Animal Behaviour 114 (2016) 261–271

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Play matters: the surprising relationship between juvenile playfulness and anxiety in later life

S. Helene Richter*, Niklas Kästner, Marie Kriwet, Sylvia Kaiser, Norbert Sachser

Department of Behavioural Biology, University of Münster, Münster, Germany

ARTICLE INFO

Article history:
Received 4 November 2015
Initial acceptance 4 January 2016
Final acceptance 18 January 2016

MS. number: 15-00943

Keywords:
animal personality
animal welfare
anxiety-like behaviour
behavioural tests
exploratory locomotion
laboratory mice
mouse individuality
play behaviour
playfulness

Besides being recognized as a potential welfare indicator, play behaviour has long been considered to have immediate and/or long-term benefits. In particular, it has been suggested that in play animals learn to cope physically and emotionally with unexpected events. Given that the propensity to play varies greatly between conspecific individuals, such interindividual variability in playfulness may be associated with differences in the animals' future behaviour, a prediction that has rarely been tested. To investigate whether different levels of playfulness in juvenility indeed coincide with behavioural differences in later life, 30 female C57BL/6J mice were subjected to the following series of behavioural observations and tests: (1) quantification of juvenile play behaviour; (2) behavioural testing in paradigms that assess anxiety-like behaviour and exploratory locomotion in an unfamiliar environment; and (3) observation of spontaneous behaviour in the familiar home cage environment. Surprisingly, a high level of juvenile playfulness was predictive of high levels of state anxiety and low levels of exploratory locomotion in later life. While this relationship existed already in adolescence, it became even more prominent in adulthood. By contrast, no substantial differences between playful and less playful mice were found with respect to home cage behaviour. While these findings may reflect better coping abilities in novel and dangerous environments in those mice that played the most during juvenility, they may also argue for the existence of different types of mice. Thus, despite genetic homogeneity and identical housing environments, preferences for either local or global use of space were observed that indicate the emergence of individuality. Concerning animal welfare, our findings suggest that play may constitute a plausible welfare indicator at the population level, but is probably less meaningful for the individual.

Despite several decades of research on the causes and consequences of play behaviour in animals, play still remains a behavioural domain of considerable fascination. In a collection of essays and short review articles, the journal *Current Biology*, on its 25th birthday, presented what the scientific community knows about play in a broad range of animal species, including mammals (Bekoff, 2015; Janik, 2015), birds (Emery & Clayton, 2015), reptiles (Burghardt, 2015) and even invertebrates (Zylinski, 2015). Under the title 'The Biology of Fun and the Fun of Biology' particular focus was put on examples of what exactly appears to be play, how these behaviours contribute to Darwinian fitness and whether they are linked to positive emotions (North, 2015).

So, what exactly is play and which components make play 'playful'? Despite its clarity in expression and our intuitive understanding of this behaviour, play has always been difficult to define. Current definitions usually assign the following characteristics to play. The behaviour does not contribute to immediate survival needs, it is intrinsically rewarding, spontaneous, voluntary and purposeless, it occurs repeatedly, but not stereotypically, and it is only performed when the animal is in a relaxed state (Burghardt, 2005; Janik, 2015). Play may further involve actions from other contexts, but differs from serious behaviours in both form (e.g. exaggerated) and timing (Bekoff, 2015; Graham & Burghardt, 2010). Alternatively, play may also evolve from creative ways of interacting with the environment, resulting in novel behavioural elements or sequences (Bateson, 2015). Furthermore, depending on the species play behaviour can occur in a variety of forms, it can be performed alone or in a social setting, and it can include objects or not (Bekoff, 2001; Tanner & Byrne, 2010). Play thus seems to be a behavioural domain of remarkable complexity and variability both within and between species that is far from being understood.

In animal welfare science, play has long been recognized as a potential indicator of the current welfare state of an animal (Biben

* Correspondence: S. H. Richter, Department of Behavioural Biology, University of Münster, Badestraße 13, 48149 Münster, Germany.
E-mail address: richterh@uni-muenster.de (S. H. Richter).

http://dx.doi.org/10.1016/j.anbehav.2016.02.003
0003-3472/

& Champoux, 1999; Blackshaw, Swain, Blackshaw, Thomas, & Gillies, 1997; Boissy et al., 2007; Held & Špinka, 2011; Oliveira, Rossi, Silva, Lau, & Barreto, 2010; Yeates & Main, 2008). Play typically occurs at times when vital necessities are covered and animals find themselves in a healthy and relaxed state, while it can disappear when their fitness is challenged. For example, castration (Thornton & Waterman-Pearson, 2002), cold weather (Newberry, Wood-Gush, & Hall, 1988) or insufficient food supply (Loy, 1970) have been described as eliminating or reducing play behaviour, while the provision of more space or more complex environments has been found to stimulate play in various species (Jensen & Kyhn, 2000; Marashi, Barnekow, Ossendorf, & Sachser, 2003; Marashi, Barnekow, & Sachser, 2004; Vinke, Van Leeuwen, & Spruijt, 2005). The absence of play thus appears to have the potential to uncover challenging conditions, in which an animal's welfare may be compromised, while the presence of play may indicate beneficial situations that induce positive emotions and good welfare (Boissy et al., 2007; Held & Špinka, 2011).

In addition to indicating a state of good welfare in a certain environment or situation, play has frequently been considered to have a variety of immediate and/or long-term benefits. Such functional benefits may comprise, for example, the development of motor coordination, the acquisition of social skills and/or the training of cognitive abilities to allow for a better performance in future real-life situations. In this context, several hypotheses have been postulated that all try to explain the adaptive value of play (e.g. Burghardt, 2005; Byers, 1998; Pellis, Pellis, & Bell, 2010). Of particular interest for the present study is the so-called 'training for the unexpected' hypothesis, stating that play promotes the ability to deal with unexpected events and cope emotionally with stressful situations (Spinka, Newberry, & Bekoff, 2001), implying both physical and psychological advantages especially in novel situations. If so, what would this mean for the individual?

Play has been shown to vary considerably within a given population with conspecific individuals of the same age and sex still differing in their propensity to play, also referred to as 'playfulness' in the scientific literature (Biben & Champoux, 1999; Brown, Klaffenböck, Nevison, & Lawrence, 2015; Eckardt et al., 2015; Held & Špinka, 2011; Poole & Fish, 1976; Siviy, Love, DeCicco, Giordano, & Seifert, 2003; Svartberg & Forkman, 2002). According to the 'training for the unexpected' hypothesis, such interindividual variability in playfulness would lead to differences in the future behaviour of these animals. In particular, a playful and hence 'trained' individual would probably be better able to cope with challenging situations than a less playful one, a prediction that has rarely been tested. The aim of the present study was therefore to assess individual differences in playfulness in juvenile mice and relate these to behavioural differences in familiar and unfamiliar situations in later life. Unlike rats, mice do not engage much in social play, such as 'play fighting' or 'tandem running', although basic components of playful attack and defence behaviours are also present (Pellis & Pasztor, 1999), but instead are known to perform different types of locomotor play (e.g. Walker & Byers, 1991; Wolff, 1981). By quantifying individual differences in locomotor play during juvenility and investigating behavioural differences in these mice during adolescence and adulthood, we sought to test the hypothesis that the individual level of juvenile playfulness is related to the behaviour shown in later life. In particular, we expected animals that vary in their propensity to play during juvenility to behave differently in three widely used behavioural paradigms that assess anxiety-like behaviour and exploratory locomotion in an unfamiliar environment. By contrast, we did not expect to find behavioural differences between playful and less playful mice within the familiar home cage environment.

METHODS

Animals and Housing Conditions

We obtained 30 freshly weaned female individuals of the inbred mouse strain C57BL/6J from a professional breeder (Charles River Laboratories, Research Models and Services, Germany GmbH, Sulzfeld, Germany) at the age of 21 days. Upon arrival, mice were randomly allocated to five super-enriched terraria, resulting in group sizes of six unrelated individuals per terrarium. For individual identification, mice were given black marks on their tails that were renewed on a weekly basis. In line with previous experiments with mice housed in super-enriched environments (Marashi et al., 2003; Marashi et al., 2004), glass terraria (100×40 cm and 34.5 cm high) were covered with a layer of wood shavings (Allspan Olympia-Einstreu, Allspan GmbH, Karlsruhe, Germany), contained two paper tissues as nesting material and had various enrichment items, such as a Macrolon type III cage (37×21 cm and 15 cm high), a second floor made of transparent plastic (45×33.5 cm), plastic stairs, a plastic inset, a wooden scaffolding and a climbing tree (Fig. 1). Food (Altromin 1324, Altromin GmbH, Lage, Germany) and water were provided ad libitum and terraria were cleaned every second week. Since this type of enrichment has been found to cause a sharp rise in aggressive behaviour in male mice (Marashi et al., 2003), only female mice were included in the present study so that they could be housed in stable social groups for the complete experiment. The housing room was maintained at a 12:12 h light:dark cycle with lights off at 1000 hours, a relative humidity of $50 \pm 10\%$, and a temperature of about 22 °C.

Ethical Note

All procedures complied with the regulations covering animal experimentation within the EU (European Communities Council DIRECTIVE 2010/63/EU) and were conducted in accordance with the institutions' animal care and use guidelines. Experiments were approved by the national and local authorities (LANUV, reference number: 84-02.05.20.12.212). Our procedures did not appear to cause any distress or pain to the animals. After the study, the animals remained in the animal facility of the institute for further behavioural experiments.

Experimental Design

To investigate whether different levels of playfulness in juvenility lead to differences in anxiety-like behaviour and exploratory locomotion in a novel test situation, but not in spontaneous behaviour in the home terrarium in later life, the experiment was subdivided into the following phases: (1) quantification of play behaviour in juvenile mice aged 24–45 days to assess individual levels of playfulness; (2) behavioural testing of mice in early adolescence (behavioural tests 1, postnatal days 48–52) and in adulthood (behavioural tests 2, postnatal days 83–87); and (3) observation of home cage behaviour in between (postnatal days 55–80; Fig. 2). Behavioural tests were conducted twice to cover different stages in life (i.e. before and after sexual maturation) and allow for generalization of findings across ages. All 30 mice were observed and tested in a single batch over a period of 10 weeks.

Quantification of Play Behaviour

To quantify play behaviour, mice were observed early in life between postnatal days 24 and 45. As the time around and after weaning has been identified as the peak age for locomotor play in