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The use of a mirror as a 'social substitute' in laboratory birds

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Abstract

A mirror has been shown to reduce stereotypies in horses housed singly, presumably as it may provide some sort of 'social stimulation'. We investigated here whether a mirror may have such a 'quietening effect' on birds kept in a laboratory, such as European starlings. We observed the reactions to a mirror of starlings of different sexes and with different social experiences. Females and pair-raised males seemed calmer, showing less movement and more comfort behaviour than socially and single-raised birds. The results are discussed in the light of the species' social organization and the effect of social experience. We conclude that a mirror might be a good way to reduce isolation-related stress in laboratory birds, but that sex and social experience of an individual have to be taken into account, as otherwise effects opposite to those wished for may be induced. ***To cite this article: L. Henry et al., C. R. Biologies 331 (2008).***

Résumé

L'utilisation d'un miroir comme « substitut social » chez des oiseaux de laboratoire. La présence d'un miroir réduit les stéréotypies chez les chevaux isolés en box, probablement en apportant une « stimulation sociale ». Dans cette étude, nous examinons si un miroir peut avoir un tel effet « apaisant » sur des oiseaux de laboratoire tels que les étourneaux sansonnets. Les réactions d'oiseaux de sexe et d'expériences sociales différentes ont été observées. En présence d'un miroir, les femelles et les mâles élevés en paire semblent apaisés, expriment moins de mouvements et plus de comportements de confort que les oiseaux élevés isolément ou en groupes sociaux. Ces résultats sont discutés en regard de l'organisation sociale de l'espèce et de l'effet de l'expérience sociale précoce. Le miroir pourrait donc être un bon moyen pour réduire le stress lié à l'isolement chez des oiseaux de laboratoire, sachant que le sexe et l'expérience sociale des individus peuvent avoir des effets opposés. ***Pour citer cet article : L. Henry et al., C. R. Biologies 331 (2008).***

Keywords: Mirror image; Starling; Animal welfare; Laboratory animals; Sex; Social experience

Mots-clés : Miroir ; Étourneau ; Bien-être animal ; Animaux de laboratoire ; Sexe ; Expérience sociale

1. Introduction

While it has been admitted for a long time that socially deprived monkeys show severe deficits in social

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competency [1–3], only recently the question has been raised for birds kept under laboratory conditions. Thus, much like companionless monkeys, hand-raised single housed birds express fear, stereotyped behaviours and flight reactions [4]. Solitary housing and the subsequent social deprivation is one of the factors that seem to be involved in the emergence of stereotypic behaviours in horses [5]. Interestingly, in this species, the use of a mirror in the box has been shown to reduce weaving and other stereotyped behaviours [6], leading to the suggestion that it could be a useful technique for reducing social frustration [5]. While studies using mirror images have focused mainly on self recognition [7] or representational abilities [8], most animals tested showed clear social responses to these images (pygmy marmosets, *Cebuella pygmaea* [9]; Patas monkey, *Erythrocebus patas* [10]; African grey parrots, *Psittacus erithacus* [8]; black-capped chickadees, *Parus atricapillus* [11]). Most species responding to mirror images are clearly interested in the mirror and some even prefer a mirror image to the presence of a living conspecific [12]. Differences between sexes have been reported for some species: Patas monkey [10] and California sea lion females *Zalophus californianus* [13]. Finally, social animals appear to show a stronger interest in their mirror image when they have been socially deprived [5,14,15]. Chicks raised in pairs react more positively to their mirror images than chicks raised socially or in isolation [16], which agrees with Anderson's report on infant monkeys [17].

In the present study, we investigated whether a mirror could also be, as in horses, a useful tool for improving welfare in laboratory birds. Most studies on song learning involve solitary housing in order to limit social (especially auditory) influences [4]. A mirror may be in such cases a way of providing some 'social stimulation' without interfering with the experiment. We test here the reactions of European starlings *Sturnus vulgaris* used in such experiments to their mirror image. Starlings are now widely used in laboratory experiments involving isolation. Their social organization outside the breeding season is based on socially affiliated groups of males and pairs of females [18] and single-housed animals often show stereotypic behaviours (pers. obs.). We expect therefore that mirror effects may vary according to sex and, as in other species, according to social experience during development, which means that the potential beneficial effect of a mirror may depend on these factors.

2. Methods

2.1. Subjects and housing conditions

Young hand-raised birds involved in a song-learning experiment were used here (for more details, see [19]). Nineteen young starlings (4–8 days old), from six different broods, had been collected from nests around the Rennes University Campus in April 1998 and hand-raised using commercial pellets (Végam, Grosset) mixed with water. They were kept in groups until June 1998, when they were divided into three experimental situations: socially-, pair- or single-raised birds. Socially raised birds (SO) corresponded to three subgroups of socially-raised birds: one adult male was placed with two to four juveniles in a $145 \times 100 \times 55$ cm aviary. The three aviaries were adjacent in the same room and therefore visual and auditory contacts were possible between subgroups. Pair-raised birds (PA) included three subgroups of pair-isolated birds ($n = 6$ birds, five males and one female: two juvenile birds were placed together in a cage ($40 \times 60 \times 52$ cm) in a soundproof chamber. Single-isolated birds (IS) included four groups of single-raised birds ($n = 4$ birds, two males and two females; each bird was placed individually in a cage ($30 \times 57 \times 43$ cm) in a soundproof chamber). The soundproof chambers and the aviaries were in two separate rooms and pair- and single-housed birds could not see any other bird, although they could hear vocal interactions from the aviaries through loudspeakers.

Birds from the same brood were not placed in the same subgroup. As the sexes of the young birds could not be assessed, groups were in some cases somewhat unbalanced. All birds were provided food (commercial pellets and apples) and water ad libitum. All birds stayed in the same housing conditions during two years. The light schedule was identical for all birds and matched the natural photoperiod, following seasonal photophase changes.

2.2. Apparatus and procedure

When two years old, the birds were transferred into an experimental room in individual cages ($102 \times 40 \times 50$ cm) that had three opaque sides and were placed side by side. Therefore, birds could hear each other, but could not see each other. The birds spent 10 days in these individual cages in order to ensure habituation before the experiment started. On the test day, each cage was first divided into two parts by an opaque screen.

Table 1
Behavioural patterns recorded during the mirror tests. Three types of activity expressed visual attention. A bird was noted to 'observe' when it looked in different directions without moving its body. On the contrary, a bird was noted to 'gaze' when it looked at the same object for more than three seconds without moving its body or its head. In this case, we noted whether the bird was looking at the mirror or if it was looking at something else ('gaze at other object')

Feeding	Eat	The bird pecks at pellets in the feeding dish or on the ground
	Drink	The bird drinks water in the drinking trough
Comfort movements	Preen	The bird preens itself
	Scratch	The bird scratches itself with its legs
	Shake	The bird ruffles its feathers and shakes them
	Stretch	The bird stretches one or both wings
Visual attention	Observe	The bird scans in several directions without moving
	Gaze at other object	The bird looks at something for more than 3 s without moving
	Gaze at mirror	The bird looks at the mirror for more than 3 s without moving
Punctual behaviours	Move head	The bird shakes its head
	Rub wings	The bird rubs its wings one against the other
	Rub tail	The bird rubs its tail feathers one against others
	Rub beak	The bird rubs its beak on a perch
	Peck	The birds peck with the beak
	Yawn	The bird yawns
	Defecate	The bird defecates
	Call	The bird calls
Mobility	Move	The bird moves on a perch, from a perch to another one or from a perch to the ground
	Fly	The bird flies in the cage
	Jump	The bird jumps on the same place
Resting	Sleep	The bird sleeps, head under feathers or sits on a perch, its legs bent under its body and eyes closed

The birds were kept in the left part of the cage. A mirror (20×10 cm) was placed in a corner of the right side of the cage. The test lasted 17 min: birds were observed for 2 min before the screen was removed and for 15 min in the presence of the mirror. Birds were videotaped during the 17-minute observation. Their behaviour was recorded by sampling all behavioural occurrences of six categories of behaviours described in Table 1 [20]. In addition, we noted every five seconds: the precise direction of the head (right, left, up, bottom, straightforward), the direction of glances (gaze at mirror, gaze at other objects), the position of the body (standing, sitting, lying, upright, horizontal, flying), the position of the beak (closed, open).

The experiment was performed in France (licence number 005283 issued by the departmental direction of veterinary services of Ille-et-Vilaine), in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.3. Data analysis

Multifactorial analyses (FCA) enabled us to visualize the types of responses performed by the different groups of birds, using custom-made software, GTABM [21]. FCA provides a graphic representation of data in a multidimensional space and detects clusters of individuals. Each axis can be interpreted by considering the factor loadings of the initial variables. A high factor loading indicated that the variable made an important contribution to the axis. Behavioural data were analysed using non-parametric statistical tests [22]: Wilcoxon signed rank tests in order to compare the birds' behaviour before and during the mirror presentation, Mann–Whitney U tests in order to compare data between groups.

3. Results

Birds clearly reacted to the mirror: they observed their environment significantly less (mean $\pm$ SD frequencies per minute: before: 2.8 ± 1.5, during mirror: 1.5 ± 1.0, Wilcoxon test, $T = 27$, $N = 19$, $p < 0.01$) and paid more attention to the mirror when it was visible (mean $\pm$ SD gaze at mirror: before: 0, during mirror: 0.8 ± 1.2, $T = 0$, $N = 19$, $p < 0.01$), while the frequency of the movement increased (mean $\pm$ SD: before:

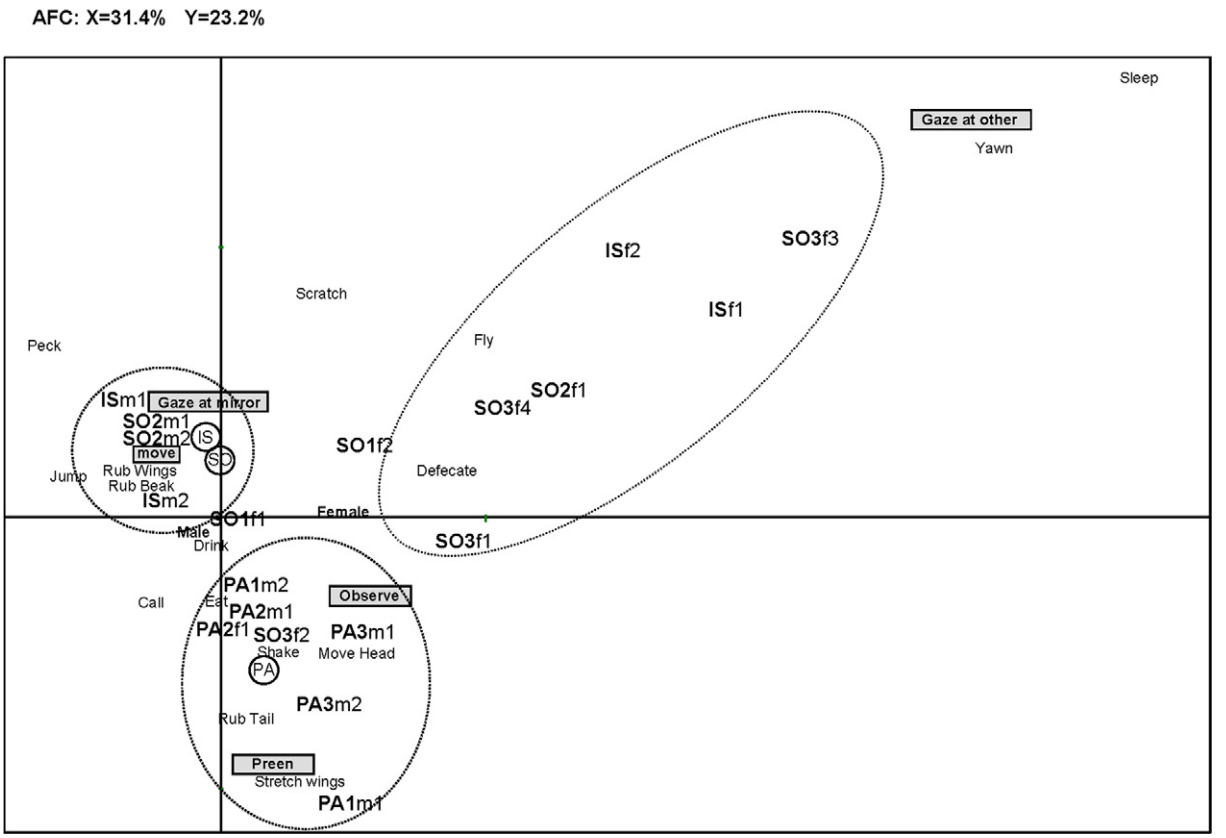

Fig. 1. FCA of behavioural patterns of male and female starlings raised under three different social conditions. Bold characters: subjects; grey boxes: behavioural patterns with the highest loading factors. Single- and socially-raised females are grouped at the top right of the graph and expressed more 'gaze at other object' than single and socially-raised males (left part of the graph) that were more mobile and 'gazed at mirror' more. Pair-raised birds are grouped at the bottom part of the graph and expressed many comfort movements such as 'preen' and 'shake' as well as feeding. Dotted-line circles were drawn according to a hierarchical classification. SO: socially-raised birds, PA: pair-raised, IS: single-raised birds.

0.4 ± 1.0, during mirror: 2.7 ± 3.2, $T = 4$, $N = 18$, $p < 0.01$).

Reactions to the mirror also clearly differed according to social experience and sex (Fig. 1, Tables 2 and 3). The first two axes of the FCA explained 54.6% of the variance. The patterns 'gaze at other object' and 'observe' opposed to 'move' had the three major loadings on axis one (0.43, 0.16 and 0.14, respectively). This axis also separated all females from single and socially raised males. 'Preen' (0.33), as opposed to 'gaze at other object' (0.17) contributed to axis 2: preening appeared more in pair-raised birds while socially and single-raised birds showed more attention towards the environment than to the mirror. Sex contributed most to axis 1, whereas social experience contributed to axis 2, separating pair-raised from socially and single-raised birds.

While males and females did not differ in their behaviour before the mirror presentation (Mann–Whitney test: $p > 0.5$ in all cases), they differed when the mirror was present. Thus, both socially and single raised females showed more 'gaze at other object', sit on perches and less 'move', 'rub wings', 'rub beak', 'preen', 'shake' and 'gaze at mirror' than males (Mann–Whitney test, $n_1 = 4$, $n_2 = 9$, $p < 0.05$ in all cases), as well as fewer changes in their body position than males (Mann–Whitney test, $n_1 = 4$, $n_2 = 9$, $p < 0.05$). In fact, females significantly increased their frequencies of 'gaze at other object' in the presence of the mirror (mean $\pm$ SD frequencies per minute: before: 0.06 ± 0.16, during mirror 0.25 ± 0.23, Wilcoxon, $T = 0$, $N = 8$, $p < 0.5$). Females therefore seemed to focus less on the mirror and to show less excitation and possibly 'derived' activities than males.

Males' reactions to the mirror differed greatly according to social experiences. All pair-raised birds greatly increased the frequency of comfort behaviours (preen, scratch, shake and stretch) in the presence of the mirror (mean $\pm$ SD frequencies per minute: before: 0.9 ± 0.25, during mirror: 1.8 ± 1.5), in contrast to sin-

Table 2
Factor loading of the behaviours

Behavioural patterns	Factor loadings of variables		
	F1	F2	F3
Observe	**0.166**	0.082	0.003
Gaze at mirror	0.032	0.095	0.001
Gaze at other object	**0.436**	**0.176**	0.124
Preen	0.004	**0.333**	0.010
Eat	0.000	0.019	0.025
Sleep	0.079	0.024	0.358
Move	**0.148**	0.082	0.008
Fly	0.020	0.012	0.009
Jump	0.001	0.000	0.000
Drink	0.001	0.002	0.012
Shake	0.002	0.038	0.003
Move head	0.014	0.039	0.008
Scratch	0.003	0.034	0.207
Rub wings	0.016	0.002	0.039
Rub tail	0.000	0.015	0.001
Rub beak	0.018	0.002	0.033
Stretch	0.000	0.020	0.001
Peck	0.012	0.012	0.001
Defecate	0.007	0.000	0.019
Yawn	0.042	0.013	0.137
Call	0.000	0.000	0.000
Total	1.000	1.000	1.000

Bold characters: behavioural patterns with major factor loadings on the first two axes. F1 = factor 1, F2 = factor 2, F3 = factor 3.

Table 3
Factor loading of the behaviours

Individuals	Factor loadings of variables		
	F1	F2	F3
ISm1	0.088	0.070	0.108
ISm1	0.040	0.003	0.001
ISf1	0.251	0.056	0.531
ISf2	0.127	0.077	0.092
PA1m1	0.035	0.412	0.025
PA1m2	0.000	0.026	0.001
PA2m1	0.000	0.045	0.004
PA3m1	0.019	0.031	0.020
PA3m2	0.011	0.098	0.001
SO1f1	0.001	0.000	0.035
SO1f2	0.011	0.005	0.010
SO2m1	0.057	0.052	0.009
SO2m2	0.057	0.032	0.005
SO2f1	0.099	0.020	0.064
SO3f1	0.012	0.000	0.004
SO3f2	0.001	0.015	0.001
SO3f3	0.162	0.050	0.084
SO4f4	0.028	0.006	0.005
Total	1.000	1.000	1.000
Supplementary variable			
PA2f1	0.001	0.078	0.174

Female PA2f1 displayed as additional variable. This individual presented a very high score for 'Rub beak' information that was hidden in the initial analysis. F1 = factor 1, F2 = factor 2, F3 = factor 3.

gle and socially raised birds (before: 2.5 ± 2.6, during mirror: 1.6 ± 0.8). Interestingly, the pair-raised birds gazed at their mirror image 16 times less often than single and socially raised males (mean $\pm$ SD frequencies per minute: pair raised: 0.2 ± 0.08 and single and socially raised: 3.3 ± 0.2) (Mann–Whitney test: $U = 0$, $n_1 = 4$, $n_2 = 5$, $p = 0.008$).

4. Discussion

While starlings clearly reacted to the presence of a mirror, their reactions differed according to both sex and social experience: the mirror appeared to induce more attention focusing and movement in single and socially raised males, while the pair-raised males and the females seemed calmer.

The sex differences observed here are reminiscent of those observed in Patas monkeys, where the males expressed tension, displacement activities and threats toward their mirror images, whereas females reacted in a much quieter way [10]. Differences in response between sexes might be related to social organization and may reflect social 'dispositions' [23]. In captive groups, starlings tend to form the same sex social affinities where females form dyads and males instead form small groups [18]. Being with only one other bird of the same sex may be a rather comforting situation.

It is interesting also to note that males' reactions depended on their social experience, but that socially and single-raised birds did not differ in this regard. It is possible that the situation may have been perceived as unusual for both categories of birds: only one 'peer' present for the socially raised birds used to several birds being present; presence of a 'conspecific' for birds used to total isolation. Further research is warranted on this interesting question as it would also be interesting to know the effects of both visual and vocal isolation on mirror reaction. In contrast, pair-isolated birds seemed to be calmed by their mirror image, as they tended to preen and to remain still. Their mirror image may have compensated for social separation from their familiar peer, as was observed for kea *Nestor notabilis* [15] and domestic fowl chicks [16]. Different studies report that a mirror quietens animals subjected to social separation as adults (horses [5,6]; vervet monkeys, *Cercopithecus aethiops* [24]; chimpanzees [25]), especially in pair-isolated animals, such as stumptail macaques *Macaca arctoides* [17]. Like starlings in our study, pair-raised, but not socially or single-raised domestic fowl chicks were calmed by the presence of a mirror [16]: they

pecked more at food and peeped less. As these authors point out, differences between the test situation and rearing conditions were lesser for pair-raised animals.

These preliminary results suggest new approaches to the welfare of laboratory animals such as social species of birds. They suggest that a mirror might be a good way to reduce isolation-related stress in laboratory birds, but that sex and social experience at least have to be taken into account, as these can reverse the response of some individuals.

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