated circadian rhythm in meal initiation. But is nocturnality in general more pronounced among LoS rats? The answer to that question ought not be based on a single instrumental response. Concluding that the pattern of lever-pressing models foraging and reflects the overall activity or quiescence of the rats in dark and light phases requires convergent evidence from another paradigm. In Experiment 3, LoS and HiS rats lived in apparatuses equipped with a running wheel for six days, with food freely available. Wheel running and grams of food intake were measured separately for the daytime hours (9:00 a.m. – 5:00 p.m.) and for the dark and transitional hours (5:30 p.m. – 8:30 a.m.). The question was whether LoS rats would show a more pronounced circadian rhythm in running than HiS rats. In Experiment 2, rats earned more pellets in the dark than in the light, and the lines did not differ in this respect. Thus, no line difference in the circadian rhythm for food intake is predicted.

### Method

**Animals.** Eighteen experimentally naïve adult female LoS and HiS rats from, respectively, four and three litters in Generation 9 were used ( $n_s = 9$ ).<sup>1</sup> Rats lived in running wheels on a 12:12 light:dark cycle (lights on at 7:00 a.m.) with continuous access to food and water.

**Apparatus.** Six stainless steel running wheels (101 cm circumference) with mechanical activity counters and an attached housing compartment (Lafayette Instruments 86041, Lafayette IN) were used. Purina 5001 rodent chow pellets or chow mash (1:1 chow:water) was provided fresh daily in a glass jar with a metal holder, with foil in the bedding tray underneath the jar to collect spillage. A water bottle was attached to the housing compartment.

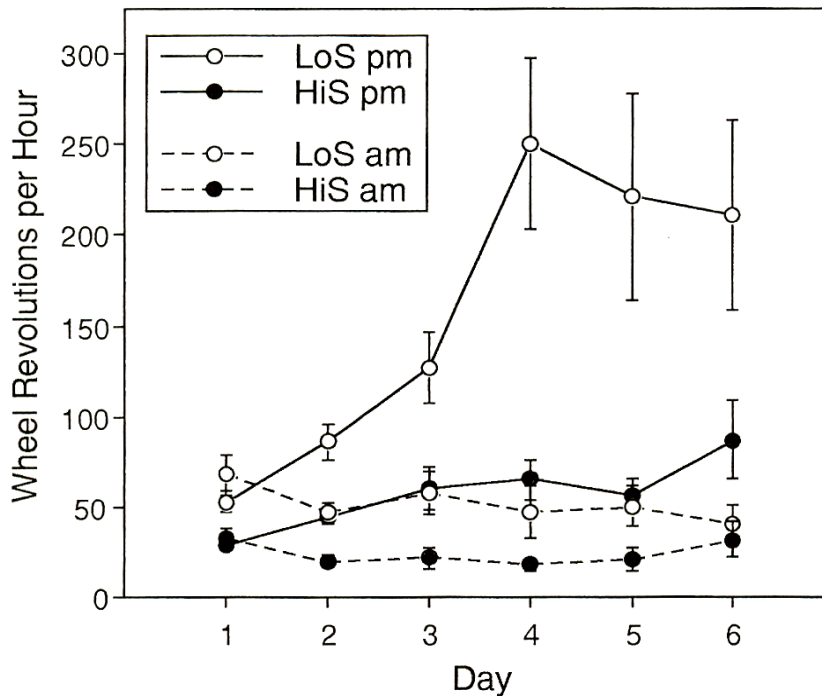
**Procedure.** The rats were weighed and placed in a running wheel apparatus. For three days, pelleted chow was freely available and the wheels were locked to allow adaptation to the new environment. For the next six days, chow mash was freely available and the wheels were unlocked. Mash was used to avoid the wheel jamming that can occur with pelleted chow. Data collection occurred at 8:30 a.m. and 5:00 p.m. daily. Body weight was measured in the morning, and food intake and wheel revolutions were recorded at both times. Water bottles were refilled and bedding was changed as needed during the 5:00 p.m. data collection period.

**Statistical analyses.** Average chow intake during the adaptation period and initial and terminal body weights in the two lines were compared with independent *t*-tests. To account for the different lengths of the light and dark/transitional phases, wheel revolutions and mash intake in each phase were transformed to per-hour averages. Running and eating in the dark and light phases were analyzed in separate mixed design ANOVAs with line (LoS, HiS), phase (dark, light), and test day (Day 1-6) as variables. Test statistics significant at  $\alpha = .05$  are reported.

## Results

Chow intake during the adaptation period did not differ between lines (LoS  $M = 20 \pm 1$  g SEM, HiS  $M = 18 \pm 2$  g SEM). Body weight did not differ between the lines either initially (LoS  $M = 294 \pm 11$  g SEM, HiS  $M = 275 \pm 10$  g SEM) or on Day 6 (LoS  $M = 291 \pm 10$  g SEM, HiS  $M = 274 \pm 8$  g SEM).

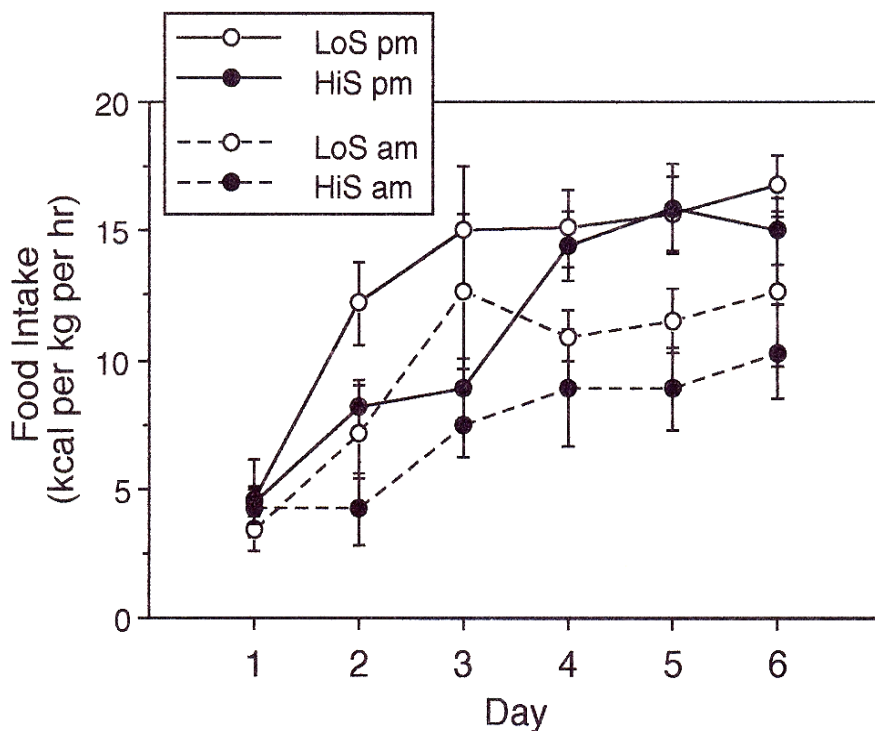
Average wheel revolutions per hour in the dark and light phases is shown in Figure 4. Both groups ran more in the dark than in the light, but the dark/light difference was larger among LoS rats than HiS rats. This difference grew over the six day test period. Every main effect and interaction in a  $2 \times 2 \times 2 \times 6$  ANOVA was significant, all  $F_s > 4$ ,  $p_s < .01$ . Of chief interest here is the line  $\times$  phase interaction,  $F(1, 16) = 8.00$ . A planned comparison of the dark/light difference averaged across test days in LoS and HiS rats was significant,  $t(16) = 2.83$ . In view of nonhomogeneity of variance, the average dark/light difference was subjected to a Mann-Whitney test, which confirmed the line difference,  $U(9, 9) = 17.00$ .



**Figure 4.** Running wheel activity (revolutions per hour) during the dark/transitional phase (pm) and the light phase (am) in LoS and HiS rats. Food was freely available.

Average hourly food intake (calories per kg of bodyweight) in the dark and light phases is shown in Figure 5. Eating increased over test days, and rats ate more in the dark phase than in the light phase. These effects were comparable in LoS and HiS rats. A  $2 \times 2 \times 2 \times 6$  ANOVA yielded significant main effects of day,  $F(5, 80) = 25.20$ , and phase,  $F(1, 16) = 11.26$ . No other effects were significant.

Both lines maintained their body weights well over the testing period; average weight change was  $-3 \pm 2$  g for LoS rats and  $-2 \pm 4$  g for HiS rats. A  $2 \times 6$  mixed design ANOVA on body weight (not shown) with line and day as variables yielded no significant effects. In addition, neither initial nor terminal body weight was correlated with food intake or running on any test day, and average light-versus-dark difference in activity remained significant when initial or terminal body weight was used as a covariate.



**Figure 5.** Food intake (calories per kg of bodyweight per hour) during the dark/transitional phase (pm) and the light phase (am) in LoS and HiS rats.

### Discussion

Relative to HiS rats, LoS rats with access to a running wheel and an unlimited supply of free food show an exaggerated circadian pattern of locomotor activity. These results indicate that the greater shift in meal frequency between dark and light phases among LoS rats in Experiment 2 was not unique to meal initiation or bar-pressing. Rather, it is part of a more general line difference in organization of behavior over the circadian cycle.

One interpretation of the line difference in wheel running is that LoS rats are more active in general. This interpretation is contradicted by other findings. First, at the end of the test period, LoS rats were not more active than HiS rats during the light phase, while the difference in the dark persisted. Second, in Experiments 1 and 2, LoS rats did not have higher bar-pressing rates than HiS rats. Third, in an earlier study (Dess & Minor, 1996), LoS rats emerged more *slowly* into a novel open field than did HiS rats, consistent with great risk reactivity; moreover, when retested later in the open field, number of line crossings did not differ between lines. Thus, there is no convergent evidence of a line difference in general locomotor activity. To the contrary, in the present study, the line differences were contingent on reinforcement schedule, time since the last meal, and/or time of day. A “hyperactivity” interpretation fails to account for, much less predict, the sensitivity of line differences to circumstance.

Running and food intake increased early in the test period, stabilizing after a few days. These increases probably reflect adaptation to the novel environment, the running wheel, and the novel texture and lower caloric density of the chow mash. Interestingly, LoS rats maintained their body weight as well as did HiS rats despite running more. LoS rats ran approximately 2500 more revolutions – about 1.6 miles “farther” – than HiS rats on each of the last three days. LoS rats’ slightly (nonsignificantly) higher caloric intake may have offset the weight loss that otherwise should accompany greater energy expenditure. However, other counterregulatory changes, such as reduced core body temperature, slower gastric emptying, or hypersomnia, also likely played a role.

Whereas male rats were used in Experiment 2, female rats were used in Experiment 3. Sex differences in wheel running might be expected on empirical (e.g. Epling & Pierce, 1996; Geary, 2001; Gentry & Wade, 1978) and theoretical (Houston, Stephens, Boyd, Harding, & McNamara, 2007) grounds. The relevant question here, however, is not whether females and males differ but whether the line difference in Experiment 3 occurs only in females. No cyclicity or untoward within-group variability is apparent. Also, as noted above, we have not yet observed a saccharin phenotype correlation unique to one sex. While circadian variation could be an exception, female rats are rats, and neither our reasoning about risk reactivity nor results to date support the idea that these results are unique to one sex.

## **General Discussion**

The present results indicate that LoS rats show better behavioral regulation of eating when food is scarce and are more dedicatedly nocturnal than HiS rats. Better behavioral regulation and greater nocturnality may seem to connote that LoS rats are behaving more adaptively than HiS rats. Our protocols do engage biobehavioral systems that evolved under selective pressure, and our lines differ in how those systems function. However, characterizing either line as better adapted or more fit would be inappropriate. The heritability of the line differences is unknown. Moreover, whether better behavioral regulation of eating or an exaggerated circadian rhythm would confer an adaptive advantage outside of the

laboratory likely would depend of a range of ecological variables. Indeed, the phenotypic variation we are studying probably exists because the expression of the variants, and its consequences for fitness, are contingent on circumstance.

The present results support the idea that greater risk reactivity among LoS rats underlies a range of behavioral line differences. The common neural mediators of these diverse effects are not known. Aversive tastes activate the autonomic nervous system (Rousmans, Robin, Dittmar, & Vernet-Maury, 2000) and sucrose consumption ameliorates stress vulnerability among adrenalectomized rats (Dallman et al., 2003), with robust strain differences occurring in the latter effect (Pecoraro et al., 2006). Thus, pathways connecting taste to hypothalamic-pituitary-adrenal (HPA) mediated stress responses, and phenotypic variation in their functioning, do exist. LoS rats are hypercorticosteronemic (VanderWeele et al., 2002), so central and peripheral regulation of HPA activity may play a role in line differences in the organization of eating.

Implications of foraging theories (McNamara & Houston, 1985) for the relationship between risk reactivity and the organization of eating also warrant consideration. Optimality and momentary maximizing both have some empirical support as foraging principles (e.g. MacDonall, Goodell, & Juliano, 2006). To the extent that rats have the capacity to operate in either manner, perhaps the energetically conservative LoS rats have a greater tendency toward optimality whereas HiS rats, consistent with their greater impulsivity (Perry et al., 2007), would tend toward momentary maximizing.

The present results also may speak to greater risk sensitivity in LoS rats. In this context, the risk to which the term *risk sensitivity* refers is uncertain food reward, and sensitivity to it is typically assessed as choice between constant and variable reinforcement when pay-offs are equal. Risk aversion is defined as preference for the former and risk proneness as preference for the latter (Bateson, 2002; Kacelnik & Bateson, 1996). Given greater risk reactivity among LoS rats, we might expect LoS rats to be more risk averse than HiS rats. According to the energy budget rule, a shift from positive to negative energy balance should increase risk-prone foraging (Hastjarjo, Silberberg, & Hursh, 1990; Ito, Takatsuru, & Saeki, 2000; Kaminski, & Ator, 2001). How energy balance affects risk proneness in LoS and HiS rats is unclear, though. LoS rats should be more reactive to negative energy balance and thus show a greater shift toward risk proneness than HiS rats, but a general tendency to avoid food uncertainty would mitigate such a shift. Parametric manipulation of food variability and energy balance might reveal different “tipping points” between risk aversion and proneness in the two lines rather than just a general tendency toward one or the other.

A particularly appropriate approach to using LoS and HiS rats to understand the relationship between risk reactivity and foraging would combine food supply- and predator-related risk. The *cognitive-emotional forager* model (Coleman, Brown, Levine, & Mellgren, 2005) incorporates trade-offs between approaching food and avoiding predation as a function of energy balance. This quantitative model would allow assessment of the relative importance of food supply, danger of predation, and hunger in the foraging strategies of LoS and HiS rats. An important complementary concept is *predatory imminence*. Fanselow &

Lester (1988) distinguished stages in the predation sequence from the perspective of prey including, in order of increasing imminence, pre-encounter, post-encounter, and circa-strike stages. The stages are associated with qualitatively different anti-predation strategies. In predator-simulating procedures, we might expect LoS rats to modify their foraging and other behaviors in ways suggestive of a higher degree of predatory imminence. A variety of stressor manipulations followed establishment of the steady-state behaviors reported here, constituting pilot work on how increased predatory imminence would affect behavior in these paradigms and whether LoS and HiS rats are affected in different ways or to different degrees. More systematic examination of those questions clearly is warranted.

The present results encourage continued attention to dispositional variables in the attempt to account for eating in ecological context. Future work with LoS and HiS rats in experimental preparations incorporating manipulation of energy balance, food quality, explicit predator-associated cues, spatial dimensions of foraging, and richer behavioral topographies will further that effort.

<sup>1</sup> A preliminary report on total running averaged over the last two days appeared in a book chapter (Dess, 2001), but running on the other four days, food intake and body weight were not reported, and light and dark phase data were not disaggregated.

## References

- Agrawal, A. A. (2003). Why omnivory? *Ecological Society of America*, **84**, 2521.
- Arcis, V., & Desor, D. (2002). Influence of environment structure and food availability on the foraging behaviour of the laboratory rat. *Behavioural Processes*, **60**, 191-198.
- Bateson, M. (2002). Recent advances in our understanding of risk-sensitive foraging preferences. *Proceedings of the Nutrition Society*, **61**, 509-516.
- Bertoglio, L. J., & Carobrez, A. P. (2002). Behavioral profile of rats submitted to session 1-session 2 in the elevated plus-maze during diurnal/nocturnal phases and under different illumination conditions. *Behavioural Brain Research*, **132**(2), 135-143.
- Brush, F. R., Del Paine, S. N., Pellegrino, L. J., Ryszewski, I. M., Dess, N. K., & Collins, P. Y. (1988). CER suppression, passive avoidance learning and stress-induced suppression of drinking in the Syracuse High (SHA) and Low (SLA) avoidance strains of rats (*Rattus norvegicus*). *Journal of Comparative Psychology*, **102**, 337-349.
- Carroll, M. E., Morgan, A. D., Anker, J. J., Perry, J. L., & Dess, N. K. (2008). Selective breeding for differential saccharin intake as an animal model of drug abuse. *Behavioural Pharmacology*, **19**(5-6), 435-460.
- Carroll, M. E., Morgan, A. D., Lynch, W. J., Campbell, U. C., & Dess, N. K. (2002). I.V. cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: Phenotype and sex differences. *Psychopharmacology*, **161**, 304-313.
- Castonguay, T. W., & Kaiser, L. L., & Stern J. S. (1986). Meal pattern analysis: Artifacts, assumptions and implications. *Brain Research Bulletin*, **17**(3), 439-443.
- Chaouloff, F., Durand, M., & Mormede, P. (1997). Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. *Behavioural Brain Research*, **85**(1), 27-35.

- Clark, L. A. (2005). Temperament as a unifying basis for personality and psychopathology. *Journal of Abnormal Psychology*, **114**(4), 505-521.
- Clifton, P. G. (1999). Meal patterning in rodents: Psychopharmacological and neuroanatomical studies. *Neuroscience & Biobehavioral Reviews*, **24**(2), 213-222.
- Clifton, P. G., & Somerville, E. M. (1994). Disturbance of meal patterning following nucleus accumbens lesions in the rat. *Brain Research*, **667**(1), 123-128.
- Clifton, P. G., Vickers, S. P., & Somerville, E. M. (1998). Little and often: Ingestive behavior patterns following hippocampal lesions in rats. *Behavioral Neuroscience*, **112**(3), 502-511.
- Collier, G., & Johnson, D. F. (1997). Who is in charge? Animal vs experimenter control. *Appetite*, **29**, 159-180.
- Coleman, S. L., Brown, V. R., Levine, D. S., & Mellgren, R. L. (2005). A neural network model of foraging decisions made under predation risk. *Cognitive, Affective & Behavioral Neuroscience*, **5**(4), 434-451.
- Dallman, M. F., Akana, S. F., Laugero, K. D., Gomez, F., Manalo S., Bell M. E., & Bhatnagar S. A. (2003). Spoonful of sugar: Feedback signals of energy stores and corticosterone regulate responses to chronic stress. *Physiology & Behavior*, **79**(1), 3-12.
- Davidson, R. J. (2002). Seven sins in the study of emotion: Correctives from affective neuroscience. *Brain and Cognition*, **52**, 129-132.
- De Jongh, R., Groenink, L., van der Gugten, J., & Olivier, B. (2003). Light-enhanced and fear-potentiated startle: Temporal characteristics and effects of  $\pm$ -helical corticotropin-releasing hormone. *Biological Psychiatry*, **54**(10), 1041-1048.
- Dess, N. K. (1991). Ingestion and emotional health. *Human Nature*, **2**, 235-269.
- Dess, N. K. (1997). Ingestion after stress: Evidence for a regulatory shift in food-rewarded operant performance. *Learning and Motivation*, **28**, 342-356.
- Dess, N. K. (2000). Responses to basic taste qualities in rats selectively bred for high versus low saccharin intake. *Physiology & Behavior*, **69**, 247-257.
- Dess, N. K. (2001). Eating, emotion, and the organization of behavior. In M. E. Carroll & J. B. Overmier (Eds.), *Animal research and human health: Advancing human welfare through behavioral science*. Washington, DC: APA.
- Dess, N. K., Arnal, J., Chapman, C. D., Siebel, S., VanderWeele, D. A., & Green, K. (2000). Exploring adaptations to famine: Rats selectively bred for differential saccharin intake differ on deprivation-induced hyperactivity and emotionality. *International Journal of Comparative Psychology*, **13**, 34-52.
- Dess, N. K., Choe, S., & Minor, T. R. (1998). The interaction of diet and stress in rats: High energy food and sucrose treatment. *Journal of Experimental Psychology: Animal Behavior Processes*, **24**, 1-12.
- Dess, N. K., & Edelhait, D. (1998). The bitter and the sweet: The human taste/stress/temperament nexus. *Biological Psychology*, **48**, 103-119.
- Dess, N. K., & Minor, T. R. (1996). Taste and emotionality in rats selectively bred for high versus low saccharin intake. *Animal Learning & Behavior*, **24**, 105-115.
- Dess, N. K., Minor, T. R., & Brewer, J. (1989). Suppression of feeding and body weight after inescapable shock: Modulation by quinine adulteration, stressor reinstatement, and controllability. *Physiology & Behavior*, **45**, 975-983.
- Dess, N. K., O'Neill, P., & Chapman, C. D. (2005). Ethanol withdrawal and proclivity are inversely related in rats selectively bred for differential saccharin intake. *Alcohol*, **37**(1), 9-22.
- Dess, N. K., & VanderWeele, D. A. (1994). Lithium chloride and inescapable, un signaled tail shock differentially affect meal patterns of rats. *Physiology & Behavior*, **56**, 203-207.

- Epling, W. F., & Pierce, W. D. (1996). *Activity anorexia: Theory, research, and treatment*. Mahwah, NJ: Lawrence Erlbaum Associates.
- Ettinger, R. H., & Staddon J. E. R. (1983). Operant regulation of feeding: A static analysis. *Behavioural Neuroscience*, **97**(4), 639-653.
- Eysenck, H. J. (1979). Personality factors in a random sample of the population. *Psychological Reports*, **44**, 1023-1027.
- Fanselow, M. S., & Lester, L. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. In R. C. Bolles & M. D. Beecher (Eds.), *Evolution and learning*. Pp. 185-211. Hillsdale, NJ: Lawrence Erlbaum.
- Fanselow, M. S., Lester, L. S., & Helmstetter F. J. (1988). Changes in feeding and foraging patterns as an antipredator defensive strategy: A laboratory simulation using aversive stimulation in a closed economy. *Journal of the Experimental Analysis of Behavior*, **50**(3), 361-374.
- Fessler, D. M. T. (2002). Pseudoparadoxical impulsivity in restrictive anorexia nervosa: A consequence of the logic of scarcity. *International Journal of Eating Disorders*, **31**(4), 376-388.
- Frankland, P. W., & Ralph, M. R. (1995). Circadian modulation in the rat acoustic startle circuit. *Behavioral Neuroscience*, **109**(1), 43-48.
- Garcia, J. (1989). Food for Tolman: Cognition and cathexis in concert. In T. Archer & L.-G. Nilsson (Eds.), *Aversion, avoidance, and anxiety: Perspectives on aversively motivated behavior*. Pp. 45-85. Hillsdale, NJ: Lawrence Erlbaum.
- Geary, N. (2001). Estradiol, CCK and satiation. *Peptides*, **22**(8), 1251-1263.
- Gentry, R., T., & Wade, G. N. (1976). Sex differences in sensitivity of food intake, body weight, and running-wheel activity to ovarian steroids in rats. *Journal of Comparative and Physiological Psychology*, **90**(8), 747-754.
- Godsil, B. P. & Fanselow, M. S. (2004). Light stimulus change evokes an activity response in the rat. *Learning & Behavior*, **32**(3), 299-310.
- Gosling, S. D. (2001). From mice to men: What can we learn about personality from animal research? *Psychological Bulletin*, **127**(1), 45-86.
- Grillon, C., Pellowski, M., Merikangas, K. R., & Davis, M. (1997). Darkness facilitates the acoustic startle in humans. *Biological Psychiatry*, **42**, 453-460.
- Guisinger, S. (2003). Adapted to flee famine: Adding an evolutionary perspective on anorexia nervosa. *Psychological Review*, **110**(4), 745-761.
- Hastjarjo, T., Silberberg, A., & Hursh, S. R. (1990). Risky choice as a function of amount and variance in food supply. *Journal of the Experimental Analysis of Behavior*, **53**, 155-161.
- Helmstetter, F. J., & Fanselow, M. S. (1993). Aversively motivated changes in meal patterns of rats in a closed economy: The effects of shock density. *Animal Learning & Behavior*, **21**(2), 168-175.
- Holeckova, E., & Chytil, F. (1963). Natural adaptation to intermittent starvation in wild Norway rats. *Physiologia Bohemoslovenica*, **12**(3), 177-181.
- Houston, A. I., & McNamara, J. M. (1989). The value of food: Effects of open and closed economies. *Animal Behaviour*, **37**(4), 546-562.
- Houston, A. I., Stephens, P. A., Boyd, I. L., Harding, K. C., & McNamara, J. M. (2007). Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioral Ecology*, **18**(1), 241-250.
- Ito, M., Takatsuru, S., & Saeki, D. (2000). Choice between constant and variable alternatives by rats: Effects of different reinforcer amounts and energy budgets. *Journal of the Experimental Analysis of Behavior*, **73**, 79-92.

- Kacelnik, A., & Bateson, M. (1996). Risky theories—the effects of variance on foraging decisions. *The American Zoologist*, **36**, 402–434.
- Kaminski B. J., & Ator N. A. (2001) Behavioral and pharmacological variables affecting risky choice in rats. *Journal of the Experimental Analysis of Behavior*, **75**(3), 275–297.
- Kraly, F. S., Cushin, B. J., & Smith G. P. (1980). Nocturnal hyperphagia in the rat is characterized by decreased postprandial satiety. *Journal of Comparative and Physiological Psychology*, **94**(2), 375-387.
- Lawrence, A. D., Murphy, F. C., & Calder, A. J. (2004) Dissociating fear and disgust: Implications for the structure of emotions. Pp. 149-171. In J. Yiend (Ed.), *Cognition, emotion and psychopathology: Theoretical, empirical and clinical directions*. New York: Cambridge University Press.
- Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, **68**, 619-640.
- Lynch, W. C., Hart, P., & Babcock, A. M. (1994). Neuropeptide Y attenuates satiety: Evidence from a detailed analysis of patterns of ingestion. *Brain Research*, **636**(1), 28-34.
- McGowan, M. K., Andrews, K. M., Kelly, J., & Grossman, S. P. (1990). Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. *Behavioral Neuroscience*, **104**(2), 373-385.
- McNamara, J. M., & Houston, A. I. (1985). Optimal foraging and learning. *Journal of Theoretical Biology*, **117**, 231-249.
- Nachman, M. (1959). The inheritance of saccharin preference. *Journal of Comparative and Physiological Psychology*, **52**, 451-457.
- Nasello, A. G., Machado, C., Bastos, J. G., & Felicio, L. F. (1998). Sudden darkness induces a high activity-low anxiety state in male and female rats. *Physiology & Behavior*, **63**(3), 451-454.
- Nemanick, R. C., Jr., & Munz, D. C. (1997). Extraversion and neuroticism, trait mood, and state affect: A hierarchical relationship? *Journal of Social Behavior & Personality*, **12**(4), 1079-1092.
- Patrick, C. J., Curtin, J. J., & Tellegen, A. (2002). Development and validation of a brief form of the Multidimensional Personality Questionnaire. *Psychological Assessment*, **14**(2), 150-163.
- Pecoraro, N., Ginsberg, A. B., Warne, J. P., Gomez, F., la Fleur, S. E., & Dallman, M. F. (2006). Diverse basal and stress-related phenotypes of Sprague Dawley rats from three vendors. *Physiology & Behavior*, **89**(4), 598-610.
- Perry, J. L., Nelson, S. E., Anderson, M. M., Morgan, A. D., & Carroll, M. E. (2007). Impulsivity (delay discounting) for food and cocaine in male and female rats selectively bred for high and low saccharin intake. *Pharmacology, Biochemistry, & Behavior*, **86**(4), 822-837.
- Poling, A., Nickel, M., & Alling, K. (1990). Free birds aren't fat: Weight gain in captured wild pigeons maintained under laboratory conditions. *Journal of the Experimental Analysis of Behavior*, **53**(3), 423-424.
- Reich, J. W., & Zautra, A. J. (2002). Arousal and the relationship between positive and negative affect: An analysis of the data of Ito, Cacioppo, and Lng (1998). *Motivation and Emotion*, **26**(3), 209-222.
- Rousmans, S., Robin, O., Dittmar, A., & Vernet-Maury, E. (2000). Autonomic nervous system responses associated with primary tastes. *Chemical Senses*, **25**, 709-718.
- Rossi, R., Driscoll, P., & Langhans, W. (1997). Differential circadian eating patterns in two psychogenetically selected strains of rats fed low-, medium-, and high-fat diets. *Behavior Genetics*, **27**(6), 565-572.

- Russell, J. A., & Barrett, L. F. (1999). Core affect, prototypical emotional episodes, and other things called emotion: Dissecting the elephant. *Journal of Personality and Social Psychology*, **76**(5), 805-819.
- Russell, J. A., & Mehrabian, A. (1977). Evidence for a three-factor theory of emotions. *Journal of Research in Personality*, **11**(3), 273-294.
- Staddon, J. E. R. (1979). Operant behavior as adaptation to constraint. *Journal of Experimental Psychology*, **108**(1), 48-67.
- Steimer, T., & Driscoll, P. (2005). Inter-individual vs line/strain differences in psychogenetically selected Roman High-(RHA) and Low-(RLA) Avoidance rats: Neuroendocrine and behavioural aspects. *Neuroscience & Biobehavioral Reviews*, **29**(1), 99-112.
- Strubbe J. H., & Woods, S. C. (2004). The timing of meals. *Psychological Review*, **111**(1), 128-141.
- Thiele, T. E., Badia Elder, N. E., Kiefer, S. W., & Dess, N. K. (1997). Continuous intraoral saccharin infusions reveal line differences between rats selectively bred for high versus low saccharin consumption. *Physiology & Behavior*, **61**, 149-152.
- Timberlake, W. (1984). Behavior regulation and learned performance: Some misapprehensions and disagreements. *Journal of the Experimental Analysis of Behavior*, **41**, 355-375.
- Timberlake, W., & Silva, K. M. (1995). Appetitive behavior in ethology, psychology, and behavioral systems. In N. S. Thompson (Ed.), *Perspectives in ethology*. (Vol. 11: Behavioral design). Pp. 211-253. New York: Plenum Press.
- VanderWeele, D. A., Dess, N. K., & Castonguay, T. W. (2002). Ingestional responses to metabolic challenges in rats selectively bred for high- and low-saccharin intake. *Physiology & Behavior*, **75**, 97-104.
- Walker, D. L., & Davis M. (2002). Light-enhanced startle: Further pharmacological and behavioral characterization. *Psychopharmacology*, **159**, 304-310.
- Watson, D., & Clark, L. A. (1984). Negative affectivity: The disposition to experience aversive emotional states. *Psychological Bulletin*, **96**(3), 465-490.
- Whishaw, I. Q., Dringenbeg, H. C., & Comery T. A. (1992). Rats (*Rattus norvegicus*) modulate eating speed and vigilance to optimize food consumption: Effects of cover, circadian rhythm, food deprivation, and individual differences. *Journal of Comparative Psychology*, **106**(4), 411-419.
- Zanutto, B. S., & Staddon, J. E. R. (2007). Bang-bang control of feeding: Role of hypothalamic and satiety signals. *PLoS Computational Biology*, **3**(5), 0924-0931.

## **The Role of Visual Cues in the Comprehension of the Human Pointing Signals in Dogs**

**Gabriella Lakatos, Antal Dóka, and Ádám Miklósi**  
*Eötvös University, Hungary*

In this study we examined the effect of the visually emphasized pointing arm in the case of the “Cross-forward pointing” gesture in dogs which proved to be difficult for them in an earlier study (Lakatos, Soproni, Dóka, & Miklósi, 2008). Our hypothesis was that if we emphasize the directionality of the visual cue using different, more contrasting coloured clothes during the tests, the dogs will be able to enhance their performance in two-way choice tasks. Our results showed that the conspicuousness of the pointing signal can affect how dogs perceive it. In contrast to our initial hypothesis pointing in a long white sleeve on a black background did not increase the dogs’ performance, while the black sleeves with nude (“white”) hands had an enhancing effect. This suggests that dogs need to see a salient body part what overhangs the median of the body silhouette and when the whole body is covered by black colour then the nude (pointing) hand appears as a conspicuous asymmetrical feature on one side of the body. Making the pointing hand less conspicuous makes the effect invariably disappear. Thus in summary we assume that the key aspect of the pointing gesture is not the directionality but the visually asymmetric cue provided by the human informant.

Recently it has been established that dogs are able to rely on the human pointing cue in two-way object choice tasks. They are also able to generalize to a certain degree from familiar pointing gestures to unfamiliar ones, and thereby they perform well on the basis of partially novel or “strange” pointing gestures (Hare & Tomasello, 1999; Hare, Brown, Williamson, & Tomasello, 2002; Lakatos et al., 2008; Miklósi, Polgárdi, Topál, & Csányi, 1998; Miklósi, Pongrácz, Lakatos, Topál, & Csányi, 2005; Soproni, Miklósi, Topál, & Csányi, 2002).

According to Butterworth and Itakura (2000) pointing production is based on vector extrapolation, thus one can assume that a corresponding version of this mechanism may be involved in the comprehension of the pointing gesture. Butterworth and Itakura (2000) have examined whether human children and adult people utilize this mechanism when they comprehend the direction indicated by pointing or head and eye movements. It was found that comprehension of pointing is unlikely to depend on extrapolating precise linear vectors along the pointing arm even in adults. Nevertheless their results showed that children perceive the directionality of the gesture and follow the direction of the arm into the visual periphery. Their results also revealed that the type of the visual effect/signal is very important, the longer lever (like the arm) carries the children’s attention further into the visual periphery than do cues based on shorter levers (head movements). In real life visual factors (i.e. the differential salience of the target) necessarily interact with these approximate cues in making definite reference (Butterworth & Itakura, 2000).

In a recent study relatively unfamiliar gestures were utilized to find the critical visual features of the pointing signal that guide the choice behaviour of

This study was supported by grants received from the Hungarian Science Fund (OTKA T043763), the Hungarian Academy of Sciences (F01/031) and an EU FP6 NEST 012787 grant. The authors are grateful to all the parents and the owners who participated in this research for their patience and support throughout the study. Correspondence concerning this article should be addressed to Gabriella Lakatos, Department of Ethology, Eötvös University Budapest, Pázmány P. sétány 1c., 1117, Hungary. (gabriella.lakatos@gmail.com).

dogs and children in different ages (Lakatos et al., 2008). By varying the pointing gesture systematically we have found that, first, the dogs' performance was above the chance level only if the gesture provided a visually protruding body part (from the point of view of the dog) on the side of the baited bowl (e.g. "Momentary distal pointing", "Long cross-pointing"), and their performance did not differ from the chance level if there was no protruding body part ("Cross-forward pointing"). Second, their performance was significantly below the chance level when the protruding body part was on the side of the incorrect bowl, and third, dogs showed some evidence of generalization in the presence of unfamiliar gestures when they responded correctly from the beginning to "gestures" made by the legs. Finally, dogs did not grasp the meaning of the pointing index finger. Taken together these results suggested that dogs relied on a common simple rule, that is, they follow the direction indicated by the protruding body part (Lakatos et al., 2008). This notion is also supported by findings that an elongated nose on a mask improved the performance of children (Butterworth & Itakura, 2000).

Agnetta, Hare, and Tomasello (2000) showed that dogs are able to use novel cues when the experimenter places an arbitrary marker in front of the target location with overt behaviour elements but they are not able to use a physical marker as a communicative cue in the absence of any overt human behaviour toward the location of the hidden food. These findings have been extended recently by Riedel, Buttelmann, Call, & Tomasello (2006) who reported that; actually, dogs attached more importance to the marker than to the hand that placed it, although the presence of the hand enhanced the dogs' performance if it appeared together with the marker. It is also possible that under such conditions dogs simply choose the side at which something happened (a visual effect: i.e. the experimenter's arm moved to that side or a marker has been placed there) in a communicative situation.

In the present study we wanted to see whether there are alternatives to the comprehension that is based on protruding body parts. For this we utilized the so called "Cross-forward pointing" gesture in the case of which the experimenter points ahead of her (from the dogs point of view there are no extruding body parts), and, accordingly, Lakatos et al. (2008) found that dogs are not able to rely on this gesture in the two choice test. Our hypothesis was that if we emphasize the visual cue (the line of the pointing arm) using different, more contrasting coloured clothes during the tests then the dogs' performance increases. This was achieved by presenting the pointing signal in a white or black sleeved black T-shirt, which resulted in clearly directional visual cues (see Figure 1).

## **Methods**

### ***Subjects***

Dogs (N=14): All individuals participated in both studies (see below), each of them participated in Study 1 for the first time, and after it was completed they participated in Study 2. Seven males, seven females; the mean age of the dogs was 4.5 years, SD was 3.6 years and the range was 1 - 13 years. Nine individuals were naive, five individuals have participated in other experiments recently, in which they had been exposed to some other kind of gestures (3 individuals: dynamic proximal and distal pointing, and momentary proximal and distal pointing gestures (Miklósi et al., 2005); two individuals: momentary long-cross pointing, momentary elbow-cross pointing; momentary cross-forward pointing and some kind of pointing performed by leg (Lakatos et al., 2008). The age of the owners was 25.07 + 7.43 years (mean + SD); range was 13 - 40; three men and eleven women.

### ***Pretraining: familiarization with the situation***

In the present experiments we used the same method as described in earlier studies (e.g. Lakatos et al., 2008; Soproni, Miklósi, Topál, & Csányi, 2001). The experimenter (G.L.) placed two bowls (brown plastic flower pots: 13 cm in diameter, 13 cm in height) 1.3-1.6 metres apart, in front of her on the floor. She put a piece of food (we used a small piece of frankfurter during the tests) into one of the bowls in the presence of the subject. The subjects could witness this hiding from a distance of 2-2.5 m with their owner standing behind them. After the experimenter put the food into the bowl, the owner allowed the dog to take the reward out from the bowl. One trial lasted about 30 seconds, and the procedure was repeated twice for each bowl to ensure that the subject knew that either bowl might contain some food.

### ***Testing***

The position of the participants was the same as above, but during the testing the subject was prevented from observing the hiding. The experimenter picked up the bowls, she put a piece of food into one of the bowls and after that she placed both bowls back onto the floor at the same time. During the pointing the experimenter was standing 0.5 m back from the middle line between the two bowls, facing the subject at a distance of 2-2.5 m. The owner was holding back the subject gently until the experimenter gave the cue. The experimenter drew the subject's attention to herself (any sounds, like clapping or/and the subject's name could be used) and presented the visual cue when the subject looked in the direction of her face. During the pointing the experimenter was looking at the subject. If the subject did not set out at the first cue, the experimenter repeated the pointing gesture again a maximum three times. The subject was allowed to choose only one bowl.

We used two types of pointing gestures in both studies. In the "Probe trial method" the experimental ("probe") trials were embedded into a background of the momentary distal pointing gestures ("control trials"); see below and see also Lakatos et al., 2008; Miklósi et al., 2005; Povinelli, Bierschwale, & Cech, 1999; Soproni et al., 2002). In the experimental trials we used the momentary Cross-forward pointing. During the *momentary distal pointing* the experimenter pointed with extended arm and index finger in the direction of the correct location by her closer hand. After signalling, which lasted about 1 second, she lowered her arm to the starting position beside her body before the subject was allowed to approach the bowls.

During the *momentary cross-forward pointing* the experimenter stepped back about 0.5 metres from the bowls and she pointed with her contra-lateral hand in the direction of the correct location, but her extended index finger did not protrude from her silhouette. After signalling (1 sec), she lowered her arm to the starting position beside her body before the subject was allowed to approach the bowls (see Figure 1 and 2).

Each test session consisted of 20 trials, which contained 10 trials of momentary distal pointing gesture and 10 probe trials (momentary cross-forward pointing). The presentations of the cues were in predetermined semi random order and were balanced for right and left side. Neither the same gesture, nor the place of the reward was applied more than two times in a row. With each dog we did two studies one after the other within one month. There were two test sessions in both studies, depending on the clothes worn by the experimenter. There was a minimum of two weeks and a maximum of one month between the two test sessions in both studies. All experimental trials were recorded on video.

It is important to note that the gestures used in this study were displayed in a "momentary" fashion, which means that the subjects could witness the emergence of the gesture but the pointing was terminated (the arm was lowered to the body side) before allowing the subjects to choose. Therefore for simplicity this characteristic will not be indicated in the names of the gestures in the following sections of the paper.

The statistical analysis was based on the number of correct choices and non-parametric procedures (One-sample Wilcoxon signed-rank test, Wilcoxon matched pairs test and Mann-Whitney test) were used.

## **Study 1**

In this study we have investigated whether the visual accentuation of the whole arm (black shirt with white sleeves) enhances the performance of the

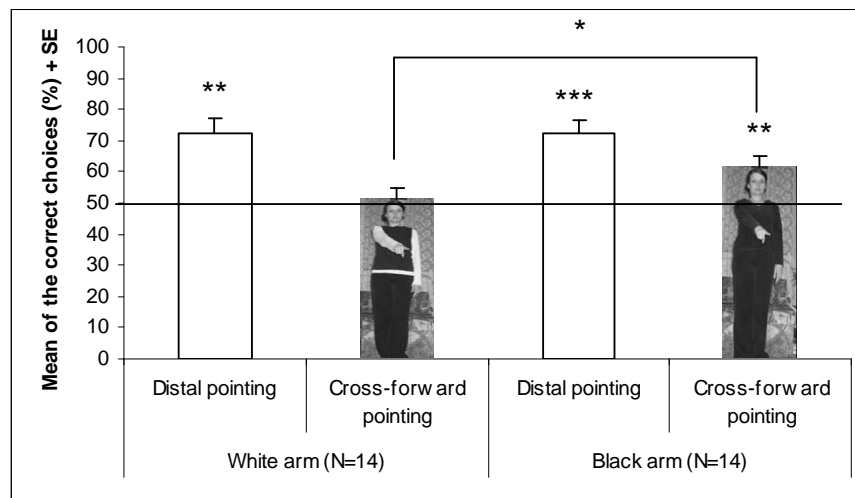
dogs in the two way choice task. We applied this modification in the case of the cross forward pointing gesture which was found to be incomprehensible by dogs (Lakatos et al., 2008) in the two-way choice test. We expected that if the dogs utilize visual cues based on the whole arm than this condition should increase their performance. As a control we camouflaged the pointing arm by dressing the whole upper body part of the experimenter in black.

### Methods

Types of the clothes worn by the experimenter (see Figure 1):

1. “Black arm”: The experimenter wore a black long-sleeved shirt, so this way the arms and the torso of the experimenter were also black, but her hands were nude (white).
2. “White arm”: The experimenter wore a white long-sleeved shirt and a black waistcoat on it, so the arms of the experimenter were white, but her torso was black. Her hands were also nude.

So in one of the two test sessions the pointing cues were presented with “Black arm” for the dogs and in the other test session with “White arm”. Half of the subjects participated in the “Black arm” session for the first time, while the other half of the subjects started the test with the “White arm” session.



**Figure 1.** Dogs’ performance in the Study 1 (Mean + SE). Dotted line represents chance level. The asterisks over the bars refer to the significant differences from the chance level (One-sample Wilcoxon signed-rank test) and to the results of the comparison of the “Black arm” and the “White arm” test (Wilcoxon matched pairs test) (\*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$ ).

### Results and Discussion

We compared the performance (mean of percentage) of the dogs to the chance level (50%) by one-sample Wilcoxon signed-rank test in both sessions in the case of both types of pointing gesture (see the results in Table 1). The dogs’ performance with the “Distal pointing” gestures differed significantly from the chance level both in the case of the “Black arm” and “White arm” sessions. In the case of the “Cross-forward pointing” their performance was at chance level in the “White arm” session, but dogs chose significantly above the chance-level in the “Black arm” session (Figure 1).

**Table 1**  
Results of the One-sample Wilcoxon signed-rank tests in Study 1.

Type of the pointing gesture	One-sample Wilcoxon signed-rank test			
	White arm		Black arm	
	<i>T</i>	<i>p</i>	<i>T</i>	<i>p</i>
Distal pointing	87.5	0.01	66.0	0.001
Cross-forward pointing	12.5	0.68	62.0	0.01

Comparing the results in the two kinds of pointing gestures used we found that in the case of the “White arm” session dogs performed better in the “Distal pointing” trials than in the “Cross-forward pointing” trials (Mann-Whitney U-test;  $U = 34.0$ ;  $p < 0.01$ ) whilst there was no such difference in the “Black arm” session between the different types of pointing gestures (Mann-Whitney U-test;  $U = 58.5$ ;  $p = 0.07$ ).

There was no sign of learning during the testing for either of the pointing gestures. Comparing the performance on the first and the second session for both type of gestures we found no significant difference (Wilcoxon matched pairs test: “Distal pointing” ( $T (-) = -35.0$ ;  $p = 0.49$ ); “Cross-forward pointing” ( $T (-) = -46.0$ ;  $p = 0.62$ )).

Thus contrary to our initial hypothesis dogs’ performance was higher in the “Cross-forward pointing” trials if the experimenter wore a black long-sleeved shirt so her arms and the torso were also black than when she was pointing dressed in a white long-sleeved shirt and a black waistcoat, when the arms of the experimenter were contrastingly white on a black background of her torso (Figure 1). Although, in the case of the “White arm” gesture the sleeve provided a directional cue, its actual directionality was ambiguous, partially because the pointing arm seemed to be almost as if it would point straight forward. Contrarily in the case of the “Black arm” clothes the experimenter’s nude pointing hand popped out conspicuously on black background on the baited side.

## Study 2

Based on the results in Study I we modified our hypothesis suggesting that if the light hand skin (“white”) provided a crucial (asymmetrical) visual cue for the dogs than pointing in black gloves should make this effect disappear. As a further control we applied the same gesture with nude (“white”) arm using an everyday T-shirt which was supposed to replicate the effect of the white arm in Study 1 and the results of our recent experiment in Lakatos et al. (2008). Thus in both cases we hypothesised that the modification of the visual features would lead to a deterioration of the dogs’ performance in the case of “cross pointing” gestures. The same group of dogs served as subjects.

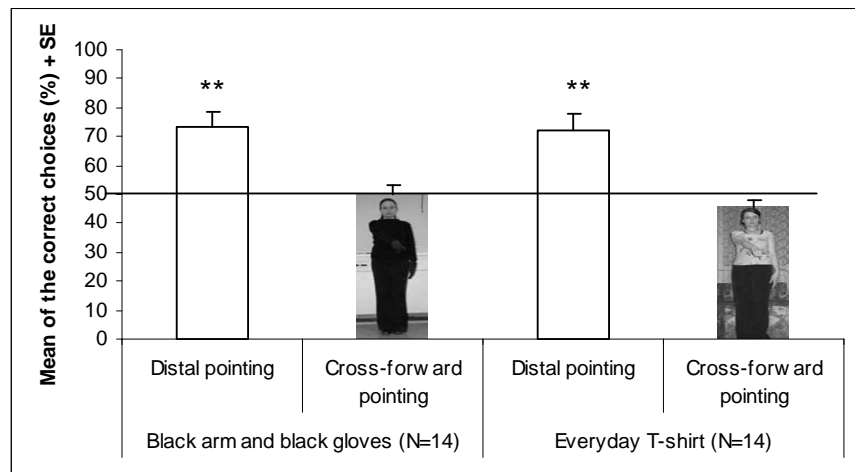
## Methods

Types of the clothes worn by the experimenter (see on Figure 2):

1. “Black arm with black gloves”: The experimenter wore a black long-sleeved shirt and black gloves, so this way the arms, the torso and the hands of the experimenter were also black.

2. “Everyday T-shirt”: The experimenter wore a lightly coloured everyday T-shirt. It could be anything, which was not similar to the other 3 types of the used clothes. Her hands were uncovered (nude).

So in one of the two test sessions the pointing cues were presented by “Black arm with black gloves” gesture for the dogs, while in the other test session the experimenter wore “Everyday T-shirt”. Half of the subjects participated in the “Black arm and back gloves” session for the first time, while the other half of the subjects started the test with the “Everyday T-shirt” session.



**Figure 2.** Dogs’ performance in the Study 1 (Mean + SE). Dotted line represents chance level. The asterisks over the bars refer to the significant differences from the chance level (One-sample Wilcoxon signed-rank test) and to the results of the comparison of the “Black arm and black gloves” and the “Everyday T-shirt” test (Wilcoxon matched pairs test) (\*  $p < .05$ , \*\*  $p < .01$ , \*\*\*  $p < .001$ ).

### Results and Discussion

We compared the performance of the dogs to the chance level by one-sample Wilcoxon signed-rank test in both sessions in the case of both types of pointing gesture (Table 2). We found that the dogs’ performance on “Distal pointing” trials differed significantly from the chance level both in the “Black arm and black gloves” session and in the “Everyday T-shirt” session. On the contrary, in the case of the “Cross-forward pointing” their performance was on the chance-level (50%) in the case of both sessions (Figure 2).

The comparison of the dogs’ performances in the different sessions in the case of each pointing gesture showed that using these kinds of clothes there were no significant difference in the dogs performance, neither in the case of the “Distal pointing” (Wilcoxon matched pairs test;  $T (+) = 19.0$ ;  $p = 0.94$ ) nor in the case of the “Cross-forward pointing” (Wilcoxon matched pairs test;  $T (+) = 28.0$ ;  $p = 0.19$ ) (Figure 2).

There was no sign of learning during the testing with either of the pointing gestures. Comparing the performance on the first and the second session for both types of gestures we found no significant difference (Wilcoxon matched pairs test; “Distal pointing” ( $T (+) = 21.5$ ;  $p = 0.64$ ); “Cross-forward pointing” ( $T (+) = 32.0$ ;  $p = 0.06$ ).

To test our new hypothesis we compared the dogs’ performance in the “Black arm and black gloves” session and in the “Everyday T-shirt” session with their performance in the “Black arm” session in Study 1. We found that

dogs performed significantly better in the case of the “Black arm” session than in either of the two sessions of Study 2 (Wilcoxon matched pairs test; “Black arm” – “Black arm with black gloves”:  $T(+) = 57.0$ ;  $p < 0.05$ ; “Black arm” – “Everyday T-shirt”:  $T(+) = 75.0$ ;  $p < 0.01$ ).

Taken together these findings suggest that dogs utilize the forward cross pointing gesture, which does not protrudes the body torso, only if it is visibly (and asymmetrically) emphasized by the pointing hand.

**Table 2**  
*Results of the One-sample Wilcoxon signed-rank tests in Study 2.*

Type of the pointing gesture	One-sample Wilcoxon signed-rank test			
	Black arm and black gloves		Common clothes	
	<i>T</i>	<i>p</i>	<i>T</i>	<i>p</i>
Distal pointing	76.0	0.01	75.5	0.01
Cross-forward pointing	23.0	0.99	-36.0	0.13

### General Discussion

In recent literature dogs have been often portrayed as being very skilful in comprehending the human pointing gestures in two way choice tasks (Hare, Call, & Tomasello, 1998; Hare et al., 2002; Miklósi et al., 1998; Miklósi et al., 2005; Soproni et al., 2002). In a recent comparative study we have found that in general the performance of the dogs is comparable to that of 1.5-2 years of children (Lakatos et al., 2008). However, we have also found that dogs were clearly inferior in the case of the forward cross pointing gesture in which from the observer’s point of view the pointing arm and hand stays within the silhouette of the body. This finding led to the prediction that the protrusion of a body part of the body torso provides the key feature of the signal. Thus in contrast to children who by the age of 3 years attend the direction of the pointing index finger, dogs of all ages choose the “correct” side on the basis of a body part which “sticks out” from the silhouette of the body (Lakatos et al., 2008).

The present study shows that making the gesture visually more conspicuous can have an enhancing effect in cases where the gesture does not stick out from the body torso. In contrast to our expectations pointing in a long white sleeve (on a black background) did not improve the dogs’ performance, however black sleeves with nude (“white”) hands had an enhancing effect. This can be understood if one takes the observer’s point of view. The important difference is that if the whole body is covered by black colour then the nude (pointing) hand appears as a conspicuous asymmetrical feature on one side of the body. Even if the forward cross pointing gesture does not protrude from the body silhouette, in this case the nude hand has visually the same effect as a body part sticking out from the body torso. It may be hypothesized that the actual colour is of less importance, and similar effect could be found using the two colours in opposite ways. Making the pointing hand less conspicuous (by using a lighter background and/or using black gloves on black background) makes the effect invariably disappear.

These results serve as further confirmation for our earlier assumption (Lakatos et al., 2008) that the pointing index finger is not informative for the

dogs. Now it seems that the most informative aspect of the gesture is not the line of the pointing arm but a clearly visible patch, which appears conspicuously at one side of the body torso. It is not relevant which body part composes this patch as Lakatos et al. (2008) demonstrated i.e. dogs go to the wrong direction because of the protruding elbow in the case of the “Elbow cross pointing”. Presumably, the most important aspect is the resulting visual asymmetry represented by the signal which will guide the dog to the appropriate direction.

These results provide a simple explanation to the ability to generalize in dogs (see also Hare et al., 1998; 2002; Hare & Tomasello, 1999; Soproni et al., 2001; 2002), even in situations when the animal is naïve. Thus by this relatively simple rule dogs are able to obtain a flexible understanding of bodily gestures which, actually, are often not points but other cues including head or eye movements. Such sensitivity could also explain why dogs can learn very rapidly to attend minute directional eye movements (Miklósi et al., 1998).

We also have to emphasize that in this study both pointing gestures were distal and momentary and hereby very difficult to process. In these cases the subjects need to remember the pointing signal until they are allowed to make their choice. In addition the gesture is relatively far from the signalled bowl, thus the signal could not be “mistaken” for a manipulation of the baited bowl (local enhancement, to find a definition see Heyes, 1994 and Fritz, Bisenberger, & Kotrschal, 2000). In the comparative perspective it is important to see that various species have difficulties to find the place of a hidden reward on the basis of human cues if the cue appears in a distance. Both chimpanzees and rhesus monkeys are not able to find the hidden food in a Wisconsin task if the signalling marker object (“beacon”) is placed more than 20 cm away from the actual location (Jenkins, 1943; Murphy & Miller, 1955). Similar observations were reported for dogs in asocial situations (Milgram et al., 1999). Further, many other species tested are able to learn easily in delayed matching-to-sample tasks but still cannot learn to choose correctly in an object choice task if they have to choose on the basis of a momentary pointing gesture (i.e. capuchin monkeys or rhesus monkeys, see Anderson, Sallaberry, & Barbier, 1995; D'Amato & Worsham, 1972; Emery, Lorincz, Perrett, Oram, & Baker, 1997; Hampton & Hampstead, 2006; Tavares & Tomaz, 2002; Washburn, Hopkins, & Rumbaugh, 1989).

The observations of the present study also point to the importance of taking into account the visual abilities of the species in comparative work, apart from the possible differences or similarities of the underlying cognitive architecture. Although there is relatively little knowledge on dogs' visual abilities (see Miller & Murphy, 1995, for the only comprehensive review on the subject; Miklósi, 2007) we know that having only two types of cones in the retina (in comparison to the trichromatic humans) their visual world is less rich in colours. Further, recent experiments have shown that dogs are inferior (in comparison to humans) in distinguishing among shades of grey (Pretterer, Bubna-Littitz, Windischbauer, Gabler, & Griebel, 2004). However, in contrast to humans dogs are more sensitive to movement in the visual domain. Coile, Pollitz, and Smith (1987) have shown that their flicker fusion frequency occurs at higher values in comparison to humans. The ability to perceive rapid movements together with a restricted grey and colour vision might have

“forced” dogs to attend to different aspects of human communicative gesturing than is utilized by our species.

Thus, in everyday situations dogs might experience problems in comprehending the pointing signal because their restricted visual abilities, and not because they are not in the position to “comprehend” the signal. In this sense the results of this study should caution others not to jump to rash conclusions on species differences in communicative skills if there is little knowledge available on the perceptual abilities of the species under study. Given the differences in the visual processing apparatus between humans and dogs, it is less surprising to find differences at the level of performance which may or may not, in addition, be the outcome of differences in the cognitive skills.

In conclusion this study demonstrated that the most informative cue for the dogs is a clearly visible patch (irrespective of the body part what makes this patch), which appears conspicuously at one side of the body torso. It seems that the most important feature of the pointing gesture for the dogs is the visual asymmetry represented by the signal which guides their attention to the appropriate direction.

### References

- Agnetta, B., Hare, B., & Tomasello, M. (2000). Cues to food location that domestic dogs of 2 different ages do and do not use. *Animal Cognition*, **3**, 107-112.
- Anderson, J. R., Sallaberry, P., & Barbier, H. (1995). Use of experimenter-given cues during object-choice tasks by capuchin monkeys. *Animal Behaviour*, **49**, 201-208.
- Butterworth, G. E. & Itakura, S. (2000). How the eyes, head and hand serve definite reference. *British Journal of Developmental Psychology*, **18**, 25-50.
- Coile, D. C., Pollitz, C. H., & Smith, J. C., (1989). Behavioral determination of critical flicker fusion in dogs. *Physiology and Behavior*, **45**, 1087-1092.
- D'Amato, M. R. & Worsham, R. W. (1972). Delayed matching in the capuchin monkey with brief sample durations. *Learning and Motivation*, **3**(3), 304-312.
- Emery, N. J., Lorincz, E. N., Perrett, D. I., Oram, M. W., & Baker, C. I. (1997). Gaze following and joint attention in Rhesus monkeys (*Macaca mulatta*). *Journal of Comparative Psychology*, **111**(3), 286-293.
- Fritz, J., Bisenberger A., & Kotschal K. (2000). Stimulus enhancement in greylag geese: Socially mediated learning of an operant task. *Animal Behaviour*, **59**, 1119-1125.
- Hampton, R. R. & Hampstead, B. M. (2006). Spontaneous behavior of a rhesus monkey (*Macaca mulatta*) during memory tests suggests memory awareness. *Behavioural Processes*, **72**(2), 184-189.
- Hare, B., Brown, M., Williamson, C., & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science*, **298**, 1634-1636.
- Hare, B., Call, J., & Tomasello, M. (1998). Communication of Food Location Between Human and Dog (*Canis familiaris*). *Evolution of Communication*, **2**, 137-159.
- Hare, B. & Tomasello, M. (1999). Domestic dogs (*Canis familiaris*) use human and conspecific social cues to locate hidden food. *Journal of Comparative Psychology*, **113**, 173-177.
- Heyes, C. M. (1994). Social learning in animals: Categories and mechanisms. *Biological Reviews*, **69**, 207-231.
- Jenkins, W. O. (1943). A spatial factor in chimpanzee learning. *Journal of Comparative Psychology*, **35**, 81-84.

- Lakatos, G., Soproni, K., Dóka, A., & Miklósi Á. (2008). A comparative approach to dogs' (*Canis familiaris*) and human infants' understanding of various forms of pointing gestures. (submitted for publication).
- Miklósi, Á., Polgárdi, R., Topál, J., & Csányi, V. (1998). Use of experimenter-given cues in dogs. *Animal Cognition*, **1**, 113-121.
- Miklósi, Á., Pongrácz, P., Lakatos, G., Topál, J., & Csányi, V. (2005). A comparative study of the use of visual communicative signals in interactions between dogs (*Canis familiaris*) and humans and cats (*Felis catus*) and humans. *Journal of Comparative Psychology*, **119**, 179-186.
- Miklósi, Á., Topál, J., & Csányi, V. (2007). Big thoughts in small brains? Dogs as model for understanding human social cognition. *NeuroReport*, **18**, 467-471.
- Milgram, N. W., Adams, B., Callahan, H., Head, E., Mackay, B., Thirlwell, C., & Cotman, C. W. (1999). Landmark discrimination learning in the dog. *Learning and Memory*, **6**, 54-61.
- Miller, P. E. & Murphy, C. J. (1995). Vision in dogs. *Journal of American Veterinary Medical Association*, **207**, 1623-1634.
- Murphy, J. V. & Miller, R. E. (1955). The effect of spatial contiguity of cue and reward in the object-quality learning of rhesus monkeys. *Journal of Comparative Psychology*, **48**, 221-224.
- Povinelli, D. J., Bierschwale, D. T., & Cech, C. G. (1999). Comprehension of seeing as a referential act in young children, but not juvenile chimpanzees. *British Journal of Developmental Psychology*, **17**, 37-60.
- Pretterer, G., Bubna-Littitz, H., Windischbauer, G., Gabler, C., & Griebel, U. (2004). Brightness discrimination in the dog. *Journal of Vision*, **4**, 241-249.
- Riedel, J., Buttelmann, D., Call, J., & Tomasello, M. (2006). Domestic dogs (*Canis familiaris*) use a physical marker to locate hidden food. *Animal Cognition*, **9**, 27-35.
- Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2001). Comprehension of human communicative signs in pet dogs (*Canis familiaris*). *Journal of Comparative Psychology*, **115**, 122-126.
- Soproni, K., Miklósi, Á., Topál, J., & Csányi, V. (2002). Dogs' (*Canis familiaris*) responsiveness to human pointing gestures. *Journal of Comparative Psychology*, **116**, 27-34.
- Tavares, M. C. H., & Tomaz C. (2002). Working memory in capuchin monkeys (*Cebus apella*). *Behavioural Brain Research*, **131**(1-2), 131-137.
- Washburn, D. A., Hopkins, W. D., & Rumbaugh, D. M. (1989). Video-Task Assessment of Learning and Memory in Macaques (*Macaca mulatta*): Effects of Stimulus Movement on Performance. *Journal of Experimental Psychology-Animal Behavior Processes*, **15**(4), 393-400.

## **A Comparative Analysis of the Preference for Dark Environments in Five Teleosts**

**Caio Maximino, Thiago Marques de Brito,  
Fernanda Dias de Moraes, and Flávia Volta Cortes de Oliveira**  
*UNESP/Bauru, Brazil*

**Iza Batista Taccolini**  
*Universidade Federal do Pará, Brazil*

**Patrícia Mendes Pereira, Rafael Colmanetti, Rafael Lozano,  
Rangel Antônio Gazolla, Raquel Tenório, Renata Inah Tavares de  
Lacerda, and Silvana Terume Koshikene Rodrigues,**  
*UNESP/Bauru, Brazil*

**Soraia Valéria de Oliveira Coelho Lameirão**  
*Universidade Federal do Pará, Brazil*

**Alvaro Antonio Assis Pontes, Cynthia Ferreira Romão,  
and Vitor Miranda Prado,**  
*UNESP/Bauru, Brazil*

**Amauri Gouveia Jr.**  
*Universidade Federal do Pará, Brazil*

The present article tried to establish dark/light preference in five different species of teleosts. We proposed, using the data obtained with this method in zebrafishes (*Danio rerio*), Cardinal-tetras (*Paracheirodon axelrodi*), lambaris (*Astyanax altiparanae*), Nile tilapias (*Oreochromis niloticus*), guppies (*Poecilia reticulata*) and banded-knife fishes (*Gymnotus carapo*), that preference for dark environments is a reliable and low-cost index of anxiety/fear in those species. A scototactic pattern of exploration was found in all species, and the pattern of locomotion in the white environment suggests its aversiveness for those species, with the exception of *G. carapo* and *O. niloticus*. A comparative analysis uncovered species differences in approach-avoidance dimensions of the task. The data are discussed in terms of the behavioral ecology of the animals and prey-predator relationships, suggesting a link with predator defense strategies in teleost.

The dark/light preference model is already established as an “ethoexperimental” anxiety model in rodents (cf. Bourin & Hascöet, 2003). It is based on the natural aversive quality of brightly-lit environments for mice, shaping

This research was funded by CNPq – Brazil. The authors would like to thank Su Guo and Silvio Morato for the invaluable contribution with theoretical support, Leonardo Pereira Antunes for proof-reading the manuscript, and Eliane Gonçalves de Freitas for the supply of Nile tilapias. Correspondence concerning this article should be addressed to Amauri Gouveia Jr., Laboratório de Neurociências e Comportamento, Centro de Ciências Biológicas, Universidade Federal do Pará r. Augusto Corrêa 01, 66075-110, Belém/PA, Brazil. (agjunior@ufpa.br).

a conflict situation in which the animal must deal with its natural tendency to explore in face of the aversiveness of the environment. The rodent dark/light preference model is an *exploration* model, in the sense that it measures locomotor activity in both environments as an index of anxiety (Green & Hodges 1991; Prut & Belzung 2001; Belzung & Griebel 2003; Hascöet, Bourin, & Dhonnchadha, 2001); there are other, non-locomotor, models of anxiety (eg., inhibitory avoidance), but those are not of concern for the objectives in this article. Locomotor models of anxiety use exploratory behavior (defined as “a species-specific behaviour pattern concerned with the gathering of information” concerning the environment: O’Keefe & Nadel, 1978, p. 242) as an index of anxiety or anxiety-like states, relating it to foraging behavior or to appraisal of novel environmental stimuli (Belzung & Griebel 2001; File, 2001). The main rationale is that exploratory behavior would correlate with neophobia, a tendency to avoid new environments (Misslin & Cigrang 1986), forming a mixed pattern of behavior that consists in gradual approaching and exploration of the new environment associated with "scanning" and "risk-assessment" behaviors.

Ethoexperimental models use variables that are akin to the concept of “antipredator apprehension” from behavioral ecology (risk assessment, defensive distance, predatory imminence continuum, risk associated suppression of competing motivational systems; Kavaliers & Choleris, 2001). Apprehension is considered to reflect a motivational state, and is defined as “any reduction in attention to other activities (e. g., foraging, mate seeking) as a result of increasing the allocation of attention to detecting and/or responding to potential predators” (Kavaliers & Choleris, 2001, p. 579). Exploratory apprehensive behavior (denoting the pattern of exploratory behavior in such situations), in naturalistic situations as well as in locomotor-based anxiety models, is a compromise between predator avoidance and the benefit of an alternative activity (Ydenberg & Dill, 1986). Blanchard and Blanchard (1988) proposed the concept of “defensive distance”, analogous to the “antipredator apprehension” delineated in behavioral ecology. Defensive distance is a “statistical appraisal” of sorts that defines the probability of threat; it is a dimension controlling the type of defensive behavior observed (explosive attack, freezing, flight, risk assessment; Blanchard & Blanchard, 1990). Apprehension is understood as a continuum, and is defined as “any reduction in attention to other activities (...) as a result of increasing the allocation of attention to detecting and/or responding to potential predators” (Kavaliers & Choleris, 2001, p. 579); various levels of apprehension “lead prey to select a certain optimal level of vigilance, that is staying alert (i.e., scanning behavior, head up) so as to detect an approaching enemy, in response to their perceptions of a predator’s whereabouts” (Kavaliers & Choleris, 2001, p. 579). Wilson, Clark, Coleman, & Dearstyne (1994) defined a “shyness-boldness” continuum which they based on a ‘propensity to take risks’, which is analogous to individual differences in antipredator behavior. Thus, in a particular situation that would require antipredator behavior, and individual that performs more risky behaviors is considered bold, whereas one which avoids risk is called shy.

In the beginning of the 1980s, Gray (1982) related the O’Keefe & Nadel (1978) model of exploratory behavior to possible anxiety-generating effects of the

exploration models. The rationale in Gray's analyses is the existence of a putative *behavioral inhibition system* that detects a conflict between two motivations – in the mentioned cases, between neophobia and a motivation to explore – and switches behavioral programs in response to this conflict. As such, the behavioral inhibition system computes trade-offs between both approach and avoidance motivations, and switches behavior in accordance to the result of this computation. There have been some suggestions that this system involves multiple structures, including (in mammals) the periaqueductal gray, the medial hypothalamus, the amygdala, the septo-hippocampal system, and the cingulate and prefrontal cortices (McNaughton & Corr, 2004). The behavioral inhibition system is involved in what is called “fear” and “anxiety”, both innate and conditioned (Antoniadis & McDonald, 2001; Misslin, 2003; Rosen, 2004); indeed, fear has been defined as “a functional defense behavior system representing a part of the innate species-specific behavioral repertoire (ethogram), basic to the survival of individuals and species” (Misslin, 2003, p. 55).

The rodent dark/light preference model has been pharmacologically validated, and is sensitive to many parametric manipulations (Hascoët et al., 2001). One given manipulation is considered to have an antianxiety-like effect – ie, it has an effect similar to that of classic antianxiety drugs – if it facilitates exploratory activity, and this effect is dependent on the baseline level of the control group. The main variable analyzed is the number of transitions between the two compartments of the apparatus; it is indexical of activity/exploration, while the habituation over time and the time spent in each compartment reflects the aversiveness of the environment. The model is based on spontaneous activity, hence not requiring prior training of a response. The main advantages are ease of use and velocity of data production.

The proposed actinopterygian dark/light preference task is a modification of an experimental manipulation used in the 1970s to establish the effects of noradrenergic substances on the scotophobic (ie, dark-avoiding) behavior of pinealectomized or scotophobin-injected goldfish (Satake & Morton, 1979). Recently, the proposed model was used to establish dark/light preference in the zebrafish *Danio rerio* (Serra, Medalha, & Matioli, 1999), the bluegill *Lepomis macrochirus*, the crucian carp *Carassius langsdorfii* (Yoshida, Nagamine, & Uematsu, 2005), the goldfish *Carassius auratus* (Gouveia Jr et al., 2005; Yoshida et al., 2005), and in the poeciliid *Brachyrhaphis episcopi* (Brown, Jones, & Braithwaite, 2005), and to screen for the neurobehavioral effects of methylmercury (Gouveia Jr. et al., unpublished) and ethanol (Gerlai, Lahav, Guo, Rosenthal, 2000) on the zebrafish. The main advantage of this task is the presentation of a clear conflict situation for the fish; however, most models that investigated innate “fear”- and “anxiety”-like behavior in fishes did not use such conflict. With the exception of predator inspection tests (eg., Budaev, 1997a; Bleakley, Martell, & Brodie III, 2006; McCartt, Lynch Jr., & Johnson, 1997), most innate anxiety tests use the exploration of an open field to measure this variable (Crawshaw, 1975; Gervai & Csányi, 1985; Kleerekoper et al., 1970; Mikheev & Andreev, 1993; Mok & Munro, 1998; Warren & Callaghan, 1976), and aim to describe individual variability in “shyness-boldness” continua (Brown & Braithwaite, 2004; Brown et

al., 2005; Budaev, 1997b; Moretz, Martins, & Robison, 2007; Ward, Thomas, Hart, & Krause, 2004; Wilson, Coleman, Clark, & Biederman, 1993). This “shyness-boldness continuum” can be mapped to Budaev's (1997a, 1998) two dimensions of “temperament” in fishes (Activity-exploration and Fear-avoidance), which, in its turn, are trait instantiations of approach-avoidance state dimensions (Craig, 1917; McNaughton & Corr, 2004). *Ex hypothesi*, these dimensions are best analysable using conflict models.

The present article analyses dark/light preference as a reliable and low-cost ethoexperimental model of exploratory behavior and anxiety-like reactions in some species of teleost fish. We propose that scototaxis (preference for dark environments) can be used to assess stress, fear and anxiety in a wide array of fish species that present similar feeding ecology. We report the data obtained with this method in zebrafishes (*Danio rerio*); Cardinal-tetras (*Paracheirodon axelrodi*) and lambaris (*Astyanax altiparanae*); Nile tilapias (*Oreochromis niloticus*); and the banded-knife fish (*Gymnotus carapo*) (all references for taxonomy were taken from Helfman, Collette, & Facey, 1997). These species all present dark-colored backs; Table 1 also presents further information on ecogeographical and ecological contexts. These species were chosen for particular reasons: zebrafish is a “model animal” in embryology and genetics; characid fishes are very common neotropical species; Nile tilapias are commercially explored animals; guppies are common subjects in behavioral ecology; and *G. carapo* is a weakly electric fish, and analysing its preference for an environment could also shed some light on its visual status. Also, the phylogenetic relations between those species are well-resolved (Helfman et al., 1997). In all experiments, methodology was the same (as described in “General methods”, below).

**Table 1**

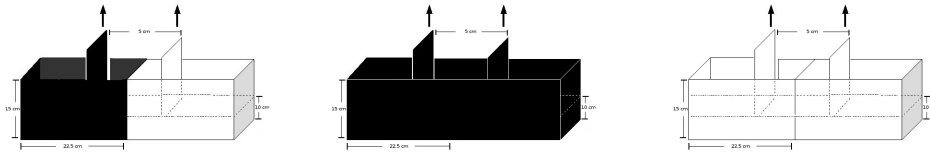
*Ecological and environmental profiles of the species chosen, with focus on ecogeography and turbidity/depth of the environment. Data was taken from FISHBASE (<http://www.fishbase.org>). Refer to text for more information.*

Family	Species	Environment	Climate	Ecogeography
Cyprinidae	<i>Danio rerio</i>	Benthopelagic	Tropical	Inhabits streams, canals, ditches, ponds and beels
Characidae	<i>Paracheirodon axelrodi</i>	Pelagic	Tropical	Inhabits middle water layers
Characidae	<i>Astyanax altiparanae</i>	Benthopelagic	Tropical	Inhabits streams, canals, ditches, ponds and beels
Gymnotidae	<i>Gymnotus carapo</i>	Benthopelagic	Subtropical	Inhabits turbid slow moving or standing waters
Poeciliidae	<i>Poecilia reticulata</i>	Benthopelagic	Tropical	Inhabits slow-flowing or still water near the margin of pools among vegetation.
Cichlidae	<i>Oreochromis niloticus</i>	Benthopelagic	Tropical	Inhabits the littoral zone of lakes, but was introduced in other environments as well.

## Methods

### Equipment

Three acrylic aquaria of equal measures (15x10x45 cm), with diverse colors according to the treatment (white (WW), black (BB), or half black/half white (BW); walls and bottom colored), with the water column kept to 10 cm. The colored material chosen was not reflective, in order to avoid the tendency of those animals which present shoaling and/or schooling tendencies to behave in relation to their own reflection. All the test aquaria contained sliding central doors, colored with the same color of the aquarium side, thereby defining a central compartment with 15x10x10 cm. For the banded-knife fish, aquaria dimensions were different. Since those animals measured  $10.0\pm 2.1$  cm at the time of testing, their test aquaria measured 15x10x55 cm (with the central compartment measuring 15x10x20 cm). During experiments, each aquaria was rotated after each trial, so as to eliminate orientation effects. Aquaria were illuminated by environmental light (60W light bulb, located at 1.80 m above the aquarium top) which kept illumination uniform and constant between trials. The aquaria are shown in Figure 1.



**Figure 1.** Schemata of the three test aquaria.

### Animal rearing

Animals were acquired in a local pet shop (zebrafish, Cardinal-tetra, guppy), in a local fisheries (lambari, banded-knifefishes), or at the fisheries in the hatchery at UNESP/São José do Rio Preto, and kept in the laboratory for at least two weeks before the experiments. All subjects were kept in collective maintenance aquaria (60x25x40 cm), with one tank per species; the water was reconstituted and buffered (Mydor Target 7.0 buffer), and the animals were acclimated for at least 7 days, with constant filtering, temperature control ( $27\pm 2$  °C), lighting (12/12 h, beginning of the cycle at 0700 pm) and feeding (Oscar Gold pellet ration). To prevent intervening motivations, all animals were fed once a day, and not fed in the day the experiment took place. Animals were not used for any other experiment besides those presented in this paper. Rearing and welfare conditions were in accordance with the standards set by the ASAB/ABS (2006) and COBEA/Brazil, and were approved by the Institution's Ethics Committee.

**Zebrafish.** 51 adult zebrafishes, of undetermined sex, were used in the experiment. Animals were acquired in a local pet shop (*AquaMundi*, Bauru/SP, Brazil), and measured  $2.63\pm 0.09$  cm at the time of the experiment.

**Cardinal-tetra.** 27 adult Cardinal-tetras, of undetermined sex, were used in the experiment. Animals were acquired in a local pet shop (*AquaMundi*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Subjects measured  $2.45\pm 1.0$  cm at the time of the experiment.

**Banded-knife fish.** 24 adult banded-knife fishes, of undetermined sex, were used in this experiment. Animals were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Subjects measured  $10.0\pm 2.1$  cm at the time of the experiment.

**Lambaris.** 24 adult lambaris, of undetermined sex, were used in this experiment. Subjects were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described above. Animals measured  $5.5\pm 0.8$  cm at the time of the experiment.

**Nile tilapias.** 60 adult Nile tilapias (30 male, 30 female), reared at the hatchery in UNESP/São José do Rio Preto, were used in this experiment. Male tilapias measured  $5.48\pm 0.65$  cm,

and females measured  $5.77 \pm 0.61$  cm, at the time of the experiment.

**Guppies.** 54 adult guppies (27 male, 27 female) were used in this experiment.

Subjects were bought in a local fisheries (*Fiu-Fiu*, Bauru/SP, Brazil), and transported to the laboratory for acclimation, as described in the “General methods” section. Female guppies measured  $2.81 \pm 0.64$  cm and male guppies measured  $3.07 \pm 0.31$  cm at the time of the experiment.

### **Procedure**

All experiments used the same procedure. Animals were randomly divided between the black/white, white/white and black/black treatments, measured, then subjected individually to a single observation session; each treatment was tested in a different aquarium, and animals were used for a single treatment. To avoid effects of repeated exposure to the apparatus, only a single session was run with each animal, and no replicates were made. Thus, the data collected refers to one session in each aquarium per species. The animals were placed in the central compartment for five minutes (habituation), after which the sliding doors were removed. The animals were then allowed to freely explore the aquarium. The session is terminated after 900 s. Total time in each environment, number of midline crossings, permanence time in each environment (total time/midline crossings), and latency for the first choice of compartment were recorded as variables. The first and third variables are measures of preference (Noakes & Baylis, 1990), while the second and fourth variables represent locomotor behavior (cf. Warren & Callaghan, 1976). It is hypothesised that preference variables are going to be affected by the type of aquaria, being significant only in the black/white aquarium, while locomotor variables will be greater in the white/white aquarium. As such, if the white environment is indeed aversive, locomotion will be heightened in the white/white aquarium, and animals will take refuge in the black environment of the black/white aquarium. Even though the confinement in the central compartment for habituation allowed for contact with both sides of the aquarium, data from those animals that did not cross the midline in the 900 s session were discarded, to prevent false positives in preference measures (Noakes & Baylis, 1990).

### **Statistical analyses**

Since normality and equal variances were not assumed, non-parametric statistics were used in all analyses. Preference variables (total time and permanence time in either compartment of an aquarium) were analysed with Wilcoxon’s signed rank tests. Motor variables (latency for first choice of compartment and number of midline crossings) were analyzed with one-way Kruskal-Wallis ANOVAs on Ranks, with aquarium as between-subjects factor, using Dunn’s post-hoc tests whenever appropriate. All *P*-values were set at 0.05. To assess species differences, two independent variables (ratio between total time in the black and the white compartments of the black/white aquarium [B:W]; and number of midline crossings in the white/white aquarium [AltW]) were analysed with one way Kruskal-Wallis ANOVAs on ranks. The same variables were used in the assessment of “shyness-boldness”, which was done using median rank values for the variables in each species. B:W was considered a proxy for preference for either environment, and AltW was considered a proxy for the aversiveness of the white environment. The data was analyzed using SigmaStat 3.1 (Systat Software, 2004).

### **Phylogenetic analysis**

To control for phylogenetic dependence effects (Blomberg & Garland, 2002), a test for phylogenetic signal was made using the PHYSIG procedure (Blomberg, Garland, & Ives, 2003). The PHYSIG procedure tests for phylogenetic signal by randomization test, computing a test statistic *K* based on a phylogenetically correct mean and mean-squared errors of the data (calculated using the variance-covariance matrix derived from the candidate tree). One trait (ratio between total time in the black compartment and total time in the white compartment of the black/white aquarium [B:W]) was analysed. Trait values were corrected for body size by computing a regression slope using phylogenetically independent contrasts (Felsenstein, 1985); the corrected value is the  $\log_{10}$  of the ratio between the original trait value and body size raised to the IC slope. Phylogenetically independent contrasts regression was made using the PDTREE.EXE module of the PDAP package (Garland et al., 1993). Branch lengths were calculated using the Philip GENDIST, with sequences

for cytochrome B mitochondrial DNA as distance parameters; sequences for cytochrome B were fetched from GENBANK. An Ornstein-Uhlenbeck model of trait evolution was assumed, and branch lengths were subsequently transformed by multiplying them by a value of  $d = 1.005$ . Transformed branch lengths were processed by the PDDIST.EXE module from the PDAP package to generate the variance-covariance matrix. After correction for body size effects and variance-covariance matrix determination, both data sets were parsed through the PHYSIG.M module of the PHYSIG package in order to determine phylogenetic signal. This should allow for selection between regular ANOVAs or Phylogenetic ANCOVAs for comparative data analysis (Garland et al., 1993).

## Results

Table 2 presents the results, across aquaria, between species for the variables analyzed.

### *Latency for first choice and number of midline crossings*

For *D. rerio*, statistical analysis showed a significant difference of latency time for the first choice of compartment ( $H[df = 2] = 9.63, p = 0.01$ ) as a function of the aquarium used for test, with smaller values in the black/black aquarium in comparison to the white/white aquarium. There was no statistical difference in the latency for first choice ( $H[df = 2] = 3.24, p = 0.20$ ) for *P. axelrodi*, *G. carapo* ( $H[df = 2] = 4.46, p = 0.11$ ), *A. altiparanae* ( $H[df = 2] = 3.14, p = 0.21$ ), female *O. niloticus* ( $H[df = 2] = 1.24, p = 0.54$ ) or female *P. reticulata* ( $H[df = 2] = 2.20, p = 0.33$ ). This same variable was significantly smaller in the black/white aquarium in male *O. niloticus* ( $H[df = 2] = 20.32, p < 0.01$ ) and male *P. reticulata* ( $H[df = 2] = 8.56, p = 0.01$ ).

The number of midline crossings was not statistically different in any of the aquaria for *D. rerio* ( $H[df = 2] = 3.847, p = 0.15$ ), male *O. niloticus* ( $H[df = 2] = 1.81, p = 0.41$ ) and male ( $H[df = 2] = 5.02, p = 0.08$ ) and female *P. reticulata* ( $H[df = 2] = 4.76, p = 0.09$ ). The white/white aquarium produced more midline crossings in *P. axelrodi* ( $H[df = 2] = 10.42, p = 0.01$ ), *G. carapo* ( $H[df = 2] = 10.67, p = 0.01$ ) and *A. altiparanae* ( $H[df = 2] = 15.52, p < 0.01$ ), while the black/black aquarium produced more locomotion in female *O. niloticus* ( $H[df = 2] = 14.22, p = 0.01$ ).

### *Total time and permanence time in each environment*

In *D. rerio*, there was no effect of aquarium in the black/black ( $W = -38, T+ = 20, T- = -58, P = 0.15$ ) and in the white/white ( $W = 56, T+ = 104.5, T- = -48.5, p = 0.19$ ) aquaria on total time measures. In the black/white aquarium more greater time spent was in the black compartment ( $W = -206, T+ = 2, T- = -208, p < 0.01$ ). Permanence time was also much greater in the black compartment in the black/white aquarium ( $W = -208, T+ = 1, T- = -209, p < 0.01$ ), but there was no difference in lateral preference in the black/black aquarium ( $W = -45, T+ = 23, T- = -68, p = 0.13$ ) or white/white aquarium ( $W = 44, T+ = 98.5, T- = -54.5, p = 0.31$ ).

*P. axelrodi* presented no lateral preference in the black/black aquarium as assessed by total time ( $W = -13, T+ = 21, T- = -34, p = 0.56$ ) and permanence time ( $W = -9, T+ = 23, T- = 32, p = 0.70$ ); the white / white aquarium generated no

**Table 2**

Variables of scototaxis in the five teleosts studied (mean±SD) in the proposed text. Refer to text for more information on each variable.

Species	Aquarium	Total time in compartment		Permanence time in compartment		Number of midline crossings	Latency to start exploration
		<i>Black/Left</i>	<i>White/Right</i>	<i>Black/Left</i>	<i>White/Right</i>		
<i>A. altiparanae</i>	Black/black	492,14±262,88	407,86±262,88	133,16±155,99	105,75±150,45	9,63±7,95	374,77±292,98
	White/white	520,61±101,39	387,38±103,67	9,19±3,83	6,42±1,67	62,88±19,95	144,17±139,33
	Black/white	628,46±75,23	333,47±119,73	12,81±5,49	6,15±1,53	56,75±24,18	167,64±131,89
<i>D. rerio</i>	Black/black	675,82±251,40	223,24±252,23	256,49±327,63	56,35±154,39	16,12±19,20	636,92±887,84
	White/white	329,65±321,45	570,35±321,45	117,97±294,02	89,99±214,25	25,47±25,27	156,71±242,59
	Black/white	845,12±92,03	41,29±72,95	208,53±113,45	13,41±17,51	180,21±134,36	544,18±392,52
<i>G. carapo</i>	Black/black	367,59±309,29	528,02±310,27	80,40±148,46	196,60±318,45	9,38±8,75	135,15±292,76
	White/white	323,70±259,86	566,72±264,11	28,38±44,36	78,73±111,75	17,50±11,62	20,34±17,60
	Black/white	626,60±114,68	253,94±100,54	20,45±17,70	6,36±2,70	45,33±22,11	140,34±265,26

<i>O. niloticus</i> , ♂	Black/black	612,11±303,23	265,67±308,63	400,86±414,64	149,28±292,63	7,22±7,53	79,67±101,21
	White/white	310,47±285,40	265,38±270,28	244,67±245,19	231,29±273,14	2,76±2,56	318,85±260,62
	Black/white	894,80±12,26	4,10±11,95	807,00±194,00	4,10±11,95	1,40±0,84	142,10±298,73
<i>O. niloticus</i> , ♀	Black/black	367,17±192,04	498,33±133,88	36,35±24,45	160,81±283,59	17,30±10,21	140,50±202,82
	White/white	320,71±398,79	555,43±384,40	248,93±335,48	498,86±383,62	2,14±1,35	112,71±131,43
	Black/white	697,50±345,71	201,00±345,55	437,42±316,77	192,38±350,12	2,50±2,42	219,40±324,72
<i>P. axelrodi</i>	Black/black	409,50±274,89	490,50±274,89	62,16±96,94	129,15±170,96	11,50±8,50	
	White/white	320,67±263,48	410,67±265,84	8,21±11,55	85,18±175,39	43,33±38,83	
	Black/white	367,23±265,25	364,64±255,16	114,47±235,34	79,31±121,32	179,10±275,87	

lateral preference, either in the total time ( $W = 1, T+ = 11, T- = -10, p = 1.0$ ) or permanence time measures ( $W = 5, T+ = 13, T- = 8, p = 0.69$ ). Time spent in the black compartment of the black/white aquarium, though, was much greater than the time spent in the white compartment, as assessed by either total time ( $W = -118, T+ = 9, T- = -127, p = 0.01$ ) or permanence time ( $W = -114, T+ = 11, T- = -125, p = 0.002$ ) variables.

In *G. carapo*, there were no differences between total time or permanence time in left or right compartments in the black/black (total time:  $W = 8, T+ = 22, T- = 14, p = 0.64$ ; permanence time:  $W = 12, T+ = 24, T- = 12, p = 0.46$ ) and white/white aquaria (total time:  $W = 6, T+ = 21, T- = -15, p = 0.74$ ; permanence time:  $W = 6, T+ = 21, T- = -15, p = 0.74$ ). Total time was greater in the black compartment of the black/white aquarium ( $W = -30, T+ = 3, T- = -33, p = 0.04$ ), but permanence time was not statistically significantly different in between compartments ( $W = -26, T+ = 5, T- = 31, p = 0.08$ ).

*A. altiparanae* presented a similar pattern of exploration as the other species; Wilcoxon's signed rank tests for total time in either black/white or left/right compartment in each aquaria resulted as follows: black/black, non-significant ( $W = -8, T+ = 14, T- = 22, p = 0.64$ ); white/white, non-significant ( $W = -24, T+ = 6, T- = -30, p = 0.11$ ); black/white, significant ( $W = -36, T+ = 0, T- = -36, p = 0.01$ ). When permanence time in either black/white or left/right compartments was tested, results were as follows: black/black, non-significant ( $W = -16, T+ = 6, T- = -22, p = 0.22$ ); white/white, non-significant ( $W = -24, T+ = 6, T- = -30, p = 0.11$ ); black/white, significant ( $W = -36, T+ = 0, T- = -36, p = 0.01$ ).

In male Nile tilapias, there was no statistically significant lateral preference in the black/black (total time:  $W = -29, T+ = 8, T- = -37, p = 0.10$ ; permanence time:  $W = -29, T+ = 8, T- = -37, p = 0.10$ ) or white/white aquaria (total time:  $W = -6, T+ = 15, T- = -21, p = 0.74$ ; permanence time:  $W = -2, T+ = 17, T- = 19, p = 0.945$ ). Animals spent significantly greater time in the black compartment in the black/white aquarium (total time:  $W = -55, T+ = 0, T- = -55, p = 0.002$ ; permanence time:  $W = -55, T+ = 0, T- = -55, p = 0.002$ ).

In the preference measures, female *O. niloticus*' behavior was similar to that of males: the black/black aquarium produced no significant lateral preference (total time:  $W = 9, T+ = 32, T- = -23, p = 0.70$ ; permanence time:  $W = 15, T+ = 35, T- = -20, p = 0.50$ ). Similarly, neither did the white/white aquarium (total time:  $W = 6, T+ = 17, T- = 11, p = 0.69$ ; permanence time:  $W = 8, T+ = 18, T- = -10, p = 0.58$ ). The black/white aquarium produced a consistent and statistically significant preference for dark environments (total time:  $W = -47, T+ = 4, T- = -51, p = 0.01$ ; permanence time:  $W = -43, T+ = 6, T- = 49, p = 0.03$ ).

No statistically significant lateral preference in the black/black (total time:  $W = -1, T+ = 7, T- = -8, p = 1.0$ ; permanence time:  $W = -1.0, T+ = 7.0, T- = -8.0, p = 1.0$ ) or white/white aquaria (total time:  $W = -9, T+ = 6, T- = -15, p = 0.44$ ; permanence time:  $W = -9, T+ = 6, T- = -15, p = 0.44$ ) was observed in male *P. reticulata*. Animals spent significantly greater time in the black compartment in the black/white aquarium (total time:  $W = -104, T+ = 8, T- = -112, p = 0.002$ ; permanence time:  $W = -102, T+ = 9, T- = -111, p = 0.002$ ).

Female *P. reticulata* did not present lateral preference in the black/black (total time,  $W = -9$ ,  $T_+ = 6$ ,  $T_- = -15$ ,  $p = 0.44$ ; permanence time,  $W = -11$ ,  $T_+ = 5$ ,  $T_- = -16$ ,  $p = 0.31$ ) or white/white aquaria (total time,  $W = -11$ ,  $T_+ = 5$ ,  $T_- = -16$ ,  $p = 0.31$ ; permanence time,  $W = -9$ ,  $T_+ = 6$ ,  $T_- = -15$ ,  $p = 0.44$ ); female guppies spent more time in the black compartment of the black/white aquarium (total time:  $W = -72$ ,  $T_+ = 24$ ,  $T_- = -96,000$ ,  $p = 0.04$ ; permanence time:  $W = -68$ ,  $T_+ = 26$ ,  $T_- = -94$ ,  $p = 0.06$ ).

### Comparative analysis

Since the PHYSIG analysis did not present a statistically significant signal, yielding p-values of 0.76 and 0.37 for traits B:W and AltW respectively (Table 3), the authors opted for a conventional ANOVA approach to compare species. The same variables analysed within-species were analysed between-species; however, since the data presented above demonstrate preference for dark environments in the species studied, only data from the black/white aquarium were used. To facilitate comparison, a proxy variable was made, comprising the ratio of time spent in the black compartment and time spent in the white compartment of the black/white aquarium (B:W). A statistically significant difference between species was found in the B:W variable ( $H_{[df=7]}=17.97$   $P=0.01$ ). As can be inferred from Table 2, the Cardinal-tetra presented much higher B:W ratios than the other species, while male and female tilapias presented smaller B:W ratios than the other species.

**Table 3**

*Parameters of the PHYSIG phylogenetic signal estimation. K is the ratio between expected  $MSE_0/MSE$  and observed  $MSE_0/MSE$  with all the parameters set. The tree branches were re-scaled using a transformation parameter  $d$  that was estimated by the Ornstein-Uhlenbeck process simulation. Refer to text for more information.*

Trait	Expected $MSE_0/MSE$	Observed $MSE_0/MSE$	K	Mean MSE permuted data	SD MSE permuted data	Skew MSE permuted data	$p$
B:W	1.13	0.46	0.41	1.53	0.58	-0.09	0.76
AltW	1.13	0.70	0.62	0.54	0.14	0.00	0.37

### Discussion

The present data allow us to analyze the parameters of the dark/light preference model in different species of fish. The preference for a dark environment was found in all of the species that were studied, even though they come from different taxa. The pattern of locomotion suggests the white environment is more aversive for those species, with the exception of male and female *O. niloticus* and female *P. reticulata*. The use of redundant variables for both preference and locomotion was intentional; further studies are needed to discriminate whether those variables have differential sensitivity to different treatments, such as parametrical manipulations in the aquaria, rearing conditions, or pharmacological screening. There was also a pattern of sexual dimorphism in

dark/light preference in the Nile tilapia and in the guppy. The magnitude of differences in preference and locomotion variables varies among the species studied, which is consistent with previous experiments with dark/light preference in fishes. This convergence points to species-specific emotional behavior in teleosts (Maximino & Gouveia Jr., *submitted*; Shaklee, 1963).

The observation that animals spent more time in the black environment of the black/white aquarium is representative of preference for dark environments. The greater degree of exploration in the white/white aquarium, as assessed by higher number of midline crossings in this aquarium for the majority of species studied, could be an index of the aversiveness of light environments; this avoidance of bright environments was observed in negative phototaxis experiments (eg. Fernö, Huse, Juell, & Bjordal, 1995; Hafeez & Quay, 1970). It is possible that, if the species did not present greater number of midline crossings, a different pattern of swimming in the white environment was present – for example, a pattern of freezing in the corners of the aquarium (observed in the guppy in Budaev's (1997a) battery of experiments). This is supported by the comparative data on B:W ratios (see below), since those species that presented smaller number of midline crossings in the white/white aquarium also tended to present smaller B:W ratios, an indication that they spent more time in the white environment of the black/white aquarium as well. However, since a detailed observation of the swimming ethogram was not possible, this derivative hypothesis cannot be answered by the present data.

The longer period of latency some animals exhibited to start exploring the white/white aquarium could also be indexical of the aversiveness of this environment, in a manner analogous to the aversiveness of open arms in the rodent elevated plus-maze model (eg., Pellow et al., 1985), a extensively used anxiety model. However, since this measure was statistically different between aquaria in only two species, it should not be suitable for analyses of locomotion and preference in this test. Since the first latency is probably a reaction to confinement stress (cf. Sadler, Pankhurst, Pankhurst, & King, 2000), it is more probable that this heightened latency to start exploring in the white/white aquarium, observed in *D. rerio* and male *O. niloticus*, is an additive effect of the modulation of this stressful/aversive environment and the confinement stress in the animals' exploratory behavior.

The fact that female *O. niloticus* presented consistent preference for dark environments, but their locomotion was heightened by the black/black aquarium, suggests that there is a sexual dimorphism in the behavior of Nile tilapia in the dark/light preference model. This greater locomotion could also be an artifact of the statistical comparisons, since it is possible that female tilapias are more prone to “freezing” in white environments. This effect could also be related to hormonal status, since this is a variable that influences rodent behavior in the dark/light apparatus (Timothy, Costall, & Smythe, 1999), and since teleosts' brains are very prone to estrogen modulation (e.g. Albert, Crampton, Thorsen, & Lovejoy, 2004; Kim, Stumpf, Sar, & Christine, 1978). There is also the possibility of species differences, unmasked by sex differences, in the pattern of swimming (see below). Female guppies, on the other hand, did not present heightened locomotion in any

of the aquaria. The preference parameters for female *O. niloticus* and *P. reticulata* tend to be more pronounced than those of males from the same species, which could be analogous to sexual dimorphism in shyness-boldness.

From a comparative point of view, the present data is very complex. Since no significant phylogenetic signal was found, it is not possible to determine if the species differences observed were due to phylogenetic or other ecological factors. Overall, however, the differences observed in the variable analyzed are not better explained by phylogenetic inertia, i.e., the tendency of closely related species to present similar phenotypes. This can be seen, for example, in the great difference observed in B:W ratios between the characids *P. axelrodi* and *A. altiparanae*. The perciform Nile tilapia, which was the outgroup for the clades chosen, presented a B:W ratio that was very similar to that of the ostariophysid *D. rerio*, *A. altiparanae* and *G. carapo*. Those differences are also not better explained by differences between wild-caught vs. laboratory-reared animals: recently wild-caught species such as *A. altiparanae* and *G. carapo* presented similar patterns as the other species, with the exception of *P. axelrodi*. Nonetheless, since all species studied presented a similar pattern of scototactic behavior, the present model should be suitable for study of fear- and anxiety-like behavior in teleosts.

It is interesting to notice that, even though *G. carapo* is a weakly electric fish, relying on electric organ discharges (EODs) to orient itself and having vestigial eyes, banded-knife fishes presented a very similar pattern of behavior in the proposed test as other species. *G. carapo* is also bigger than the other species studied, including the perciforms used (*P. reticulata* and *O. niloticus*); since perciforms are highly visually-guided (Kotrschal, van Staaden, & Huber, 1998), it is probable that banded-knife fishes present at least vestigial vision, being able to discriminate contrast.

This pattern of scototactic (ie, darkness-seeking) behavior in different taxa of teleosts could be understood as an adaptation of those species in terms of a crypsis-based defense against predation (e.g. Fuiman & Magurran, 1994; Shaklee, 1963), allowing for the inclusion of this model in the analysis of anxiety/fear systems made by McNaughton & Corr (2004). Prey often respond to a predator's presence (or its possible presence) by increasing the use of refuges (Abrams, 1986, 1984; Blanchard & Blanchard, 1988; Blanchard, et al., 1993; Lima & Dill, 1990); the preference for dark environments observed in the present work is interpreted in this sense. In the bluegill sunfish *Lepomis macrochirus*, the preference for dark environments is conditioned by the light levels in the bright compartment, as well as the presence of a predator in any compartment (McCart et al., 1997), suggesting that this strategy is based on a trade-off between neophobia/predator avoidance, in one hand, and other environmental variables, including the possibility of crypsis-based defense behavior. The fact that, in the zebrafish, this behavioral pattern is altered by acute treatment with classic anti-anxiety drugs (Su Guo & Billy Lau, 2006, *personal communication*), as well as ethanol (Gerlai et al., 2000), presents further support for the proposition that this apparatus generates unconditioned anxiety-like responses in teleosts. Since there are many functional similarities in the central nervous system of teleosts and other vertebrates, at least in the systems that regulate emotional responses (Striedter, 2005), it is probable that the

behavioral pattern found in these species is mediated by monoaminergic and amino acid neurotransmitters (Su Guo & Billy Lau, 2006, *personal communication*).

Taken together, these data demonstrate 1) The presence of a preference for dark environments in the species studied; 2) The aversiveness of the white environment for all species, except *O. niloticus*; 3) Species differences in the preference for darkness; and 4) The suitability of this model for cross-species comparison on “boldness-shyness” and emotional reactivity traits. In conjunction with the observations that antianxiety substances alter the behavior of zebrafish in the black/white box, the authors conclude that this model could be suitable for studying emotion-like behaviors in teleost fishes.

## References

- Albert, J. S., Crampton, W. G. R., Thorsen, D. H., & Lovejoy, N. R. (2004). Phylogenetic systematics and historical biogeography of the Neotropical electric fish *Gymnotus carapo* (Teleostei: Gymnotidae). *Systematics and Biodiversity* **2**, 375-417.
- Antoniadis, E. A. & McDonald, R. J. (2001). Amygdala, hippocampus, and unconditioned fear. *Experimental Brain Research* **138**, 200-209.
- ASAB/ABS (2006). Guidelines for the treatment of animals in behavioural research and teaching. *Animal Behaviour* **71**, 245-253.
- Belzung, C. & Griebel, G (2001). Measuring normal and pathological anxiety-like behaviour in mice: A review. *Behavioural Brain Research* **125**, 141-149.
- Blanchard, R.J. & Blanchard, D. C. (1990). Anti-predator defense as models of animal fear and anxiety. In: P. F. Brain, S. Parmigiani, R. J. Blanchard, D. Mainardi, editors. *Fear and Defence*, pp. 89-108. Chur: Harwood Academic Publishers.
- Blanchard, R.J., Yudko, E.B., Rodgers, R.J., & Blanchard, D.C. (1993). Defense system psychopharmacology: An ethological approach to the pharmacology of fear and anxiety. *Behavioral Brain Research*, **20**, 155-165.
- Bleakley, B. H., Martell, C. M., & Brodie III, E. D. (2006). Variation in anti-predator behavior among five strains of inbred guppies, *Poecilia reticulata*. *Behavior Genetics* **36**, 783-791.
- Blomberg, S. P. & Garland, T. Jr. (2002). Tempo and mode in evolution: Phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology* **15**, 899-910.
- Blomberg, S. P., Garland, T. Jr., & Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evolution* **57**, 717-745.
- Bourin, M. & Hascoët, M. (2003). The mouse light/dark box test. *European Journal of Pharmacology* **463**, 55-65.
- Brown, C. & Braithwaite, V. A. (2004). Size matters: A test of boldness in eight populations of the poeciliid *Brachyraphis episcopi*. *Animal Behaviour* **68**, 1325-1329.
- Brown, C., Jones, F., & Braithwaite, V. (2005). In situ examination of boldness-shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. *Animal Behaviour* **70**, 1003-1009.
- Budaev, S. V. (1997a). “Personality” in the guppy (*Poecilia reticulata*): A correlational study of exploratory behavior and social tendency. *Journal of Comparative Psychology* **111**, 399-411.
- Budaev, S. V. (1997b). Alternative styles in the European wrasse, *Symphodus ocellatus*: Boldness-related schooling tendency. *Environmental Biology of Fishes* **49**, 71-78.

- Budaev, S. V. (1998). How many dimensions are needed to describe temperament in animals: A factor reanalysis of two data sets. *International Journal of Comparative Psychology* **11**, 17-29.
- Craig, W. (1917). Appetites and aversions as constituents of instincts. *Proceedings of the National Academy of Sciences USA* **3**, 685-688.
- Crawshaw, L. I. (1975). Twenty-four hour records of body temperature and activity in bluegill sunfish (*Lepomis macrochirus*) and brown bullheads (*Ictalurus nebulosus*). *Comparative Biochemistry and Physiology* **51A**, 11-14.
- Felsenstein J (1985). Phylogenies and the comparative method. *American Naturalist* **125**, 1-15.
- Fernö, A., Huse, I., Juell, J.-E. & Bjordal, Å (1995). Vertical distribution of Atlantic salmon (*Salmo salar* L.) in net pens: Trade-off between surface light avoidance and food attraction. *Aquaculture* **132**, 285-296.
- File, S. E. (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioral Brain Research* **125**, 151-157.
- Fuiman, L. A. & Magurran, A. E. (1994). Development of predator defences in fishes. *Reviews in Fish Biology and Fisheries* **4**, 145-183.
- Gerlai, R., Lahav, M., Guo, S., & Rosenthal, A. (2000). Drinks like a fish: Zebra fish (*Danio rerio*) as a behavior genetic model to study alcohol effects. *Pharmacology, Biochemistry and Behavior* **67**, 773-782.
- Gervai, J. & Csányi, V. (1985). Behavior-genetic analysis of the paradise fish, *Macropodus opercularis*. I. Characterization of the behavioral responses of inbred strains in novel environments: A factor analysis. *Behavior Genetics* **15**, 503-519.
- Gouveia Jr., A., Lima, S. M. A., Bastos da Silva, A. C., Oliveira, F. V. C., Pereira, P. M., Rodrigues, S. T. K., Ribeiro, C. A. O., Ribas, S., Costa, J. R. M., Vicenzi, T. R., Silva Filho, M., Maximino, C., & Ventura, D. S. F. *Methylmercury LD50 and its effects on a fish anxiety model*. Unpublished manuscript.
- Gray, J. A. (1982). *The Neuropsychology of Anxiety: An Enquiry Into the Functions of the Septo-hippocampal System*. New York: Oxford University Press.
- Green, S. & Hodges, H. (1991). Animal models of anxiety. In: *Behavioral Models in Psychopathology: Theoretical, Industrial and Clinical Perspectives* (Ed. by P. Willner), pp. 21-49. Cambridge: Cambridge University Press.
- Hafeez, M. A. & Quay, W. B. (1970). The role of the pineal organ in the control of phototaxis and body coloration in rainbow trout (*Salmo gairdneri*, Richardson). *Journal of Comparative Physiology A* **68**, 403-416.
- Hascoët, M., Bourin, M., & Dhonnchadha B. A N. (2001). The mouse light-dark paradigm: A review. *Progress in Neuropsychopharmacology and Biological Psychiatry* **25**, 141-166.
- Helfman, G. S., Collette, B. B., & Facey EF. (1997). *The Diversity of Fishes*. Berlin: Blackwell Publishing.
- Kavaliers, M. & Choleris, E. (2001). Antipredator responses and defensive behavior: Ecological and ethological approaches for the neurosciences. *Neuroscience and Biobehavioral Reviews* **25**, 577-586.
- Kleerekoper, H., Timms, A. M., Westlake, G. F., Davy, F. B., Malar, T., & Anderson, V. M. (1970). An analysis of locomotor behaviour of goldfish (*Carassius auratus*). *Animal Behaviour* **18**, 317-330.
- Kim, Y. S., Stumpf, W. E., Sar, M., & Christine, M. (1978). Estrogen and androgen target cells in the brain of fishes, reptiles and birds: Phylogeny and ontogeny. *Integrative and Comparative Biology* **18**, 425-433.
- Kotrschal, K., van Staaden, M. J., & Huber, R. (1998). Fish brains: Evolution and environmental relationships. *Reviews in Fish Biology and Fisheries*, **8**, 373-408.

- Lima, S. L., Dill, L. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology* **68**, 619-640.
- Maximino, C. & Gouveia Jr., A. *Phylogenetical distribution of scototaxis in 11 teleost species*. Unpublished manuscript.
- McCartt, A. L., Lynch Jr., W. E., & Johnson, D. L. (1997). How light, a predator, and experience influence bluegill use of shade and schooling. *Environmental Biology of Fishes* **49**, 79-87.
- McNaughton, N. & Corr, P. J. (2004). A two-dimensional neuropsychology of defense: Fear/anxiety and defensive distance. *Neuroscience and Biobehavioral Reviews* **28**, 285-305.
- Mikheev, V. N. & Andreev, O. A. (1993). Two-phase exploration of a novel environment in the guppy, *Poecilia reticulata*. *Journal of Fish Biology* **42**, 375-383.
- Misslin, R. (2003). The defense system of fear: Behavior and neurocircuitry. *Neurophysiologie Clinique* **33**, 55-66.
- Misslin, R. & Cigrang, M. (1986). Does neophobia necessarily imply fear or anxiety? *Behavioural Processes* **12**, 45-50.
- Mok, E. Y. & Munro, A. D. (1998). Effects of dopaminergic drugs on locomotor activity in teleost fish of the genus *Oreochromis* (Cichlidae): Involvement of the telencephalon. *Physiology and Behavior* **64**, 227-234.
- Moretz, J. A., Martins, E. P., & Robison, B. D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral Ecology*
- Noakes, D. L. G. & Baylis, J. R. (1990). Behavior. In: C. B. Schreck & P. B. Moyle (eds.), *Methods for Fish Biology*, pp. 555-584. Bethesda: American Fisheries Society.
- O'Keefe, J. & Nadel, L. (1978). *The Hippocampus as a Cognitive Map*. Oxford: Clarendon Press.
- Prut, L. & Belzung, C. (2003). The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: A review. *European Journal of Pharmacology* **463**, 3-33.
- Rosen, J. B. (2004). The neurobiology of conditioned and unconditioned fear: A neurobehavioral system analysis of the amygdala. *Behavioral and Cognitive Neuroscience Reviews* **3**, 23-41.
- Sadler, J., Pankhurst, N. W., Pankhurst, P. M., & King, H. (2000). Physiological stress responses to confinement in diploid and triploid Atlantic salmon. *Journal of Fish Biology* **56**, 506-518.
- Satake, N. & Morton, B. E. (1979). Scotophobin A causes dark avoidance in goldfish by elevating pineal N-acetylserotonin. *Pharmacology, Biochemistry and Behavior* **10**, 449-456.
- Serra, E. L., Medalha, C. C., & Matioli R. (1999). Natural preference of zebrafish (*Danio rerio*) for a dark environment. *Brazilian Journal of Medical and Biological Research* **32**, 1551-1553.
- Shaklee, A. B. (1963). Comparative studies of temperament: Fear responses in different species of fish. *Journal of Genetic Psychology* **102**, 295-310.
- Striedter, G. F. (2005). *Principles of Brain Evolution*. Sunderland: Sinauer Associates.
- Timothy, C., Costall, B., & Smythe JW. (1999). Effects of SCH23390 and raclopride on anxiety-like behavior in rats tested in the black-white box. *Pharmacology, Biochemistry and Behavior* **62**, 323-327.
- Ward, A. J. W., Thomas, P., Hart, P. J. B., & Krause, J. (2004). Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* **55**, 561-568.
- Warren, E. W. & Callaghan, S. (1976). Individual differences in response to an open field test by the guppy *Poecilia reticulata* (Peters). *Journal of Fish Biology* **7**, 105-113.

- Wilson, D. S., Coleman, K., Clark, A. B., & Biederman, L. (1993). Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): An ecological study of a psychological trait. *Journal of Comparative Psychology* **107**, 250-260.
- Wilson, D. S., Clark, A. B., Coleman, K., & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. *Trends in Ecology and Evolution* **9**, 442-446.
- Ydenberg, R. C. & Dill, L. M. (1986). The economics of fleeing from predators. *Advances in the Study of Behavior* **16**, 229-249.
- Yoshida, M., Nagamine, M., & Uematsu, K (2005). Comparison of behavioral responses to a novel environment between three teleosts, bluegill *Lepomis macrochirus*, crucian carp *Carassius langsdorfii*, and goldfish *Carassius auratus*. *Fisheries Science*, **71**, 314-319.

## **A Brief Report: The Use of Experimenter-Given Cues by South American Sea Lions**

**Lauren E. Highfill**  
*Eckerd College, U.S.A.*

**Harald Schwammer**  
*Tiergarten Schönbrunn, Austria*

**Stan A. Kuczaj**  
*University of Southern Mississippi, U.S.A.*

South American sea lions (*Otaria byrona*) were tested in an object choice task in which they had to use one of the following experimenter-given cues to choose the correct object for a reward: (1) the experimenter pointed and gazed at the object, (2) the experimenter pointed at the object, (3) the experimenter gazed at the object, (4) the experimenter placed a marker on the object, or (5) the experimenter presented a replica of the target object. The sea lions were able to successfully use three of these five cues. These results demonstrate that relatively little experience with human trainers was necessary for the subjects to perceive and act on select attentional cues given by a human experimenter. These results indicate that sea lions are able to interpret certain untrained communicative cues successfully.

Humans often use pointing and gazing to communicate their attention to something of interest. The ability to attribute attention based on such communicative cues has also been studied in a variety of nonhuman species (e.g., chimpanzees: Call, Hare & Tomasello, 1998; dogs: McKinley & Sambrook, 2000; dolphins: Herman, et al., 1999; Tschudin, et al., 2001; fur seals: Scheumann & Call, 2001; horses: McKinley & Sambrook, 2000). Within this comparative approach, two communicative behaviors have received special attention: point and gaze direction. Unlike symbols, these cues are not totally arbitrary but instead contain a resemblance to the action they represent (Tschudin, 2001). For example, Tomasello and colleagues (1998) found that individuals from each of five primate species were capable of following the gaze of conspecifics to an outside object: chimpanzees (*Pan troglodytes*), sooty mangabeys (*Cercocebus atys torquatus*), rhesus macaques (*Macaca mulatta*), stump-tail macaques (*M. arctoides*), and pigtail macaques (*M. nemestrina*). The experimenters had one individual look at a food item on display and then observed the reaction of a second individual (the subject) that was looking in the direction of the first individual. They found that subjects from all five species consistently followed the gaze of conspecifics to the food. Another study indicated that 12 of 16 domestic dogs were able to use pointing gestures performed by humans to successfully choose between two cups for a food reward (McKinley & Sambrook, 2000).

One experimental model often used by researchers to study the comprehension of communicative signs is the object-choice task (e.g. Call et al., 1998; Kaminski et al., 2005; McKinley & Sambrook, 2000). In this paradigm, an

We thank Ludwig Fessl and Doris Preininger for their invaluable help with this project. Correspondence concerning this article should be addressed to Lauren Highfill, Department of Psychology, Eckerd College, 5400 54<sup>th</sup> Avenue South, St. Petersburg, FL, 33711, U.S.A. (highfile@eckerd.edu).

animal must use a variety of communicative signals to choose the location of a food reward. Surprisingly, a number of primate species have performed poorly on this type of task (Itakura & Anderson, 1996). However, Itakura and Tanaka (1998) found that chimpanzees and one orangutan were successful at the object-choice task when it required them to attend to a number of gestural cues presented by humans (e.g. tapping, pointing, gazing, and glancing). Success in such tasks is not limited to the great apes. For example, McKinley and Sambrook (2000) reported that horses have a limited ability to use communicative cues given by humans, and that dogs have substantial success in such situations. Their research indicated that dogs can successfully use human pointing, head orientation and eye gaze as communicative cues. Similar results were found with marine mammal species. Bottlenose dolphins were able to use human pointing gestures for selecting one of three objects, including objects located behind the subject (Herman et al., 1999). Fur seals were able to use point and gaze, point only, gaze only, and asymmetrical point and gaze (experimenter sits closer to non-target object) to correctly choose between two objects for a reward (Scheumann & Call, 2004).

One criticism of these studies has concerned the fact that most subjects studied are living under human care, and so have had ample opportunity to observe humans using pointing or gazing. For example, domestic dogs may be exposed to many pointing gestures when living within a human family setting. Also, some apes that have been studied had extensive human contact (see Miklósi & Soproni, 2005, for a review), suggesting that an individual's previous social experience influences the extent to which an animal can comprehend points and gazes. It can be difficult to evaluate the amount of inadvertent previous exposure an enculturated animal might have because pointing is such a natural behavior in humans. This is especially true for animals involved in behavioral training (e.g. dolphins: Herman et al., 1999; Tschudin et al., 2001 or seals: Scheumann & Call, 2001). As a result, some species have been tested using communicative cues that would not have observed in normal human interactions. Novel cues have included placing a marker (i.e. a wooden block) on top of the correct target or displaying a replica of the correct target (Tomasello, Call, & Hare, 1998; Tschudin et al., 2001; Scheumann & Call, 2004).

The goal of the current study was to investigate whether South American sea lions (*O. byrona*) would be able to use communicative cues in the absence of formal training to correctly choose a target within an object-choice task. To date, there have been no studies addressing this ability in sea lions. This study investigated a number of cues varying in difficulty, none of which had been explicitly trained. However, the sea lions engaged in daily interaction with humans, so some of the cues to be investigated, such as pointing, may have been produced by their human caretakers. In addition to the normally occurring cues that the sea lions may have observed while humans interacted with one another, the current study also examined sea lion comprehension of novel communicative cues. Previous studies with other species have indicated limited success with these more difficult communicative cues, especially, marker and replica. However, past research would suggest that sea lions are prime candidates for the replica study due to their previous success on match-to-sample tasks (Kastak & Schusterman, 1994).

## Method

### *Subjects*

Three female South American sea lions (*O. byrona*) housed at Tiergarten Schönbrunn in Vienna, Austria participated in this study. Carmen and Moneta, both approximately 10 years old, were born in the wild whereas Zwerg was born in captivity and was 8 years old. The sea lions were housed with four other sea lions in an outdoor pool, which allowed access to a set of indoor rooms. All three subjects' training experience was limited. Carmen and Zwerg had only been trained to perform 2 veterinary procedures, which were to open their mouth and show their flippers. In addition to these two veterinary procedures, Moneta had also been trained to stand vertically against a door.

### *Procedure*

During the experimental sessions, the subjects were separated from the other animals. Two identical objects were placed on the ground equidistant from the subject (approximately 1.2 m on either side of subject). A 0.60 m tall cylindrical block of wood (approximately 0.75 m in diameter) was placed in the center of the two objects and acted as a pedestal for the subjects. Each subject was initially trained to simply target an object (touch an object with her nose). During the experimental sessions, the subjects were asked to touch the target object designated by one of the communicative cues used by the trainer. Again, none of these cues had been trained. If the subject touched the correct object, the trainer reinforced her with a fish or with tactile interaction. A fish reward was always offered first, however if the subject rejected the food reward, the trainer would provide tactile reinforcement. If the subject touched the wrong object or failed to respond to the experimenter's cue, the trainer said "no" and the reinforcement was withheld. At the beginning of the experimental sessions, the trainer requested that the sea lion stand by the cylindrical block of wood and look in the direction of the trainer. All three subjects would place their front flippers on the block. All three subjects would automatically come back to the block of wood after making their choice. If the choice was correct, the reward was provided while the subject was back at the block of wood.

There were a total of five conditions. For conditions 1-4, the target items were two identical and novel plastic jugs (approximately 5 L each).

**Condition 1.** Point and Gaze: The trainer extended his arm and index finger toward the correct target while orienting his head and eyes in the direction of the correct target.

**Condition 2.** Point only: The trainer only extended his arm and index finger toward the correct target while keeping his body and eyes forward.

**Condition 3.** Gaze only: The trainer only oriented his head and eyes in the direction of the correct target, while keeping his arms straight-down by his sides.

**Condition 4.** Marker: A marker (a red circle) was placed on the correct target object. Also, the trainer sham-marked the non-target object to reduce the effects of local enhancement. After the marker was placed, the trainer kept his body and eyes facing forward.

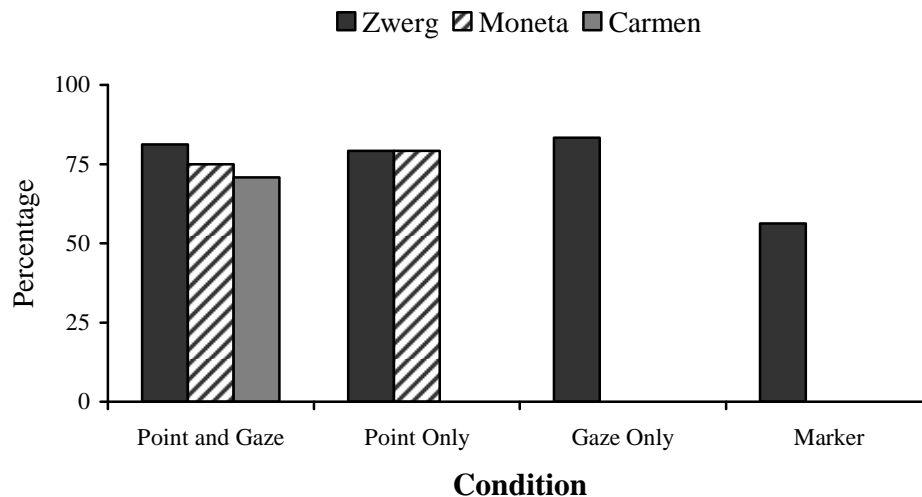
**Condition 5.** Replica: For this condition, the targets were replaced with qualitatively different objects (a flowerpot and a metal plate). Again, both objects were novel to the subject. The trainer kept his body and eyes facing forward while displaying a replica of the correct target object in front of him.

One experimental session consisted of 12 trials (six indicating the correct target to the right, six indicating the correct target to the left), plus two controls in which the experimenter exhibited no communicative gesture while looking directly at the subject. The location of the correct target object within each session was randomized with restrictions, so that right and left was indicated an equal number of times. The inter-trial interval was dependent on the time it took the subject to finish her reward (range: 30 – 60 sec). Each condition was tested over four experimental sessions for a total of 48 trials per condition. Zwerg was the only subject to complete all five conditions. The conditions

were presented in the following order: point and gaze, point only, gaze only, marker, and replica. Moneta fully completed the point and gaze condition and partially completed the point only condition. She was tested over six experimental sessions, for a total of 48 trials for the point and gaze condition, and 24 trials for the point only condition. Her participation was terminated because Europe endured a heat wave during July 2006, and all of the sea lions became lethargic, resulting in her loss of willingness to participate. Carmen only completed the point and gaze condition. She was tested over four experimental sessions, for a total of 48 trials for the point and gaze condition. Her participation was terminated after she gave birth to her pup.

## Results

Figure 1 presents the percentage of correct choices made by Zwerg across 48 trials for each of the five conditions. She performed above chance in the point and gaze, point only, and gaze only conditions (binomial test:  $p < 0.01$  in all cases, with 50% chance). However, she did not perform above chance in the marker or replica conditions. In addition, on five occasions, Zwerg's incorrect response to a communicative cue was not by touching the non-indicated object. For two of the point and gaze sessions, Zwerg touched the trainer's hand and for one of the point and gaze sessions and two of the gaze only sessions, Zwerg gave no response.



**Figure 1.** Percentage correct by Zwerg across 48 trials for the six conditions; Percentage correct by Moneta across 48 trials of Point and Gaze and across 24 trials of Point Only; Percentage correct by Carmen across 48 trials of Point and Gaze

Figure 1 also presents the percentage of correct choices made by Moneta across 48 trials for the point and gaze condition and 24 trials for the point only condition. She performed above chance for both of these conditions (binomial test:  $p < 0.01$ , with 50% chance). On one occasion, Moneta gave no response to the point and gaze cue.

Figure 1 also presents the percentage of correct choices made by Carmen across 48 trials for the point and gaze condition. She performed above chance for this condition (binomial test:  $p < 0.01$ , with 50% chance).

None of the subjects made any choices when presented with no communicative cue (control trials). All three subjects simply remained in starting position at the block of wood.

## **Discussion**

Three South American sea lions were able to correctly choose an object indicated by the human communicative gestures, point and gaze. Two of these sea lions participated in trials in which the gesture of point only was used to indicate an object. Again, these subjects were able to use this gesture correctly. One sea lion, Zwerg, was the only subject to participate in all five conditions. She was able to correctly choose an object indicated by three human communicative cues (point and gaze, point only and gaze only). However, her performance dropped to chance levels when other communicative cues (marker and replica) were used to indicate an object. Her results are similar to those found with other marine mammals (grey seals: Shapiro, Janik, & Slater, 2003; fur seals: Scheumann & Call, 2004; dolphins: Herman et al., 1999; Tschudin et al., 2001). It is possible that Zwerg would have been able to use the replica and marker cues had she been exposed to additional trials, as was the case for dolphins (Tschudin et al., 2001).

The results of the present study are consistent with those of previous research, suggesting that some marine mammals can use various human communicative cues in the absence of explicit training to do so. The mechanisms that underlie these abilities are unclear. Much of the previous research involving marine mammals has studied subjects with an extensive history of behavioral training (dolphins: Herman et al., 1999; Tschudin et al., 2001; fur seals: Scheumann & Call, 2004). This training could have influenced these subjects' ability to apprehend and comprehend certain human communicative cues. For example, Scheumann and Call (2004) reported that their subjects may have been rewarded for swimming in the direction indicated by the experimenter's arm to find a toy in their pool. They further suggested that their subjects may have generalized this performance to the object-choice testing situation. In the current study, we purposely examined a sea lion with relatively little behavioral training, in order to reduce the chance of previous exposure to human pointing and gazing. However, it is still possible that the sea lion observed trainers pointing and gazing with each other. Furthermore, when the trainers fed this group of sea lions the fish were tossed towards the sea lions. Following the path of the fish is very rewarding for these animals, so the sea lions in this study may have learned to attend to the direction of a trainer's moving arm. Consequently, we cannot be certain of the roles experience with humans played in the subjects' successful performance during point and gaze trials. Nonetheless, it is clear that being trained to attend to human pointing and gazing is not necessary for comprehension of these cues to emerge. In conclusion, this study provided evidence that sea lions are capable of correctly using certain human-given cues without formal training. Of course, much more remains to be learned about the mechanisms responsible for this ability.

## References

- Call, J., Hare, B. A., & Tomasello, M. (1998). Chimpanzee gaze following in an object-choice task. *Animal Cognition*, **1**, 89-99.
- Herman, L. M., Abichandani, S. L., Elhajj, A. N., Herman, E. Y. K., Sanchez, J. L., & Pack, A. A. (1999). Dolphins (*Tursiops truncatus*) comprehend the referential character of the human pointing gesture. *Journal of Comparative Psychology*, **113**, 347-364.
- Itakura, S., & Anderson, J. R. (1996). Learning to use experimenter-given cues during an object-choice task by a capuchin monkey. *Current Psychology of Cognition*, **15**(1), 103-112.
- Kaminski, J., Riedel, J., Call, J., & Tomasello, M. (2005). Domestic goats, *Capra hircus*, follow gaze direction and use social cues in an object choice task, *Animal Behaviour*, **69**, 11-18.
- Kastak D., & Schusterman, R.J. (1994) Transfer of visual identity matching-to-sample in two California sea lions (*Zalophus californianus*). *Animal Learning and Behavior*, **22**, 427-435.
- McKinley, J., & Sambrook, T.D. (2000). Use of human-given cues by domestic dogs (*Canis familiaris*) and horses (*Equus caballus*). *Animal Cognition*, **3**, 13-22.
- Miklósi, A., & Soproni, K. (2006). A comparative analysis of animals' understanding of the human pointing gesture. *Animal Cognition*, **9**(2), 81-93.
- Shapiro, A. D., Janik, V. M., & Slater, P. J. B. (2003). A gray seal's (*Halichoerus grypus*) responses to experimenter-given pointing and directional cues. *Journal of Comparative Psychology*, **117**(4), 355-362.
- Scheumman, M., & Call, J. (2004). The use of experimenter-given cues by South African fur seals (*Arctocephalus pusillus*). *Animal Cognition*, **7**, 224-230.
- Tomasello, M., Call, J., & Hare, B. (1998). Five primate species follow the visual gaze of conspecifics. *Animal Behaviour*, **55**, 1063-1069.
- Tschudin, A., Call, J., Dunbar, R., Harris, G., & van der Elst, C. (2001). Comprehension of signs by dolphins (*Tursiops truncatus*). *Journal of Comparative Psychology*, **115**(1), 100-105.