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Effect of pre- *versus* post-weaning environmental disturbances on social behaviour in mice

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ABSTRACT

Environmental factors are involved to various degrees in psychiatric diseases. Unfortunately, early-life manipulations have been rarely investigated in mice. Interestingly, given the advances in genetics, combination of environmental and genetic factors to get construct validity is now possible. Herein, spontaneous activity, anxiety-like behaviour, social behaviour and short term spatial working memory were assessed in mice after maternal separation or social isolation. Of note, social withdrawal was observed in both models suggesting that this aspect needs to be better considered in future studies, particularly in testing new treatments for schizophrenia.

Although a strong genetic component has been evidenced in schizophrenia, its inheritance mode is complex and involves contribution of multiple genes and environmental factors [5,8]. The multiple facets of this complex psychiatric disorder lead to the consistent conclusion that there is no perfect paradigm to model schizophrenic disorders in rodents. Nonetheless, exposure of rats to environmental adversities, such as early maternal separation or social isolation, is admitted to negatively affect brain development and adult behaviour. Although the molecular mechanisms involved in the negative effects of environmental changes are unclear, similar environmental disturbances in children may contribute to the development of psychiatric disorders, such as schizophrenia, in genetically predisposed individuals. This refers to the so-called "three-hit" hypothesis [10]. Thus, even though no single animal model can possibly combine all the factors associated with schizophrenia, such neurodevelopmental models are very helpful to understand the neurobiological aetiology of schizophrenia. Furthermore, using such non-pharmacological animal models is powerful to assess efficacy of novel therapeutic agents. In schizophrenia, social disturbances are linked to emotional flattening, social isolation, and interpersonal oddity [2]. Social and emotion-related behavioural deficits that mimic negative symptoms of schizophrenia are crucial for functional outcome of the disease [12,20], but do not respond to current antipsychotic treatments [18,22].

The rat has been the gold-standard rodent species in experimental research for a long time, but with the recent advances in genetics, mouse use became more and more important. Possibility is now given to combine transgenic with environmental neurodevelopmental modulation, and thus to improve construct validity [10]. However, although long term consequences of maternal separation and isolation rearing have been extensively studied in rats, not so much is known in mice. Furthermore, priority is usually given to positive symptoms while negative symptoms like social disturbances are often neglected [23].

The aim of our work was therefore to explore sociability in two models of early and late environmental disturbances in mice, *i.e.* maternal deprivation (pre-weaning) and isolation rearing (post-weaning).

NMRI mice were housed in standard conditions ($21 \pm 1\,^{\circ}C$, $55 \pm 10\%$ humidity, cage size: $42\,cm \times 29\,cm \times 15\,cm$) with free access to food and water. Animals came from 18 different litters housed in individual cages and reduced to 8 pups per litter the day of birth. For each experimental group, 2–3 male pups were randomly picked among the 18 different litters. Maternal separation (pre-weaning P9; $n = 13$) consisted in removing the dam when the pups were 9-days old and placing it in a cage nearby for 24 h. After weaning (21-days old), young mice were group-housed. Socially isolated mice (post-weaning isolated; $n = 16$) were individually housed from the day of weaning. Control mice ($n = 16$) were left undisturbed, and placed at weaning in groups of 6–9 animals. All procedures were in accordance with the French legislation and the regional ethical committee.

From the age of 120 days, all animals underwent a battery of behavioural tests in the following order: anxiety-related

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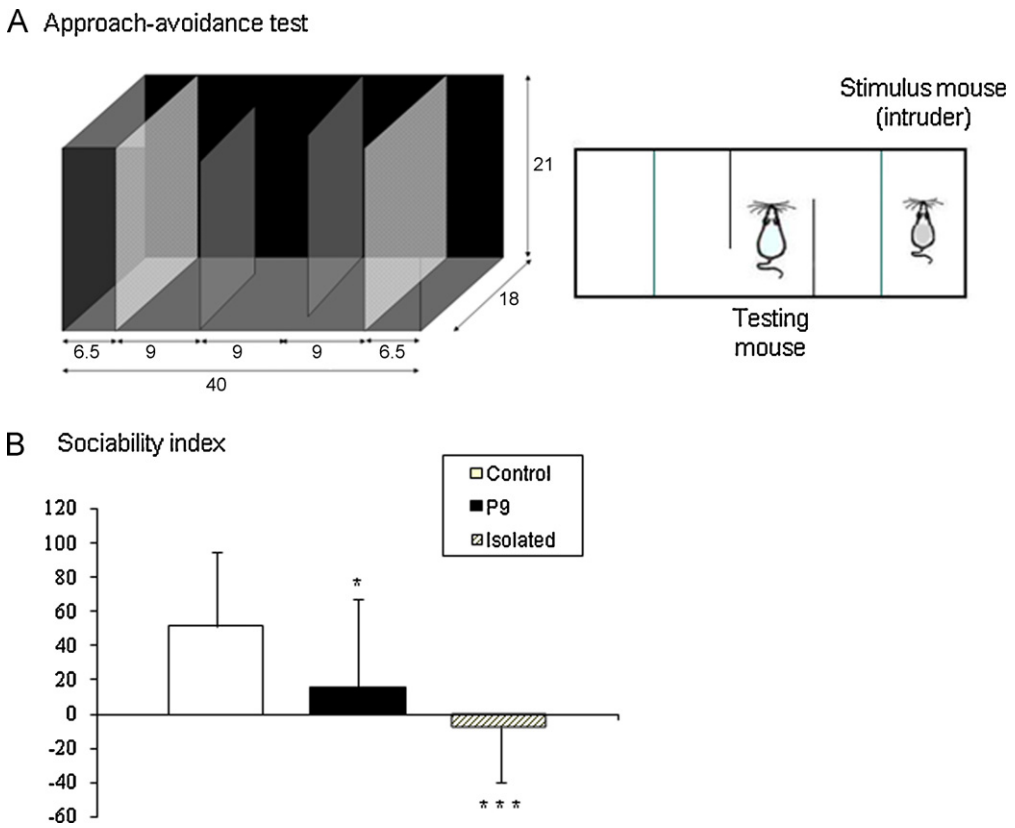

Fig. 1. (A) Schema of the social approach-avoidance apparatus (dimensions are expressed in cm). (B) Sociability index: time spent close to the stimulus mouse minus time spent in the opposite compartment from the stimulus mouse. Different from control group ($^{*}p < 0.05$, $^{***}p < 0.001$).

behaviour, spontaneous activity in open-field, spontaneous activity in a photoactimeter, spontaneous alternation, approach-avoidance. Anxiety-like behaviour was tested in an elevated-plus maze (open arm: 5 cm × 30 cm; closed arm: 5 cm × 30 cm × 15 cm) by collecting number of entries and time spent in the arms during a 5-min session. Spontaneous global activity was assessed during a 30-min session in an automated photo-actimeter containing closed enclosures (21 cm × 26 cm × 10 cm), and open-field (50 cm × 50 cm × 35 cm) activity was assessed during 5 min. Working memory performances were assessed by spontaneous alternation behaviour in a Y-maze (each arm: 7 cm × 21 cm × 15 cm; duration: 5-min). Social motivation was assessed in a social approach-avoidance task adapted from [7]. The apparatus (Fig. 1A) was a black box containing a testing compartment (27 cm × 18cm × 21 cm) with three interconnected chambers and a compartment for the stimulus animal (6.5cm × 18cm × 21 cm), the two being separated by a transparent plastic perforated wall to ensure visual and olfactive communication. The tested mouse was placed in the centre of the testing compartment and the stimulus mouse was placed in the other compartment for 5-min. The stimulus animal was a 45 days old mouse, different for each trial. Time spent in each compartment was collected for calculation of the sociability index. ANOVAs, Fisher PLSD, and univariate *t*-test were used for statistical analysis (Statitview®).

Spontaneous activity was differentially affected according to the testing environment. In the closed enclosure of the photoactimeter, there was no significant difference (Table 1). On the contrary, in the open-field, isolated mice displayed a significant increase in horizontal activity compared to controls ($p < 0.0001$), while P9 mice did not display any difference. Percentage of activity in the central zone was not different between groups, but the number of rearing significantly increased in isolated ($p < 0.01$), but not in P9 animals. Anxiety-like behaviour was affected in the two models: percentage of time in open arms was significantly reduced in P9 ($p < 0.05$) and in isolated mice ($p < 0.05$). Spontaneous alternation percentage was affected in isolated mice ($p < 0.001$; Table 1). There was no difference between P9 and controls. Moreover, comparison with reference value of 50% confirmed a deficit in isolated but not in P9 mice. Social approach-avoidance towards a stimulus mouse was affected in both models ($p < 0.01$ and $p < 0.001$ for P9 and isolated animals, respectively; Fig. 1B). The difference between time spent close to *versus* far from the stimulus mouse showed that, while control mice displayed a significant preference to stay close to the stimulus ($p < 0.05$), P9 and isolated mice were not significantly attracted by it (comparison with the reference value of 0, univariate *t*-test).

Modelling schizophrenia in rodent has gathered much attention to develop and assess efficacy of new potential therapeutics,

Table 1
Behavioural parameters.

	Control	P9	Isolated
Spontaneous locomotor activity			
Actimeter			
Beam interruptions	517 ± 32	530 ± 56	627 ± 57
Open-field			
Total crossed squares	187 ± 11	202 ± 9	306 ± 13[a]
% central	22 ± 2	26 ± 2	24 ± 1
Rearing	24 ± 2	29 ± 3	33 ± 2
Anxiety-related behaviour			
% time in open arms	44 ± 2	31 ± 3[a]	33 ± 2[a]
Spontaneous alternation			
Arm entries	36 ± 1	34 ± 2	52 ± 2[a]
% alternation	64 ± 2[b]	67 ± 2[b]	54 ± 2[a]

[a] Different from control group.
[b] Different from reference value (see text for details).