Differential outcomes of unilateral interferences at birth

Alice de Boyer des Roches¹, Virginie Durier^{1,*},
Marie-Annick Richard-Yris¹, Catherine Blois-
Heulin¹, Mohammed Ezzaoui²,
Martine Hausberger¹ and Severine Henry¹

¹UMR 6552 Ethologie Animale et Humaine, CNRS-Université de
Rennes 1, Campus de Beaulieu, 35042 Rennes Cedex, France

²F.N.A.R.C.—Haras National de Sidi Thabet, 2020 Sidi Thabet, Tunisia

*Author for correspondence (virginie.durier@univ-rennes1.fr).

Behavioural modifications, including modifications of emotional reactivity, can occur following early experience such as handling (manual rubbing). Here, we investigated the effects of unilateral tactile stimulation at an early stage on emotional reactions later on. We handled newborn foals intensively on one side of their body. This early unilateral tactile experience had medium-term effects: the reactions of foals to a human approach, when they were 10 days old, differed according to the side stimulated at birth. Fewer right-handled foals accepted contact with humans, they delayed first contact longer and they evaded approaching humans sooner than did non-handled and left-handled foals. These results raise questions concerning the organization of neonatal care in animals and humans.

Keywords: behaviour; early experience; development; emotion; side bias

1. INTRODUCTION

Early age is a crucial period for animals' neurological and behavioural development, which is strongly influenced by both individual genetic programmes and environmental conditions [1]. Brief events during early life may have strong impacts on the organization and/or development of the brain asymmetry. Early exposure of chick embryos to light prior to hatching induces lateralization of several types of visual behaviour, as revealed by pebbles–grains tasks (review in [2]). Handling rat pups for few minutes each day during their first 20 days of life influences their stress responses after weaning, by increasing their exploratory behaviour in an open-field. This procedure, coupled with left or right neocortical ablation, revealed a hemispheric dominance for responses after early interference (review in [3]). Other studies evidence lateralization of emotion processing in brain structures [4].

Motor or perceptual laterality exists in some species, even before birth [2,5,6]. Sensitivity to tactile stimulation seems to be especially high during very early stages. For instance, the side that is subject to tactile

stimulation influences subsequent emotional states of human newborns [7]. The fact that early interference can induce long-lasting modifications raises questions concerning the possibility that unilateral stimulation could lead to differential reactions during development.

Here, we tested the hypothesis that unilateral stimulation at an early stage would induce different emotional responses to a stressful situation. We handled newborn foals intensively on one side of their body. Bilateral handling procedures of horses at birth have been shown to induce long-term behavioural modifications, including later reluctance to allow human approaches [8]. Similarly, bilateral forced stroking of foals aged from few hours to 5 days old decreases the acceptance of human contact when they are 15 days old [9]. Horses are an interesting model, as they show laterality patterns [10–12], among which visual laterality clearly indicates a hemispheric specialization for emotional processing [13], and standardized tests evaluating their emotionality have been developed [14].

2. MATERIAL AND METHODS

(a) Animals and experimental groups

Subjects were 28 purebred Arab foals (*Equus caballus*), (14 females and 14 males), born at the 'national stud farm of Sidi Thabet' (Tunisia). Mares were stabled in a 4 × 4 m foaling stall before parturition. Delivery was not assisted and newborn foals received minimal care (wiping off foetal membranes and applying antiseptic to their umbilical stump). Each dam–foal dyad was kept indoors in their familiar box for the first three weeks after parturition. Human contacts were mostly restricted to feeding. Water and roughage were available ad libitum.

At birth, each foal was allocated to one group on the basis of foaling date, sex and sire:

- a 'right-handled group' ('Rhand'; $n = 10$, four males and six females): handled intensively on the right side of their body;
- a 'left-handled group' ('Lhand'; $n = 9$, five males and four females): handled intensively on the left side of their body; and
- a 'control group' ('control'; $n = 9$, five males and four females): not handled after birth but exposed to a motionless person standing for 1 h within the box.

Owing to the limited number of available foals for this experiment, only these three experimental conditions could be tested. A previous study on bilaterally handled foals had revealed long-term effects of the procedure [8].

The neonatal handling procedure (inspired from Miller's 1991 'imprint training', cited in [8]) was performed immediately after birth, before foals stood up and suckled. The foal was maintained in a recumbent position, while the experimenter rubbed it intensively (tactile stimulation) on one side of its body for 60 min. The procedure was always performed by the same experienced handler. The dam was held and not allowed physical contact with her foal.

(b) Behavioural tests

From 10 to 15 days old, foals were exposed daily to two 'approach tests', one per side, to estimate their reactions to the familiar handler [14]. The first side of approach was assigned randomly. The experimenter entered the box and placed herself at 1.5 m from the foal, she then tried to approach and touch it on its shoulder, approaching from its side. The test lasted 60 s at the most. Contact was never forced and foals were free to avoid the experimenter. We recorded the latency required to touch foals (contact latency), as well as the latency at which the foal first moved away from the experimenter (avoidance latency). When a foal had not been touched or had not moved away within 60 s, maximum scores (60 s) were recorded.

To avoid any interference of the mare during testing, an unfamiliar person entered the box at the beginning of the test, fitted the dam with a halter and held her, avoiding standing between the mare and her foal.

The experiment was performed in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC).

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rsbl.2010.0979> or via <http://rsbl.royalsocietypublishing.org>.

Figure 1. Ten day old foals' emotional reactions in approach tests. (a) Frequencies at which control, Lhand and Rhand foals could be touched whatever the side of approach; (b) latencies before first contact by the experimenter; (c) latencies before first avoidance of the experimenter. Level of significance: one symbol = $p < 0.05$, two = $p < 0.01$ and three = $p < 0.001$.

(c) Statistical analyses

Data analysed were the numbers of occurrences and durations. Non-parametric statistics were used. As no differences between male and female data were observed in any of the tests, all data were pooled (Mann–Whitney test, $p > 0.05$).

3. RESULTS

Ten day old foals' reactions to humans differed clearly according to the early treatment. Thus, the responses of foals in the approach test were distributed along a gradient from control foals to Lhand and to Rhand foals, the former being the easiest to approach by a human and the latter showing the highest reluctance with respect to direct human contact (figure 1a). Significantly fewer Rhand foals than other foals could be touched (Fisher test: Rhand–Lhand and Rhand–control: $p < 0.001$). In addition, the time required to touch them was significantly longer (Kruskal–Wallis test: $p < 0.01$; Mann–Whitney U -test:

Rhand–control and Rhand–Lhand: $p < 0.01$; Lhand–control: $p = 0.59$; figure 1b), but the latency of avoidance response was shorter than for control foals (Rhand–control: $p < 0.05$; Rhand–Lhand: $p = 0.08$; figure 1c).

Rhand foals accepted human contact more reluctantly than did the other foals, whatever the side that was approached by human, as fewer of them could be touched (Fisher tests: Rhand–control: left and right: $p < 0.01$; Rhand–Lhand: right: $p < 0.05$; left: $p = 0.18$; figure 2a). The time required to establish contact and avoidance latency when approached on the left differed between Rhand foals and controls (Mann–Whitney U -test: contact from right or left: $p < 0.05$; avoidance from left: $p < 0.05$ or right: $p = 0.57$; figure 2b,c). Contact and avoidance latencies differed between Rhand and Lhand foals only when they were approached on their right side (Mann–Whitney U -test: contact or avoidance from right: $p < 0.05$ or left: $p = 0.11$ and $p = 0.64$; figure 2). Indeed, Lhand foals accepted human approach on the right side more readily than on the left side (avoidance latency: Wilcoxon test: right: $p < 0.05$).

The approach test was repeated every day from the 10th to the 15th day. From the 11th to the last (15th) days, Rhand foals' reactions no longer differed from those of Lhand and control foals, both in terms of contact latencies and of avoidance latencies (see electronic supplementary material, S1).

4. DISCUSSION

Previous experiments had revealed that bilateral handling or forced stroking at an early stage after birth could induce avoidance of contact with a familiar or unfamiliar human, either shortly after the negative event or months later [8,9]. In the present study, foals were handled unilaterally at birth. This early tactile experience had at least medium-term effects, as the reactions of these foals to human approach when 10 days old differed according to the side of stimulation. Non-handled foals appeared to accept human approach and contact more than did right-handled foals, which were very reluctant to be touched. Left-handled foals presented intermediate reactions as they avoided human approach when it occurred on their left side. Thus, a short (1 h) early interference can impair emotional development durably. Repetition of neutral interactions (1 min of non-invasive presence) restored the alterations.

The negative effects of handling could be explained by the intense stress generated by the procedure the foals were subjected to immediately after birth (foals struggled a lot while being restrained on the floor). In addition to this stress, they were deprived of the maternal care they could expect at that time. This creates an insecure attachment to their mare that impairs their subsequent social relationships with conspecifics [8]. Forced contacts during the days following birth also has consequences on the perception a foal has of humans [9].

In our experiment, tactile stimulation was lateralized. Vision was coupled with touch as foals could see the experimenter when they were handled.

Figure 2. Foals' emotional reactions in approach tests in relation to side of approach. (a) Frequencies of control, Lhand and Rhand foals that could be touched according to the side of approach; (b) latencies before first contact by the experimenter; (c) latencies before first avoidance of the experimenter. Level of significance: one symbol = $p < 0.05$, two = $p < 0.01$ and three = $p < 0.001$. Black bars = left; grey bars = right.

We assume that the emotional states created by vision and touch differed in relation to the side stimulated, and induced different reactions in the perception of human approaches.

Individuals of several species use either their right or their left eye in relation to what they perceive and the

related behaviour. Predator avoidance differs when a predator approaches from the left or from the right [2,15]. Similarly, one eye is often preferred for conspecific or individual recognition (fishes [16], chicks [17], quail [18] and toads [19]) or for scanning situations with different emotional values [13,20]. Concerning lateralized effects of tactile stimulation, few examples exist, mainly in humans. Stimulation of newborns' peri-oral area elicits head turning and cardiac acceleration more often when applied on the right than on the left side [7]. This reveals differential sensitivity between sides of the face.

The differential responses to human approach according to the side handled were the result of visual and tactile interferences at birth. The emotional value of each form of stimulation differs from one another, so are their behavioural consequences. Chicks' right eyes (and left hemisphere) are involved in the elaboration of categories facilitating rapid learning of what they perceive. The left eye is attracted to novelty and learning with this eye is slow [2]. One can assume that, in our experiment, the foals rapidly associated humans and handling when it occurred on their right side, while the procedure on their left side did not induce a negative perception of humans.

To conclude, attention should be given to the symmetry of stimulation and their consequences in all experiments involving early handling. Practices around birth in humans and animals may be adjusted by taking care of the way stimulation is provided to the newborn.

We are grateful to the 'Haras National de Sidi Thabet' for allowing us to work with their horses and the staff for its help and understanding. We are also grateful to the COST ('Haras Nationaux') for their financial support.

- 1 Chapillon, P., Patin, V., Roy, V., Vincent, A. & Caston, J. 2002 Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Dev. Psychol.* **41**, 373–387.
- 2 Rogers, L. J. 2008 Development and function of lateralization in the avian brain. *Brain Res. Bull.* **76**, 235–244. (doi:10.1016/j.brainresbull.2008.02.001)
- 3 Denenberg, V. H. 1981 Hemispheric laterality in animals and the effects of early experience. *Behav. Brain Sci.* **4**, 1–21. (doi:10.1017/S0140525X00007330)
- 4 Demaree, H. A., Everhart, D. E., Youngstrom, E. A. & Harrison, D. W. 2005 Brain lateralization of emotional processing: historical roots and a future incorporating 'dominance'. *Behav. Cogn. Neurosci. Rev.* **4**, 3–20. (doi:10.1177/1534582305276837)
- 5 Hepper, P. G., Wells, D. L. & Lynch, C. 2005 Prenatal thumb sucking is related to postnatal handedness. *Neuropsychologia* **43**, 313–315. (doi:10.1016/j.neuropsychologia.2004.08.009)
- 6 Rönqvist, L. & Hopkins, B. 1998 Head position preference in the human newborn: a new look. *Child Dev.* **69**, 13–23.
- 7 Hammer, M. & Turkewitz, G. 1974 A sensory basis for the lateral difference in the newborn infant's response to somesthetic stimulation. *J. Exp. Child Psychol.* **18**, 304–312. (doi:10.1016/0022-0965(74)90110-6)
- 8 Henry, S., Richard-Yris, M.-A., Tordjman, S. & Hausberger, M. 2009 Neonatal handling affects durably bonding and social development. *PLoS ONE* **4**, e5216. (doi:10.1371/journal.pone.0005216)

- 9 Henry, S., Richard-Yris, M. A. & Hausberger, M. 2006 Influence of various early human–foal interferences on subsequent human–foal relationship. *Dev. Psychobiol.* **48**, 712–718. (doi:10.1002/dev.20189)
- 10 Austin, N. P. & Rogers, L. J. 2007 Asymmetry of flight and escape turning responses in horses. *Laterality* **12**, 464–474.
- 11 Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M. & Lemasson, A. 2009 Socially dependent auditory laterality in domestic horses (*Equus caballus*). *Anim. Cogn.* **12**, 611–619. (doi:10.1007/s10071-009-0220-5)
- 12 McGreevy, P. D. & Rogers, L. J. 2005 Motor and sensory laterality in thoroughbred horses. *Appl. Anim. Behav. Sci.* **92**, 337–352. (doi:10.1016/j.applanim.2004.11.012)
- 13 De Boyer Des Roches, A., Richard-Yris, M.-A., Henry, S., Ezzaoui, M. & Hausberger, M. 2008 Laterality and emotions: visual laterality in the domestic horse (*Equus caballus*) differs with objects' emotional value. *Physiol. Behav.* **94**, 487.
- 14 Henry, S., Hemery, D., Richard, M. A. & Hausberger, M. 2005 Human–mare relationships and behaviour of foals toward humans. *Appl. Anim. Behav. Sci.* **93**, 341–362. (doi:10.1016/j.applanim.2005.01.008)
- 15 Lippolis, G., Bisazza, A., Rogers, L. J. & Vallortigara, G. 2002 Lateralisation of predator avoidance responses in three species of toads. *Laterality* **7**, 163–183.
- 16 Sovrano, V. A., Bisazza, A. & Vallortigara, G. 2001 Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiol. Behav.* **74**, 237–244. (doi:10.1016/S0031-9384(01)00552-2)
- 17 Vallortigara, G., Regolin, L. & Pagni, P. 1999 Detour behaviour, imprinting and visual lateralization in the domestic chick. *Cogn. Brain Res.* **7**, 307–320. (doi:10.1016/S0926-6410(98)00033-0)
- 18 Zucca, P. & Sovrano, V. A. 2008 Animal lateralization and social recognition: quails use their left visual hemifield when approaching a companion and their right visual hemifield when approaching a stranger. *Cortex* **44**, 13–20. (doi:10.1016/j.cortex.2006.01.002)
- 19 Vallortigara, G., Rogers, L. J., Bisazza, A., Lippolis, G. & Robins, A. 1998 Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport* **9**, 3341–3344. (doi:10.1097/00001756-199810050-00035)
- 20 Farmer, K., Krueger, K. & Byrne, R. W. 2010 Visual laterality in the domestic horse (*Equus caballus*) interacting with humans. *Anim. Cogn.* **13**, 229–238. (doi:10.1007/s10071-009-0260-x)