Assessment of individual differences in behavioural inhibition in mice across different testing paradigms and in a homecage-test situation (IntelliCage)

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Mice of the strain 129X1/SvJ, a major ES cell donor, are considered to be dull due to their passivity and poor performance on a variety of behavioral paradigms. It has been proposed, however, that this hypoactivity is due to a neophobic reaction to the test settings, rather than to a limited cognitive ability(Homanics *et al.*, 1999), (Dockstader & Van der Kooy, 2001)

We run a preliminary series of tests (elevated plus maze, Làt maze, light/dark box) on 55 mice, we found a bimodal distribution of exploratory measures within a cutoff of 10 min, with 30% to 60% of the mice strongly inhibiting locomotion in any arena. When re-exposed to the same testing arena, most mice consistently replicated their locomotor responses, albeit a sort of inverse habituation (i.e. increase of activity upon retesting) was observed for some of them. Measures of locomotor activity appeared to be consistent both within and across arenas, mice refraining to move in a given setting being also hypoactive in other settings.

In the attempt of generating two recombinant lines differing for their coping attitudes we bred to each other mice selected for high (respectively low) latencies to enter the novel compartments in a free exploration paradigm (FEP) (Misslin *et al.*, 1982). The FEP consists of a grey PVC box divided in 6 compartments, measuring 10x10 cm. The mouse is allowed to habituate to three communicating compartments during 24 hours; thereafter, access to three adjacent compartments is made available by opening of guillotine doors. Latency to enter the novel compartments, percent of time spent in the novel side and risk assessment events are measured on a 10 minute time lapse from door opening.

This psychogenetic selection produced so far two F3 generations quite differing in locomotor activity, rather than on measures of anxiety, as assessed in the open-field, elevated plus maze and FEP. Quantification of locomotor tracks produced by EthoVision revealed similar between-session habituation in the two groups of mice, yet different within-session habituation profiles, suggesting that the psychogenetic selection specifically affected locomotion, or a form of anxiety, yet not memory abilities. Indeed, measures of anxiety taken from different tests did not correlate to each other; however, activity did, the less active mice moving poorly on virtually any arena.

Exploratory attitudes appear to correlate inversely to the stress response: ten minutes after door openings in the FEP, mice were either (1) allowed to continue exploration of the novel compartments, or (2) re-confined in the familiar compartments, or (3) forced and confined in the novel compartments. Afetr 10 minutes, blood was samples from the tail vein and plasma corticosterone assessed by RIA. Results show that corticosterone levels were inversely correlated to the amount of locomotion in response to the opening of the doors.

To understand whether this locomotor inhibition had to be ascribed to a form of neophobia, we studied the exploration and place learning patterns of F3 inhibited and non-inhibited mice in the Intellicage™.

The Intellicage is a sort of automated operant conditioning meant to evaluate associative learning in a social, homecage setting: the access to water bottles, set in the corners of the cage, is gated by a tunnel and doors, and can be programmed in such a way to impose an operant schedule of visits and nosepokes to each individual mouse, recognized by means of transponders. Because "wrong" responses can also be "punished" with an air puff, the device promises application also in the study of avoidance learning and fear response. We put 4 inhibited and 7 non-inhibited mice together in two Intellicages, and programmed the corners in such a way that half of the mice (2 inhibited and 3 non-inhibited) received an airpuff when visiting corner 1, while the other half (2 inhibited and 4 non-inhibited) received an airpuff when visiting corner 3. Corners 2 and 4 gave free access to water to all mice.

Latencies to first visit the corners of Intellicage are higher for the F3 offspring of inhibited mice: the same animals, however, readily learn to avoid punished corners. By 3 days, all mice habituate and differences in activity between inhibited and non-inhibited animals become undetectable. Surprisingly, all mice, no matter whether punished in corner 1 or 3, learned to avoid both punished corners. Apparently, inhibited mice learned even faster than the non-inhibited to avoid the punished corners.

These results suggest that the poor proficiency of the 129X1 mice is largely due to a form of trait anxiety that results in a strong locomotor inhibition, rather than to impaired learning and memory abilities.

In order to further assess the effect of the testing environment vs homecage on measures of anxiety, the study has been extended to a 5HTR1 mutant mouse line, where elements of locomotor inhibition make difficult the interpretation of measures of anxiety.

References

1. Dockstader, C. L. & Van der Kooy, D. (2001). Mouse strain differences in opiate reward learning are explained by differences in anxiety, not reward or learning. *Journal of Neuroscience*, **21**, 9077-9081.
2. Homanics, G. E., Quinlan, J. J., & Firestone, L. L. (1999). Pharmacologic and behavioral responses of inbred C57BL/6J and strain 129/SvJ mouse lines. *Pharmacol.Biochem.Behav*, **63**, 21-26.
3. Misslin, R., Herzog, F., Koch, B., & Ropartz, P. (1982). Effects of isolation, handling and novelty on the pituitary--adrenal response in the mouse. *Psychoneuroendocrinology*, **7**, 217-221.