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Azure-winged magpies fail to understand the principle of mirror imaging



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ABSTRACT

Mirror self-recognition (MSR) is considered a crucial step in the emergence of self-cognition. The MSR paradigm has become a standard method for evaluating self-cognition in several species. For example, Eurasian magpies and Indian house crows have passed the mark test for self-cognition, whereas efforts to find MSR in other corvid species have failed. However, no literature has conducted MSR tests on azure-winged magpies, a species of corvids. Therefore, the current research aimed to investigate the MSR behaviours of azure-winged magpies upon looking into a mirror for the first time. The study included four tests: (1) mirror preference and standardised mirror exploration, (2) single vertical mirror test, (3) mark test and (4) mirror-triggered search test. The azurewinged magpies displayed immense curiosity towards the mirror and their images in the mirror in Test 1&2. In the subsequent mark tests, they failed to recognise themselves in the mirror and regarded their images as conspecifics. Behaviour analysis showed no significant difference between marked and unmarked behaviours. Finally they seemed to infer the presence of bait from the image in the mirror, but were found to fail to understand that the location of the bait in the mirror was the same as that in the real world. For a better insight into the MSR behaviour of azure-winged magpies, research studies involving prolonged mirror exposure and training are recommended.

1. Introduction

The mirror self-recognition (MSR) paradigm has emerged as a standard method for evaluating self-awareness in many species since the pioneering work by Gallup (Gallup, 1970), although numerous researchers have debated whether animals that show MSR possess human-like self-awareness (Heyes, 1994, 1995; Bard et al., 2006). The ability to recognise oneself in a mirror is rare in the animal kingdom. Most of the animals exposed to the mirror test displayed various social behaviours (such as aggressive behaviour) and continued to do so during repeated testing (Prior et al., 2008). The ability to recognise oneself in a mirror is often assessed empirically by exposing animals (previously marked on the head or elsewhere on a spot they can see only in the mirror) to a mirror and assessing their behaviour. If the tested animals respond with mark-directed or self-directed behaviours (e.g. touching the mark on their body, demonstrating that they can see it and realise that the mark it is on themselves) after exposure to the mirror, then the animal passes the mirror-mark test (Ma et al., 2015).

Fairly clear evidence of self-recognition has been obtained in some non-human primate species, although mirror-directed behaviour is much less stable in these species than in human (Swartz and Evans, 1991; Povinelli et al., 1993). Some studies have provided clear evidence of self-awareness in chimpanzees (Pan troglodytes)(Gallup, 1970), orangutans (Pongo pygmaeus) (Lethmate and Dücker, 1973) and bonobos (P. paniscus) (Walraven et al., 1995). A few gorillas (Gorilla gorilla) (Ledbetter and Basen, 1982; Hyatt, 1998; Posada and Colell, 2007) have shown MSR, although the findings are less robust than those reported for other ape species. Also, video evidence showing compelling selfrecognition in gorillas is markedly lacking. No strong evidence exists to show that gibbons (Hylobates lar), siamangs (H. syndactylus) (Anderson and Gallup, 2015) and other primates are capable of self-recognition (Ujhelyi et al., 2000). An exhaustive review of the existing self-recognition literature on primates has been conducted by Anderson and Gallup (2015). Furthermore, some non-primates have shown mark-directed performance in the same mark test taken by primates. One Bottlenose dolphins (Tursiops truncates) (Reiss and Marino, 2001), one Asian elephant (Elephas maximus) (Plotnik et al., 2006), two Eurasian magpies (Pica pica) (Prior et al., 2008) and four Indian house crows (Corvus splendens) (Buniyaadi et al., 2019) have reportedly passed the mark test.

As it has been clearly shown that higher cognitive abilities are not restricted to the brains of primates, some novel self-recognition studies have moved beyond just phylogenetic causes to focus on ecological explanations. Horowitz (2017) has recently claimed the approximation

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Received 13 September 2019; Received in revised form 5 May 2020; Accepted 28 May 2020 Available online 30 May 2020 0376-6357/ © 2020 Elsevier B.V. All rights reserved. of self-recognition in dogs using an 'olfactory mirror', i.e. the dog's own urine. Horowitz's study translates the MSR study for dogs (who primarily rely on olfaction) who have shown interest in their own odours, implying the dogs' recognition of the odour that came from them. Gallup and Anderson (2018) have reported their critique of the olfactory mirror with dogs. A task exploiting an ecologically relevant behaviour has been used to assess the self-recognition of Clark's nutcrackers (*Nucifraga columbiana*) (Clary and Kelly, 2016) and California scrub jays (*Aphelocoma californica*) (Clary et al., 2019). When presented with a blurry, rather than clear mirror, Clark's nutcrackers show more mark-directed behaviours and cache more often, suggesting that they interpret the blurry image as their own rather than a conspecific. These tested California scrub jays do not show increased caching and cache protection behaviours in the presence of a mirror and no mark-directed behaviours in the mark test.

Numerous studies have reported that animals that do not conclusively pass the mark test show other interesting and intermediate mirror-induced responses, such as mirror-triggered search and peekaboo (Gallup, 1970; Anderson, 1984; Pepperberg et al., 1995; Suddendorf and Collier-Baker, 2009). Mirror-triggered search is a basic task for exploring whether animals can find hidden food (that is visible in the mirror but invisible directly) using the mirror as a cue (Menzel et al., 1985; Anderson, 1986; Povinelli, 1989). Animals can find hidden food at a fixed location by exploiting the correlation between an object and its reflection; however, they do not need to understand that the object is being reflected by the mirror or that the mirror is used to guide their actions (Pepperberg et al., 1995). Contrarily, the mirror-mediated spatial location task requires a highly complicated cognitive competence (Menzel et al., 1985; Anderson, 1986; Povinelli, 1989; Pepperberg et al., 1995) because the subjects must use the mirror without any trial and error attempts to find the reward hidden in one of several locations. Subjects must understand the correspondence between the location of the reward in real space and the information in mirror. A study in 2011 demonstrated that New Caledonian crows (C. moneduloides) learnt to associate a mirror image of meat with finding the bait in its actual location (Medina et al., 2011). Macaques (Macaca fuscata) can use a mirror to reach hidden food that is only visible with a mirror. In addition, a more elaborative mirror-guided reaching task (Menzel et al., 1985; Anderson, 1986) was designed to test whether animals could synchronise mirrors with their own body movements.

Previous studies have suggested that some bird species have evolved highly cognitive skills similar to those of humans and apes (Lefebvre et al., 2002; Deregnaucourt and Bovet, 2016). These skills are manifested by these birds by completing different tasks, such as using tools and predicting the behaviour of conspecifics using their episodic-like memory and own experience (Duffey, 1993; Emery and Clayton, 2004). Mirror-directed behaviour has been studied in different species of birds, both in the wild (e.g. chickadees (Parus atricapillus) (Censky and Ficken, 1982) and glaucous-winged gulls (Larus glaucescens) (Stout, 1969)) and in the laboratory (e.g. blue grouse (Dendragapus obscurus) (Stirling, 1968), budgerigars (Melopsittacus undulates) (Gallup and Capper, 1970), African grey parrots (Psittacus erithacus) (Pepperberg et al., 1995), New Caledonian crows (Medina et al., 2011), jungle crows (C. macrorhynchos) (Kusayama et al., 2000) and jackdaws (C. monedula) (Soler et al., 2014)). Most of these birds have responded to their self-image in the mirror with different social behaviours, i.e. treating the mirror image as if viewing a conspecific, exhibiting aggressive behaviour and exhibiting displays of courtship. A flock of flamingos (Phoeniconais minor) has displayed a very interesting behaviour of marching in front of the mirror (Pickering and Duverge, 1992).

Several corvid species have been subjected to mirror tests and displayed a range of different reactions to the mirror. The studies on captive jungle and New Caledonian crows have revealed that these crows consider their mirror images as their conspecifics and show no self-recognition behaviour in the mirror-exposure test (Kusayama et al., 2000; Medina et al., 2011). Carrion (*C. corone*) and hooded crows (*C.* *cornix*) exhibit exploratory behaviours rather than social behaviours on their first encounters with a mirror, but none of the crows show significant mark-directed behaviours (Vanhooland et al., 2019). Surprisingly, two out of five Eurasian magpies have passed the mark test after cumulative exposure to mirrors (Prior et al., 2008). Thereafter, four of six Indian house crows have been reported to have the ability of self-recognition in a study that followed a procedure similar to the one described in the Eurasian magpie study (Prior et al., 2008; Buniyaadi et al., 2019). A recent study has shown that jackdaws fail the mark test (Soler et al., 2014). They show a mark-directed behaviour in the mirror that is similar to their behaviour without a mirror. Researchers hypothesise that the magpies and the jackdaws may have detected the mark by tactile sense. Meanwhile, researchers also suggest the adoption of appropriate marking methods in future marking tests, such as paint that does not agglomerate the feathers.

Azure-winged magpies (Cyanopica cyana), a corvid species attracted lots of attention from researchers, are found in Eastern Asia (Yamagishi and Fujioka, 2007; Ren et al., 2016; Wang et al., 2019). The corvid species was the first to experimentally show proactive pro-sociality, which is considered a human hallmark (Horn et al., 2016). MSR is thought to correlate with higher forms of empathy and altruistic behaviour. We inferred that azure-winged magpies would be good candidates for mirror test because of their proactive pro-sociality. In this study, we tested and observed the responses of seven hand-raised azurewinged magpies to mirrors through four tests. (1) Mirror preference and standardised mirror exploration (Test 1): In this test, we assessed the preference for mirrors and the quantified mirror-directed behaviours of the subjects. (2) Single vertical mirror test (Test 2): We investigated the mirror-directed behaviours of each subject. (3) Mark test (Test 3): Each subject was marked (in the throat area) to explore whether a markdirected behaviour in front of the mirror would be exhibited. (4) Mirror-triggered search test (Test 4): This test was used to investigate whether the subjects would learn to use the mirror to find hidden food. Similar to the experimental procedure of the study on European magpies (Prior et al., 2008), the first three tests were conducted to investigate whether azure-winged magpies would show MSR. Most of the subjects were unwilling to approach the experimental cages because of neophobia. Therefore, their home and experimental cages were connected to eliminate their fear and encourage them to display their behaviour in their normal state.

2. Materials and methods

2.1. Subjects

Seven azure-winged magpies (named Daniel, Emily, Fatty, Joyce, Tiny, Alina and Neil) served as the subjects throughout the study. The subjects were suitable for the study because they had not encountered mirrors before. The magpies were housed in a seven-cage indoor aviary. All cages were provided with perching space, branches and cribs. The subjects were in auditory and visual contact with their conspecies before the experimental procedures began, but they were single-housed in home cages. All subjects were juveniles (6 months of age) of unknown sex and had been hand-raised at the Animal Behavior and Conservation Laboratory (School of Life Sciences, Nanjing University). A detailed description of the birds is shown in **Table S1**.

2.2. Procedure and apparatus

The experiments were conducted between December 2017 and February 2018. The magpies were left to become accustomed to the mirror and human presence for seven days before any experimental procedures began. In all experimental sessions, the home and experimental cages were placed together, and the doors were connected. To ensure that the subjects remained testable, a subject went through two experimental sessions at most on the same day. Once the subject was



Fig. 1. Top view of experimental apparatus in Test 1 (unit: cm).

transferred to the experimental space, the experimenter quickly left the space. Food and water were placed in the home cage. In the entire experiment, mirrors were thoroughly cleaned before each session. In the mark test, the tested bird was held by one of the experimenters and blindfolded so that another experimenter could mark them (throat region) quickly. The handling procedure was the same in each of the experimental conditions.

2.2.1. Mirror preference and standardised mirror exploration (Test 1)

For a quantitative estimation of interest in the mirror, a cage (60 cm \times 40 cm \times 40 cm) with two opposite compartments was used. One compartment was equipped with a mirror, and the other showed the non-reflective back of a mirror of the same size (Fig. 1). A partition with two overlapping walls divided the compartments so that the birds could move freely between the compartments but could not see from one compartment into another. The time the birds spent in each compartment was measured, and the occurrences of their contingent and social behaviours were counted based on the videotapes.

2.2.2. Single vertical mirror test (Test 2)

A 28-cm wide and 28-cm high mirror was placed in the middle of a $60 \text{ cm} \times 40 \text{ cm} \times 40 \text{ cm}$ experimental cage, allowing the birds to move around the mirror. Meanwhile, the tested birds could freely explore the experimental cage (Fig. 2). The behaviours of the tested birds were recorded by a video system.



Fig. 2. Top view of experimental apparatus in Test 2 and 3 (unit: cm).

2.2.3. Mark test (Test 3)

In the mark test, each subject was involved in three sessions with the following conditions: (1) red mark on chest, (2) red mark without a mirror and (3) red mark with a mirror. In the first condition (red mark on chest), visible marks on the subjects' chests were used to find out whether birds showed any mark-directed behaviours if the powder was on a visible part of their body (the subjects were not offered mirrors). The subjects were marked by red vegetable powder, which was flavourless and did not agglomerate the feathers. The first session was conducted in subjects' home cages. In the latter two conditions, coloured marks were fixed below the beak onto the throat region, as in the study on European magpies (Prior et al., 2008). A previous study showed that pigeons were blind to this area even during strong convergent eye movements (Jahnke, 1984). We assumed that our birds were similar to the pigeons. The second and the third sessions were conducted in the experiment cages that were used in Test 2. However, the mirror was replaced by a non-reflected plate instead in the second session. The behaviour of the tested birds was recorded by a video system.

2.2.4. Mirror-triggered search test (Test 4)

This test was conducted in the same experimental cages used in Tests 2. A dam-board was installed in the experimental cage so that the birds could not see the hidden food directly. The location of the dam-board was confirmed after several adjustments. The dam board was not moving and it was a visual barrier. Mealworms, as the hidden food, were hung by a 6-cm-long red string above the dam-board. Each bird received 10 trials in total, i.e. 5 trials with mealworms and 5 trials with an empty string (Figs. 3, 4 and S1). The baiting in each trial was random. To ensure the interest of the birds in mealworms, the test was conducted after 2 h of food deprivation. The behaviour of the tested birds was recorded by a video system.

2.3. Behaviour and data analysis

Immediately after the subjects were transferred to the experiment space, the behaviours of the tested birds were recorded by the video system. After testing, we recorded all observable behaviours and defined the detailed behaviour ethogram of the birds (Table 1). Similar behaviours have been reported in children (Amsterdam, 1972), chimpanzees (Lin et al., 1992), gibbons (Suddendorf and Collier-Baker, 2009), macaques (Straumann and Anderson, 1991), parrots (Pepperberg et al., 1995), chickadees (Censky and Ficken, 1982),



Fig. 3. Top view of experimental apparatus in Test 4 (unit: cm).



Fig. 4. Lateral view of experimental apparatus in Test 4 (unit: cm).

finches and parakeets (Gallup and Capper, 1970), Eurasian magpies (Prior et al., 2008), jungle crows (Kusayama et al., 2000) and New Caledonian crows (Medina et al., 2011). Two observers recorded behaviours of the tested birds independently and defined the controversial behaviour together. Inter-rater reliability was measured with Spearman rank correlation test (Test 1: $r_s = 0.995$; Test 2: $r_s = 0.989$; Test 3: $r_s = 0.988$; all P < 0.001). In the experimental sessions, the experimental duration was divided into two parts: duration in the living cage and duration in the experimental cage. The time that was spent at the gates of the two cages was included in the duration in the experimental cage.

We calculated the frequencies of behaviours exhibited in front of the mirror and in front of the non-reflective surface in Test 1. The durations the subjects spent in front of the mirror and the back of the mirror were compared by paired-samples T test. We calculated the behaviour frequency (per hour) in front of the mirror of each test session in Test 2. The behaviour frequency of the first session and the fifth session were compared using the Wilcoxon signed-rank test to see if the birds became habituated to the mirror. The average behaviour frequency in Test 2 and mark session in Test 3 were also compared by the Wilcoxon signed-rank test. With Fisher's exact test, we tested the probability of searching for bait depending on whether the bait was available or not in the individual level in Test 4.

All statistical analyses were conducted using IBM SPSS Statistics 22. The data of experimental duration are shown as the mean \pm SEM. Alpha was set at 0.05, and all tests were two-tailed.

3. Results

3.1. Mirror preference and standardised mirror exploration (Test 1)

Seven subjects participated in five sessions of Test 1 on separate days. The apparatus of the experimental cage was invisible directly, so several of the subjects were unwilling to enter the experimental cage because of neophobia. Four subjects stayed in their home cages throughout the five sessions. Finally, only three of the seven birds

 Table 1

 Mirror responses and definitions



Fig. 5. Percentage of time the birds spend in different spaces.

entered the experimental cage. Each bird underwent five consecutive trials for approximately 30 ± 7.74 min. on separate days.

The durations in the experimental cage varied, and the corresponding proportions were 56.71 % (Fatty), 57.17 % (Daniel) and 44.01 % (Joyce). We found that the subjects spent longer time in front of the mirror (Fatty: 37.06 %; Daniel: 46.82 %; Joyce: 26.80 %) and less time at the back of the mirror (Fatty: 8.38 %; Daniel: 0.03 %; Joyce: 1.93 %). The durations the subjects spent in front of the mirror and the back of the mirror were compared by paired-samples T test (T = 4.946, df = 2, P = 0.039). Fig. 5 presents the time spent in different positions. We recorded 400 behavioural responses when the subjects stayed in front of the mirror, and Fig. 6 shows the frequency of responses to a mirror. The three subjects showed mirror-directed behaviours, particularly social and exploratory behaviours (see Videos 1 and 2). Given the equipment limitation, the birds could not look behind the mirror in this test. Notably, the three birds had a high interest in the mirror.

3.2. Single vertical mirror test (Test 2)

Seven subjects participated in five sessions of Test 2 on separate days. Five test sessions of 30 ± 7.29 -min duration (a total of approximately 150 min) were allotted for each of the birds on separate days. We found that Emily preferred to stay in its living cage and showed few mirror-directed behaviours apart from observing the mirror. Therefore, we did not include this bird in the following analysis. The time spent in the experimental cage of each subject was different, and the corresponding proportions were 38.98 % (Fatty), 55.67 % (Daniel), 20.85 % (Joyce), 19.16 % (Tiny), 23.35 % (Alina), 29.64 % (Neil) and 6.31 % (Emily).

Mirror-directed behaviours were defined as behaviours that occurred when the subjects were immediately in front of the mirror. The numbers of mirror-directed behaviours in each session were counted, and a total of 777 responses were recorded in Test 2. On several

Description
Bird jumps at the mirror image, usually with claws up and wings flapping (Videos 1 and 2)
Bird bows its head in submission with a slight call and continuous flapping of wings (Video 3)
Bird flaps its wings rapidly in front of the mirror, sometimes accompanied by a slight call
Bird pecks at the surface of the mirror or the mirror image (not associated attack) (Video 4)
Bird stands in front of the mirror, staring at the mirror image for two or more seconds (Video 5)
Bird moves from the front area to the back area of the mirror (Video 6)
Bird walks around the mirror continuously (Video 7)
Bird preens its feathers in front of the mirror



Fig. 6. Frequency (per hour) of responses to mirror in Test 1.

occasions, the birds jumped to the mirror as soon as they saw their image in the mirror but usually returned to their living cage after the exploratory stage. The initial exploration of the mirror included approaching, pecking and looking behind the mirror. Five of the seven subjects displayed social behaviours at least once. The frequencies of responses to a mirror of six of the seven (except Emily) subjects are presented by Fig. 7.

To investigate whether the frequency of social and exploratory behaviours decreased during the experimental sessions, we compared each response to the mirror in the first session and the fifth session on separate days. The results indicated no significant differences between the first and fifth sessions (Observing the mirror image: Z = -0.314, P = 0.753; Pecking: Z = -0.946, P = 0.344 ; Aggressive displays: Z = -0.447, P = 0.655; Spreading the wings: Z = -1.000, P = 0.317; Submissive displays: Z = -1.000, P = 0.317; Look behind mirror: Z = -1.461, P = 0.144; Walking around the mirror: Z = 0.000, P = 1.000).

3.3. Mark test (Test 3)

Seven individuals participated in the mark test. Each bird was involved in three different sessions (approximately 30 ± 8.29 min. per session) with the following conditions: (1) red mark on chest, (2) red mark without a mirror and (3) red mark with a mirror. We found that the subjects showed mark-directed behaviours in the first condition. Emily preferred to stay in its living cage and showed few mirror-directed behaviours apart from observing the mirror. Therefore, we did not include this bird in the following analysis. We observed no remarkable mark-directed behaviours in the red-mark-mirror condition (detailed results are shown in Table 2) and the red-mark-no-mirror condition (the detailed results were not analysed because no mirrordirected and mark-directed behaviours were observed). Meanwhile, no significant differences were observed in the mirror-directed behaviours between the red-mark-mirror (Test 3) and no-mark-mirror conditions (Test 2) (Observing the mirror image: Z = -1.014, P = 0.310; Pecking: Z = -1.363, P = 0.715; Aggressive displays: Z = -0.365, P = 0.715; Spreading the wings: Z = -1.461, P = 0.144; Submissive displays: Z = -0.447, P = 0.655; Look behind mirror: Z = -0.943, P = 0.345).

3.4. Mirror-triggered search test (Test 4)

Five birds participated in the mirror-triggered search test. The birds' behaviours were classified into two categories: searching for food and no search response. According to the initial searching position, "searching for food" further divided into "looking behind the mirror

directly"," looking for the bait aimlessly" and "jumping up to search the location of bait". If a subject neither searched the correct position of the bait nor searched the position of the mirror's back, the behaviour was grouped into "looking for the bait aimlessly". The detailed results are shown in Table 3. With Fisher's exact test, we tested the probability of searching for bait depending on whether the bait was available or not in the individual level. Four out of five birds searched for food significantly more often in those trials where food was present, compared to the trials where no food was present (Fatty: P = 0.004; Daniel: P = 0.083; Joyce: P = 0.004; Tiny: P = 0.004; Neil: P = 0.024). However, most search response were not directed to the actual location of the food, but were aimless or directed to the back of the mirror.

4. Discussions

Upon exposure to a mirror for the first time, animals commonly respond in one of three ways. (1) They regard their image in the mirror as a conspecific or another animal, thus exhibiting different social behaviours (such as aggressive and submissive behaviours) towards it or looking behind the mirror to search for the conspecific. (2) They perceive their mirror images as illusory and ignore them, showing interest only in the smooth surface (Ma et al., 2015). (3) They recognise themselves in the mirror and then decorate or groom themselves in front of the mirror or show self-directed behaviours by using the mirror to respond to themselves (Parker et al., 1994). In Tests 1 and 2 of this study, the azure-winged magpies showed social and exploratory behaviours. The duration spent by the subjects in front of the mirror (in Test 1) indicated that the corvids took a keen interest in the mirror and their images in the mirror. Compared to the time they spent in front of the non-reflective back of the mirror, they spent time significantly longer in front of the mirror. When first confronted with the mirror in Test 1, the azure-winged magpies displayed social behaviours, that is to say, the azure-wing magpies perceived their mirror images as a conspecific. Their social behavioural responses (e.g. aggressive and submissive behaviours) and exploratory behaviours (e.g. pecking, looking at the mirror image and looking behind the mirror) to their mirror images continued during the entire experimental sessions of Test 2, with no decreasing trend. Meanwhile, the azure-winged magpies did not display any of the self-contingent behavioural responses (Subjects moved their head or the whole body back and forth in front of the mirror in a systematic way) previously reported in Eurasian magpies (Prior et al., 2008). The current study is consistent with previous studies on e.g. jungle (Kusayama et al., 2000) and New Caledonian crows (Medina et al., 2011), which also did not exhibit self-directed behaviours. Eurasian magpies (Prior et al., 2008) and Indian house crows (Buniyaadi et al., 2019), that showed mark-directed behaviours, were provided larger exploratory space than our subjects. It is possible that subjects show different exploratory behaviours in larger space. Chimpanzees and orangutans showed a rapid decline of social behaviours and displayed self-directed behaviours after prolonged mirror exposure (Gallup, 1970; Suarez and Gallup, 1981). Considering the current study, longer exploratory time and larger exploratory space in front of a mirror should be provided to corvids in future studies.

Although no bird exhibited self-directed behaviours in Test 2, we still conducted the mark test because a mark could provide a new visual stimulus which may inspire subjects to perform different exploratory behaviours. When developing the marking procedure, we did not pay more attention to the weight of the vegetable powder used to mark the subjects. In contrast, attention was given to the healthy and unscented dye used to mark the recruited subjects. When the subjects were marked on the chest, where they could directly see the mark without the mirror, they showed mark-directed behaviours. The subjects considered the powder mark as 'dirt' that should be removed; thus, the powder can be used as a mark. However, no evidence of mark-directed behaviours in front of the mirror was recorded among all the five subjects that participated in the mark test when the mark was placed in



Fig. 7. Frequency (per hour) of responses to mirror in Test 2.

a location that was invisible without a mirror. Prior et al. (2008) placed a sticker (16 μ g) as a mark under the beak of Eurasian magpies. They observed that two out of the five subjects could remove the sticker in front of the mirror. Soler et al. (2014) used a heavier sticker (2375 ± 129 μ g) and reported that jackdaws failed to pass the mirror

mark test because the subjects showed mark-directed behaviours not only in front of mirror but also without a mirror. The marks used by Soler et al. were 150 times heavier than those used by Prior et al. This weight might explain why Soler et al. observed an effect of the mark irrespective of the condition. Thus, using an inappropriate marking

Table 2

Frequency	(per	hour)	of	responses	to	mirror	in	red	l-mark-mirror	cond	ition	and	no	-mark-mi	rror	condition.
1 2	~ 1			1												

1 2	4 <i>7</i> 1					
Bird	Observing the mirror image Red mark / NO mark	Pecking Red mark / NO mark	Aggressive displays Red mark / NO mark	Spreading the wings Red mark / NO mark	Submissive displays Red mark / NO mark	Looking behind mirror Red mark / NO mark
Daniel	6.98/10.40	29.30/17.44	0.00/0.00	9.77/3.82	2.79/0.24	19.53/29.28
Fatty	8.00/22.26	36.00/21.31	8.00/0.28	2.00/1.09	0.00/0.56	22.00/11.91
Joyce	2.00/4.52	22.00/18.52	0.00/0.60	0.00/0.40	0.00/0.00	0.00/3.88
Tiny	9.00/18.50	2.00/1.40	0.00/0.00	0.00/0.00	0.00/0.00	0.00/3.50
Alina	15.33/13.80	7.33/14.58	0.00/1.44	7.33/1.68	0.00/0.00	0.00/0.24
Neil	15.60/15.54	4.80/2.72	2.40/1.02	0.00/0.00	0.00/0.00	0.00/2.65
Emily	24.75/20.40	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00	0.00/0.00

Table 3

First responses of the subjects in Test 4.

Subject	Response	With bait	Without bait
Fatty	Looking behind the mirror directly	2	0
	Looking for the bait aimlessly	3	0
	No search responses	0	5
Daniel	Looking behind the mirror directly	4	0
	Jumping up to search the location of bait	1	2
	No search responses	0	3
Joyce	Looking for the bait aimlessly	5	0
	No search responses	0	5
Tiny	Looking for the bait aimlessly	4	0
	Jumping up to search the location of bait	1	0
	No search responses	0	5
Neil	Looking behind the mirror directly	4	1
	Looking for the bait aimlessly	1	0
	No search responses	0	4

method might cause tactile sensation on the test subjects. In this study, azure-winged magpies were involved in mark test for the first time. The results from the three mark-test conditions showed that marking with powder was appropriate method though the subjects did not perform mark-directed behaviours.

The mark test is only one piece of evidence of mirror-induced selfcognition in animals. Despite their poor performance in the mark test, the azure-winged magpies displayed exploratory behaviour towards their mirror image, as if looking at a conspecific, similar to the actions of primates (Straumann and Anderson, 1991; Ujhelyi et al., 2000; Suddendorf and Collier-Baker, 2009), children (Parker et al., 1994) and other corvids (Prior et al., 2008; Medina et al., 2011; Soler et al., 2014; Deregnaucourt and Bovet, 2016). These subjects searched behind the mirror during the mirror sessions, especially Daniel and Joyce. However, we did not observe the 'peekaboo' behaviours (the bird stares at the mirror image and then quickly moves its head out of view and then back within 3 s) reported in carrion crows (Vanhooland et al., 2019), New Caledonian crows (Medina et al., 2011) and primate infants (Robert, 1986) from the magpies. African grey parrots have also been observed to search behind mirrors, with the juveniles performing better than the adults (Pepperberg et al., 1995).

The last test (mirror-triggered search test, Test 4) was conducted to test whether azure-winged magpies could use the mirror image of the bait as a cue and locate the bait with the help of the mirror. In this study, five subjects preferred looking behind the mirror or looking for the bait aimlessly when food was present. That is to say, searching behaviours were triggered by the mirror image of the bait and the mirror provided visual cues. The subjects did not search for food specifically when no bait was available, indicating that the birds used the mirror to obtain information about the presence of the bait. Even so, the subjects in our study did not search for the food in the correct location, thereby indicating that they could not understand that the bait in the mirror was the same as that in the real world. Mirror-triggered search is the most basic task in which mirrors are used to find hidden objects (Povinelli, 1989; Pepperberg et al., 1995). Numerous studies reported a more difficult observation, i.e. the mirror-mediated spatial location required a more sophisticated cognitive ability. The subjects must understand the concept of mirror reflections, that is, they must realize that the object reflected in the mirror corresponded to an object in the real world. Pepperberg et al. (1995) took a different view by positing that an individual might understand that the objects reflected in the mirror had the same spatial location in the environment but might not understand that the reflected objects are the same as the real objects. The study on wild caught New Caledonian crows revealed that all individuals [4] successfully learnt to use a horizontal mirror to locate hidden food in the four-box spatial location task in the training stage (20 or 30 trials) (Medina et al., 2011). The author concluded that New Caledonian crows learnt to associate the mirror image of the food with finding the food in the compartment. The New Caledonian crows employed in their study were captured from the wild; therefore, they might have some experience with horizontal reflections of themselves because of drinking water from pools and other open sources of water. Therefore, subjects could probably learn to associate the mirror images of objects with finding the objects in the real world despite their lack of understanding of the concept of reflection. In another cognitive study, azurewinged magpies showed good learning capacity: some subjects failed in easy tasks but succeeded in later different tasks (Wang et al., 2019). Whether azure-winged magpies could, with training, ultimately succeed in more complex mirror test (mirror-mediated spatial location) remains to be seen.

Self-recognition has been observed in animals with relatively large brains, such as apes (Gallup, 1970), and highly evolved social cognition, such as dolphins (Reiss and Marino, 2001) and elephants (Plotnik et al., 2006), whereas solitary animals, such as giant pandas (Ma et al., 2015), fail the mark test. Corvids are especially well known for performing amazing cognitive tasks (Ujfalussy et al., 2013; Wang et al., 2019) and have been referred to as "feathered apes"(Emery and Clayton, 2004). Therefore, corvids have become model birds for exploring animals' cognition, especially considering that Eurasian magpies passed the mark test for self-cognition (Prior et al., 2008). To the best of our knowledge, this study is the first to investigate the mirrordirected behaviours of hand-raised azure-winged magpies. The azurewinged magpies displayed immense curiosity towards the mirror and their images in the mirror in Test 1&2. In the subsequent mark tests, they failed to pass the mark test and regarded their images as conspecifics. Further, the subjects in our study could not understand that the bait reflected in the mirror was the same as that in the real world. The subjects may use the mirror image as a cue to start searching the hidden bait rather than to obtain accurate location information of the hidden bait. This study provides valuable additional information on the mirror-understanding abilities of a further corvid species: the azurewinged magpie. Additional research involving prolonged mirror exposure and training is suggested to further explore the mirror self-recognition behaviours of azure-winged magpies.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Ethical standards

The study was conducted according to the Ethics Review Committee of Nanjing University (No. 2009-116). All applicable international, national and institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants.

Author contributions

LW, YL, ZL designed the study, LW, YL, HW, YZ, HY did the experiments, LW, YL, ZL analyzed the data, LW drafted the manuscript, SU and ZL reviewed and polished the manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.beproc.2020.104155.

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