Predictability and randomness of paw choices are critical elements in the behavioural plasticity of mouse paw preference

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Lateralized paw usage of mice, *Mus musculus*, is a learned behaviour, based on a gradual reinforcement of randomly occurring weak asymmetries in paw choice early in training. The reinforcement relies on strain-dependent, short-term and long-term memory. We characterized the skills of information accumulation by quantifying the predictability of each reach of initially naïve mice from past behaviour in two training sessions of 50 reaches, separated by a 1-week interval. We studied six mouse strains, including 9XCA and BTBR with absent corpus callosum and severely reduced hippocampal commissure, and compared them to a null model with random, unbiased paw preference. We found that each paw choice was based on a limited, strain-specific number of previous choices. Also, there was a limited, strain-specific degree of predictability of each choice. Consequently, there was a strain-specific degree of randomness that was not lost with training. After 1 week for consolidation of memory of learned biases, paw choices became more predictable and made use of fewer previous choices, except in 9XCA and BTBR; nevertheless, a degree of randomness remained. We conclude that paw choices are regulated by short-term memory of a small number of previous choices and by long-term memory that affects future behaviour patterns and decreases, but does not remove, the usage of short-term memory. Both short-term and long-term memory skills are strain dependent. Importantly, a degree of randomness is not removed by training and this may be a critical element for behavioural plasticity in paw preference in changing environments, supplying constant adaptability in paw preferences.

Paw preference behaviour in mice, *Mus musculus*, has the feature of symmetry at the level of the population, but strong asymmetries at the level of individuals. Laboratory strains of mice have characteristically different patterns of paw preference that have remained consistent across many generations and in different laboratories, since the initial description of the behaviour in a single-paw reaching test (Collins, 1968, 1969). Studies in right- and left-biased test chambers demonstrated that mice learn a direction of paw preference as they reach for food with their forepaws (Biddle & Eales, 1999). Therefore, the differences in patterns of paw preference must arise from strain differences in a genetically regulated system of learning and memory. The challenge for genetic analysis of paw preference behaviour has been to understand the mechanism of learning and memory in different strains and to identify measurable elements that are genetically regulated and, hence, that give rise to different patterns of paw (or hand)

preference (McManus, et al., 1988; Palmer, 2002, 2003, 2012; Rogers, 2009).

Early assessments of the learning and memory of paw preference behaviour have been reviewed in Biddle and Eales (2013). Briefly, kinetic analysis in right- and left-biased test chambers uncovered the learning response to the number of training reaches, the decay of memory between tests, the importance of memory by blocking memory consolidation with a protein synthesis inhibitor, and the range of phenotypic, hence, genotypic differences in paw preference learning ability between mouse strains (Biddle & Eales, 2006). Subsequently, agent-based simulations reproduced the dynamic patterns of paw preference between strains by using 'learning rate' as the heritable trait (Ribeiro, Lloyd-Price, Eales, & Biddle, 2010). From this, it was determined that several strains, previously identified as 'nonlearners', actually have significant learning ability. Further, the simulations allowed a definition of the expected behaviour of nonlearner model mice and a prediction of the limits of paw preference learning in unbiased test chambers (Ribeiro, Eales, & Biddle, 2011). Finally, studies showed that the acquisition of biases is severely hampered, particularly in the long-term, in strains with absent corpus callosum and severely reduced

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hippocampal commissure, which suggests that memory is essential in the generation and consolidation of biases in paw preference in mice (Ribeiro, Eales, & Biddle, 2013).

Ribeiro et al. (2011) characterized long-term learning and memory of paw preference in unbiased test chambers by comparing the biases in paw preference in two tests that were separated by a 1-week interval. Most mice exhibited heavier biases in the second test and in the same direction as the weaker biases in their first test. This was evidence that paw preference is an adaptive behaviour, based on learning. Moreover, mouse strains differed in degree of bias. Namely, in some strains (e.g. C57BL/6J) most mice were heavily biased and the number of 'heavily biased' mice increased in the second test, whereas in other strains (e.g. CDS/Lay) many mice remained unbiased in both tests and, more importantly, the fraction of heavily biased mice increased only weakly from the first to the second test. This result was strong evidence that the degree of learning of paw preference is genetically regulated and it is detectable in tests using unbiased chambers.

We also characterized strain-dependent short-term memory skills in the system of learning and memory in paw preference behaviour (Ribeiro et al., 2011). A positive autocorrelation for any lag between two paw choices in the test session demonstrated that the reaching behaviour of mice was not fully random; rather, it was based on previous events. Also, this positive autocorrelation decreased with increasing lag between successive paw reaches within a test session, which demonstrated that mice gradually learned a direction of paw preference from reach to reach, during a session. For this, mice ought to pay more attention to their recent paw choices than to their distant past choices. Finally, mouse strains differed in both mean positive autocorrelation and rate of decrease in autocorrelation with increasing lag between reaches (Ribeiro et al., 2011), which further established that there were heritable differences in short-term memory skills in a test session as well as in the long-term memory skills between sessions. These observations might reflect a reciprocal and antagonistic relationship between short-term and long-term memory acquisitions. Whereas long-term memory skills are required to make use of past learning in future reaching events, individuals that retain more information from immediate past reaches than from distant past reaches can adapt their behaviour more rapidly to changing environments.

Rates of adaptation to changing environments are being studied in many biological systems. A recent study on genetic networks suggests that sensing changes in the environment provides evolutionarily selective advantages in rapidly fluctuating environments (Ribeiro, 2008). In the case of paw preference behaviour, we suggested that a probabilistic paw choice creates a wide distribution of right-paw and left-paw usage among genetically identical individuals of an inbred mouse strain and differences in the patterns of paw preference between different genotypes of mice, such as from different inbred strains, could provide mice with more robustness to environmental changes and create a selective advantage for some strains in specific environments (Ribeiro et al., 2010). Further, different rates of adaptation of paw preference would lead to different fitness, depending on the rate of fluctuation of the environment, as discussed in other systems (Acar, Mettetal, & van Oudenaarden, 2008; Eldar & Elowitz, 2010; Hill & Zhang, 2004). In the context of probabilistic paw preference behaviour, different rates of adaptation are manifest in the variance of the phenotypic distributions of paw preference of different genotypes (i.e. differences in patterns of right- and left-paw preference), rather than as changes in mean values of right- or left-paw usage (Biddle & Eales, 2011; Ribeiro et al., 2010).

Autocorrelation analysis of successive paw reaches (Ribeiro et al., 2011) provided the first clear evidence for heritable differences in short-term memory of paw preference learning between strains as well as for the occurrence of behavioural modification from reach to reach, during a training session. Namely, it showed that the positive autocorrelation that is found between successive paw reaches decreases with increasing lag between the reaches (Ribeiro et al., 2011). Nevertheless, so far, there is no method to determine how many previous reaches influence the decision of which paw to use at each reach (and, thus, the predictability of the choices), nor is there a means to quantify the degree of influence that these past choices have on each paw choice during the course of a test session. These quantities would allow us to measure and evaluate the system of short-term memory processes associated with learning a paw preference and, thus, to compare the genetically regulated, short-term and long-term learning abilities of different mouse strains. They may also help us meet the challenge of genetic analysis of paw preference behaviour with identified measurable elements of the system that are genetically regulated.

We confronted this issue in the present study by analysing the information entropy in the time series of paw choices that were made by previously untested, naïve mice of various inbred strains in unbiased U-world or UW test chambers. We asked three questions concerning the behaviour of naïve mice during their first training session. (1) How many previous reaches are used in decision making? (2) How predictable is each paw choice, given the knowledge of all past paw choices? (3) What is the magnitude of the strain differences in these two properties? With the data from the second training session of these mice, 1 week after their first session, we asked the following questions. (1) How much are the properties of paw choice in the second training session modified by the information from the first session? (2) How much do the strains differ in degree of behaviour modification between sessions? (3) Are the effects of both short- and long-term memory of previous reaches visible in the behaviour in the second session?

From the answers, we quantify the amount of paw preference learning that occurs during a training session and provide a measure of the maximum degree of predictability of paw choice and, consequently, of the remaining degree of stochasticity (i.e. uncertainty) in paw preference. Finally, we also quantify the number of previous reaches that are required to achieve maximum predictability, which can be used as a measure of the number of previous reaches used by the short-term memory mechanisms to affect subsequent decisions. We used an unbiased test chamber because it has several advantages compared to biased ones. First, there is no physical or other reason for a bias in paw choice to appear where none existed, other than the existence of procedural learning from previous choices. Second, it allowed us to show that there are no biases at the population level, which suggests absence of constitutive biases. Third, we expected that the speed of learning a bias in paw preference in the absence of a physical reason for preferring a paw would be slower than in a biased test chamber, which may facilitate its detection in the case of strong learners.

METHODS

As in our previous work, we use the word 'memory' to mean 'implicit memory' or an unconscious form of memory (Roediger, 1990; Schacter, 1987) and 'learning' to imply 'procedural learning' or a behavioural change by an acquisition of implicit memory (Nissen & Bullemer, 1987).

Mouse Strains, Housing and Husbandry

We studied paw preference in the inbred strains C57BL/6JBid, C3H/HeSnJ.PafBid, DBA/2JBid and CBA/FaCamBid. We also used the