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Research report

Differences in extinction of conditioned fear in C57BL/6 substrains are unrelated to expression of α-synuclein

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Abstract

C57BL/6 mice are commonly used as background strains for genetically modified mice, and little attention is usually paid to the notification of the specific substrain. However, it is known that C57BL/6NCrl (B6N) and C57BL/6JOlaHsd (B6JOla) mice differ in the course of extinction of conditioned fear (Stiedl O, Radulovic J, Lohmann R, Birkenfeld K, Palve M, Kammermeier J, et al. Strain and substrain differences in context- and tone-dependent fear conditioning of inbred mice. Behav Brain Res 1999;104:1–12), as well as in the expression of α-synuclein (Specht CG, Schoepfer R. Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. BMC Neurosci 2001;2:11). We tested for a causal relationship between the two findings by employing B6N (expressing α-synuclein), B6JOla (not expressing α-syn) and the third strain C57BL/6JCrl (B6Jax, expressing α-syn). We show that α-syn does not account for differences in extinction in a fear conditioning task, as its expression did not covary with the decrease of freezing on repeated non-reinforced tone and context exposure in the three strains: B6Jax exhibited fastest extinction followed by B6JOla. In contrast, B6N showed persistent fear over the course of extinction training. The differences in extinction between B6JOla and B6N were unrelated to sensorimotor processing (pain threshold and basal tone reaction) and innate fear (light–dark test). However, B6Jax displayed less innate fear than B6JOla and B6N. Our results of marked differences in innate and conditioned fear in three B6 substrains illustrate the necessity of a strict adherence to an exact mouse strain nomenclature.

Keywords: C57BL/6JOlaHsd; C57BL/6JCrl; C57BL/6NCrl; Fear conditioning; Mice; Inbred strains; Extinction; Light–dark avoidance; Anxiety; Innate fear

1. Introduction

Pavlovian fear conditioning is a well established experimental model. While the anatomical structures and molecular mechanisms underlying acquisition of the CS–US association are well described [14], there are still many open questions regarding the neurobiological basis of extinction [18]. Aberrations in the extinction of conditioned fear have been reported in psychiatric disorders like social [12] or specific [17] phobias and post-traumatic stress disorder [4,8], illnesses whose pathomechanisms are still unknown. Therefore studying the substrates of extinction in animal models is a matter of interest for both basic and clinical research.

Biochemical comparison of animals that differ solely in a distinct behavioural feature represents a promising approach for characterising neurobiological correlates of the respective behavioural phenotype [13]. The two inbred mouse strains C57BL/6NCrl (B6N) and C57BL/6JOlaHsd (B6JOla) differ in their course of extinction of conditioned fear but not in acquisition of fear memory, with B6N showing a slower decline of fear in response to the CS than B6JOla [20]. Naive B6N and B6JOla mice, in contrast, exhibit only minor differences in anxiety-related behaviour at best [21]. It is probably for this similarity in innate emotionality that in the literature the exact nomenclature of C57BL/6 substrains is often not indicated, although the two substrains are far from being similar in respect to the processing of aversive experience. In fact, the latter differences might provide an opportunity to selectively examine biological correlates of fear extinction. In this

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study, we undertook a first approach of searching for biological substrates of the diverging extinction course in the two B6 strains.

Descending from the same breeding stock, B6N and B6JOla mice should be genetically similar. Nevertheless, Specht and Schoepfer (2001) described a spontaneous ablation of the alpha-synuclein (α-syn) gene in B6JOla. α-syn is a presynaptically localised protein that has been implicated in synaptic transmission and the aetiology of neurodegenerative disorders such as Alzheimer's and Parkinson's disease (for reviews see [6,15]). Accordingly, two mouse models with genetic ablation of α-syn displayed alterations in synaptic transmission [1,3] that have been interpreted as indicators of an inhibitory role of α-syn in activity-dependent modulation of dopamine neurotransmission [1] and of a significant contribution of α-syn to the genesis and maintenance of "reserve" or "resting" pools of presynaptic vesicles in CA1 neurones of the dorsal hippocampus [3]. Based on the putative consequences of α-syn ablation on synaptic transmission in glutamatergic and dopaminergic neurons, we wondered whether the lack of α-syn in B6JOla mice might account for the accelerated extinction of conditioned fear in these animals compared to B6N mice. If the lack of α-syn had similar consequences on excitatory synaptic transmission implicated in the expression and extinction of conditioned fear [18], as in CA1 neurons in α-syn knock-out mice [3], a prolonged stimulation by the tone might result in a faster "exhaustion" of the stimulus–response system. As a consequence freezing would be expected to more rapidly decline in animals without α-syn (B6JOla) than in animals with an intact α-syn gene (B6N).

Therefore, additionally to B6JOla and B6N we analysed the extinction course of a third BL/6 strain (C57BL/6JCrl, B6Jax) that is known to express α-syn similarly to B6N mice. We paired a neutral tone (CS) to a footshock and tested the fear reaction to the CS and to the conditioning context over a course of 1–3 min on days 1, 6, 7 and 21 after conditioning. In this way we assessed short-term (within-session) and long-term (between-session) extinction of auditory-cued and contextual fear as well as context dependency of extinction and spontaneous recovery [18]. In addition we controlled for effects of innate fear, pain threshold and sensorimotor stimulus processing on fear conditioning by examining in naive animals the reaction to electric currents of increasing intensity (pain threshold), to the tone that is used as CS and to a light–dark avoidance task.

2. Materials and methods

All experiments were approved by the Committee on Animal Health and Care of the local governmental body and performed in strict compliance with the EEC recommendations for the care and use of laboratory animals (86/609/CEE). Light–dark avoidance testing and fear conditioning were performed within the same animals, with the light–dark test preceding fear conditioning by 2 days. The remaining experiments included independent groups of animals. Within the experiments of fear conditioning and innate reaction to the CS tone each mouse went through each phase of the testing procedure in a repeated measure design. B6Jax mice were tested 7 months after the testing of B6JOla and B6N.

2.1. Animals

A total of 36 C57BL/6JOlaHsd (Harlan Winkelmann, Bochern, Germany; B6JOla), 35 C57BL/6NCrl (Charles River Germany GmbH, Sulzfeld, Germany; B6N) and 12 C57BL/6JCrl (originally Jackson Laboratory, provided by Charles River, Germany GmbH, Sulzfeld, Germany; B6Jax) male mice were tested at an age of 8–13 weeks. Animals were separated immediately after arrival from the supplier and housed singly with an inverse 12:12 h light–dark schedule (lights on at 8 pm) in standard macrolon cages (type 2) for 14 days before the start of experiments. They were allowed free access to food and water. Experiments were performed during the activity period of the animals. Except for light–dark testing, all experiments took place in the same room, where a sound- and light-tight curtain separated experimental compartments and home cages.

2.2. Apparatus

2.2.1. Fear conditioning, innate tone reaction, pain threshold

Experiments were performed in three contexts: (1) the conditioning chamber (ENV-307A, MED Associated, St. Albans, VT, USA), cubic-shaped with two metal walls and two transparent Plexiglas walls, a house light (ENV-215M, MED Associates; 0.6 lx), a metal grid for shock application (ENV-407; Shocker/Scrambler: ENV-414, MED Associates), (2) test context 1, cylindrically shaped and made of transparent Plexiglas (Ø 15 cm × H 30 cm) with a house light (ENV-215M, MED Associates; 0.3 lx) and (3) test context 2 with the shape of a hexagonal prism with opaque, rough surfaced Plexiglas walls except for one transparent side facing the camera (total dimensions: L 15 cm × W 13 cm × H 30 cm) with a stimulus light as a house light (ENV-221M, MED Associates; 12 lx). The conditioning context was cleaned with 70% ethanol, test context 1 with 1% acetic acid and test context 2 with 0.05% isoamylacetate (banana smell). All contexts were located in soundproof isolation cubicles (ENV-018M, MED Associates) that were additionally isolated with acoustic foam (Conrad Electronics, Hirschau, Germany). The tone was generated by audio stimulus generators (ANL-926, MED Associates) and applied by speakers (DTW 110 NG, Visaton, Haan, Germany) mounted to the ceiling of the isolation cubicle over the respective contexts, which were open at the top. Experiments were controlled by a PC and the software MED-PC for Windows v1.17 via interfaces (DIG 715) and the respective control panels (SG 215, all MED Associates). Contexts were cleaned thoroughly after each trial and bedding was changed.