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Short communication

# Stereotypic behaviour in standard non-enriched cages is an alternative to depression-like responses in C57BL/6 mice

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## HIGHLIGHTS

- We compared home-cage and Porsolt test behaviour in enriched and non-enriched mice.
- Non-enriched mice spent more time *still but awake* in the home cage..
- High levels of *still but awake* predicted longer float times.
- Non-enriched mice were also more stereotypic.
- Being stereotypic and spending time *still but awake* were inversely correlated.

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## ABSTRACT

Depressive-like forms of waking inactivity have been recently observed in laboratory primates and horses. We tested the hypotheses that being awake but motionless within the home-cage is a depression-like symptom in mice, and that in impoverished housing, it represents an alternative response to stereotypic behaviour. We raised C57BL/6 ('C57') and DBA/2 ('DBA') females to adulthood in non-enriched (n = 62 mice) or enriched (n = 60 mice) cages, observing home-cage behaviour during the active (dark) phases. We predicted that being still but awake would be reduced by environmental enrichment; more pronounced in C57s, as the strain most prone to learned helplessness; negatively related to stereotypic behaviour; and positively related to immobility in Forced Swim Tests (FST). Compared to enriched mice, non-enriched subjects did spend more time spent being inactive but awake, especially if they displayed relatively little stereotypic behaviour. C57 mice also spent more time awake but motionless than DBAs. Furthermore, even after statistically controlling for housing type and strain, this behaviour very strongly tended to predict increased immobility in the FST, while high levels of stereotypic behaviours in contrast predicted low immobility in the FST. Being awake but motionless is thus a reaction to non-enriched housing that seems to be an alternative to stereotypic behaviour, and could reflect depression-like states.



Clinically depressed patients often show reduced activity, taking less exercise, and engaging less in both social and non-social activities [1,2]. They can also show learned helplessness, wherein 'highly desired outcomes are believed improbable or highly aversive outcomes are believed probable, and the individual comes to expect that no response in his repertoire will change their likelihood' [3]. Aetiologically, a common trigger is chronic stress (*e.g.* aversive life events or chronic pain/illness [4,5]). Such symptoms may well not be unique to humans [6,7]; indeed, modelling them in rodents and primates is common in biomedical research [8]. Furthermore, depression-like inactivity has been reported in some animals after aversive life events and/or chronic stress (*e.g.* horses, dogs, cats, elephants, non-human primates; reviewed [9]).

This study aimed to test the hypothesis that a specific form of inactivity in mice is a depression-like symptom: being still but awake in the home-cage during the active (dark) phase, thus apparently alert but nonetheless motionless. This behaviour was previously observed in C57BL/6 (henceforth 'C57′) mice, especially in standard, non-enriched laboratory cages compared to large,

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enriched, highly preferred enclosures [10] (although this study did not correct for the enriched mice being harder to observe). Unusual forms of inactivity, that proved reversible with imipramine, were also reported in C57 mice exposed to repeated defeat stress [11]. Furthermore, C57s appear prone to helplessness (*e.g.* in Forced Swim Tests and after exposure to inescapable shocks), in contrast to DBA/2 mice for example (henceforth 'DBAs') which instead react to stress with hyper-activity and stereotypy [12].

We tested the hypothesis that this specific form of inactivity is a depression-like symptom by assessing whether 'still but awake' behaviour is diminished by stress-reducing enrichment, even after statistically controlling for time spent out of sight; is performed more by C57s than by DBAs; and positively co-varies with immobility in Forced Swim Tests, a well-accepted indication of helplessness [8,13,14]. We also investigated its relationship with stereotypic behaviour (SB) to test a second hypothesis: that it and SB are alternative behavioural responses to chronic stress [12].

All procedures were approved by the University of Guelph Animal Care Committee (AUP 1398) and complied with Canadian Council on Animal Care guidelines. Unrelated adult female C57 and DBA mice in two cohorts were purchased from Charles River Laboratories (Quebec), and differentially housed from three to five weeks into adulthood. Food (Harlan® Teklad Global Diet, Mississauga, ON, Canada) and water were *ad libitum*. Rooms were kept at 21 °C and 48% relative humidity, on a 12-h reverse light schedule (with lights out at 10 am).

*Cohort 1*: Ninety mice were randomly assigned into mixed strain trios of two C57 mice and one DBA mouse per cage (previously shown not to affect welfare or strain-typical phenotypes: [15]), one C57 per cage being ear-notched after receiving Carprofen. Half were housed in non-enriched ('NE') cages, half in larger enriched ('EE') cages, as described in Ref. [16]. Enrichments were biologically relevant items (*e.g.* allowing hiding and nest-building), selected from publications indicating they enhanced mouse welfare *e.g.* [10]. In each cage, they included: a plastic igloo mouse house & 'fast-trac' running wheel combo (Bio Serv®); a black polyvinyl chloride PVC tunnel (10 cm × 4 cm; also used for handling, see below); two paper cups; a Nestlet; one tissue; two square pieces of cotton fabric (each 4 × 4 cm); a pinecone (from one of several diverse conifer species); a sponge (roughly 5 × 3 × 5 cm); a sock 'hammock' (a 12 × 12 cm piece of sock hung from the cage lid via cable ties); and two cotton balls. EE mice were also always handled using their familiar PVC tunnel, not directly by hand, to further reduce stress [17]. Two mice were lost to malocclusion, such that final sample sizes were 58 C57s (29 EE, 29 NE) and 30 DBAs (15 EE, 15 NE).

*Cohort 2*: These mice were part of another experiment that further validated mixed strain housing, here for trios consisting of one C57, one DBA and one BALB/c mouse per cage [18] (this last strain being excluded from our analyses as absent from Cohort 1). Hous-

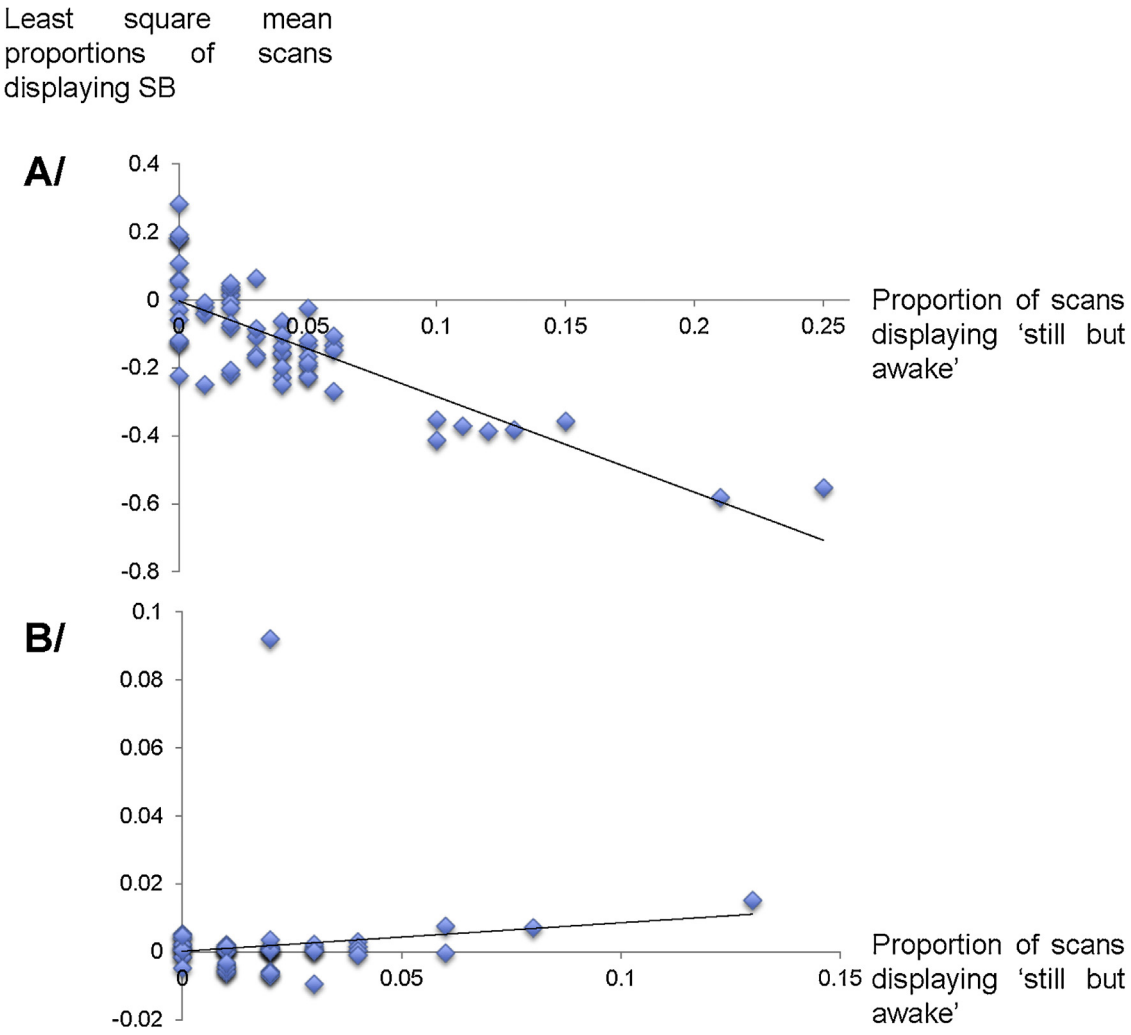


**Fig. 1.** Relationship between time spent still but awake and stereotypic behaviour (SB), controlling for Cohort, Strain, and proportion of scans out of sight. A/non-enriched housing; B/enriched housing. The presented values reflect the model taking into account these other factors, which causes some values to be negative.