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# Influence of a chronic ultramild stress procedure on decision-making in mice

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**Objective:** To test the influence of a chronic ultra mild stress (CUMS) procedure, based solely on socio-environmental stressors, on cognitive-behavioural function in mice. **Design:** Behavioural study. **Participants:** B6D2F1 mice. **Interventions:** Mice were exposed to various stressors and then tested using a decision-making task. **Results:** We observed that stress facilitated "choice" behaviour, with an absence of "no choice" behaviour. Stress also facilitated a more rapid capacity to process information, a decrease in the level of evaluation of the choice situation and less hesitation. These stress-related consequences on decision making may be attributed to a higher level of distractability in the stressed mice. **Conclusions:** The CUMS model may be useful for the study of stress-related disorders by proposing a new method for assessing gene-environment interactions in cognitive-affective behaviours.

**Objectif :** Vérifier l'effet d'une procédure fondée sur le stress chronique et ultra léger (CUMS — *chronic ultra mild stress*), qui repose seulement sur des facteurs de stress socio-environnementaux, sur la fonction cognitive comportementale chez des souris. **Conception :** Étude de comportement. **Participants :** Souris B6D2F1. **Interventions :** On a exposé les souris à divers facteurs de stress pour les tester ensuite au moyen d'une tâche décisionnelle. **Résultats :** Nous avons observé que le stress facilitait le comportement de «choix» et l'absence de comportement «sans choix». Le stress a aussi accéléré la capacité de traitement de l'information, réduit le niveau d'évaluation de la situation de choix et réduit l'hésitation. Ces conséquences des facteurs liés au stress sur la prise de décisions peuvent être attribuées au fait que les souris stressées sont plus distractibles. **Conclusions :** Le modèle CUMS peut être utile pour l'étude de troubles liés au stress en proposant une nouvelle façon d'évaluer les interactions génétiques et environnementales dans les comportements cognitifs-affectifs.

## Introduction

Stress, either acute or chronic, or severe or moderate in intensity, is a predisposing factor for the development of a wide variety of behavioural and pathophysiological disturbances, including a range of psychiatric, endocrine and inflammatory disorders.[1-7] Stress-related disorders are heterogeneous, and it is now recognized that they are multifactorial, resulting from multiple interactions between individual factors (genetic influ-

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ences as well as nongenetic maladaptive cognitive and/or neurochemical coping mechanisms) and superimposed environmental conditions (stress but also socioenvironmental and familial factors).[2,4,6,8-10] However, the degree to which a given pathology is conditioned by individual or environmental determinants is unclear.

Stress-based studies in laboratory animals may help researchers to understand the mechanisms involved in the pathogenesis and treatment of stress-related disorders in humans. Most of the studies in this field have been conducted on rats. Studies on mice may, however, be particularly promising. The availability of more than 200 inbred strains of mice and the development of transgenic and knock-out mice, provide the opportunity to study genetic differences, the role of specific genetic and neurochemical alterations, and environmental factors, in the development of the pathophysiological states promoted by stressful conditions.

Among the experimental stress-procedures employed in rodents, the chronic mild stress (CMS) model developed by Willner et al[11] may be particularly adapted for a model of stress-related disorders. The CMS model involves chronic exposure to various stressors of minimal intensity and is commonly employed as a model of the melancholic subtype of depression.[11-14] Indeed, a wide range of studies in humans have pointed to the role of chronic low-grade stress in the etiology of depression.[4,5,15-18] CMS has been shown to be effective in inducing *anhedonia*, a core symptom of depression, reflected by a generalized decrease in reward sensitivity in various behavioural paradigms (reviewed by Willner[13]) but also of other biological and behavioural markers of human depression, including architectural sleep abnormalities,[20,21] disturbances in circadian rhythms related to locomotor activity,[22] diminished sexual activity[23] and alterations in body weight.[24] These disturbances can be reversed by a variety of chronic antidepressant treatments during continued application of stress.[25] With the investigation of the mechanisms of antidepressant action as the main objective, a many studies have shown that CMS can induce anhedonia. However, the impact of CMS exposure on the development of experimental anhedonia varies considerably among studies, with differences in the magnitude and persistence of the effect (including an absence of effect). These considerations indicate the potential of the CMS model, not as a specific model of depression but rather as a model of stress-related disorders in general.[26-28] The CMS model may, therefore, be of value for investigation of other behavioural disturbances, over and above pure depressive symptomatology.

Therefore, we decided to apply the CMS model to mice, with the investigation of cognitive-behavioural function as the main objective. The CMS model involved the chronic exposure to various stressors of minimal intensity, each of them being neither necessary nor sufficient to induce profound and long-lasting disturbances.[29] Thus, the essential features of the effectiveness of the CMS model are the chronicity and the variety of the ultra-mild stressors, rather than the characteristics of the individual stressors employed.[29] Our chronic stress schedule was adapted from that employed by Moreau et al,[30] which is simpler than the one originally developed by Willner et al.[11] Moreau et al's CMS regimen differs principally by the inclusion of periods in which the animal is left undisturbed in its home cage and by the inclusion of repeated periods of confinement. To prevent habituation/adaptation that may be observed after repeated exposure to the same stressor,[31] in our stress regimen (which we refer to as the chronic ultramild stress [CUMS] procedure) we reduced the number of periods of confinement and augmented the variety of the stressors employed, all of which were taken from the different CMS procedures employed by Willner et al.[32]

In our procedure, we omitted food or water deprivation. First, because we considered this manipulation as a severe stressor, as suggested by Willner et al,[32-34] and second, because food or water deprivation influences behavioural performance in a variety of tasks, particularly exploratory behaviour, and thus is not suited for studies of congnitive behavioural function in animals.[35] In addition, in contrast to most of the studies on the CMS model, we did not employ isolated animals as controls. Several studies in male rodents have demonstrated that this factor increases the vulnerability to subsequent stress situations[36,37] and that this manipulation may be stressful for control subjects and may interfere with the effect of CMS. It has been demonstrated that isolating the control group may not abolish the effect of CMS exposure on sucrose intake,[29,38] and a greater effect of CMS has been observed in singly-housed stressed animals.[29] The most important reason, however, was that we used female mice, and it has been shown that individually-housed female rats exhibit a stress response, reflected by increased levels of corticosterone, compared with group-housed females.[39] This effect is not observed in males,[39] indicating that isolation is a more severe stressor for females than for males. A previous experiment compared spontaneous motor activity measured in an actographic apparatus of grouped-housed, single-housed and stressed mice. The results (unpublished data) showed that isolated animals exhibit levels of activity between those of the group-housed and stressed mice. Therefore, we considered isolation rearing as a social-stressor component of our stress regimen.

To examine the effect of CUMS on cognitive-behavioural function, we devised a decision-making (DM) task, based on observation of the spontaneous behavioural reaction to environmental stimuli in a conflict-choice exploration model. DM is a cognitive process underlying volitional behaviour and problem-solving abilities. It allows an organism, whether human or animal, to choose, at the right time, the best action in the given environmental context.[40] In a given problem situation, making a decision is choosing 1 of the available response options, correct or not, adapted to the situation or not. Independent of the ability to make a choice (DM ability), the DM process is the process underlying the selection (choice) of a given response; the choice results from interactions between the essential components of the DM process, (i.e., motivation, emotion, attention and working memory).40 The choice not only depends on the assessment of information relating to the current situation and that obtained from past experience, but also depends on the value assigned by the subject to characteristics of the situation depending on its internal state (motivational/emotional) at the time it encountered the situation.[40–43]

To test the influence of CMS on DM behaviour, we observed both the choice made and the behaviour preceding the choice. For this, we used a principal component analysis based on matrix correlation between the different variables, which has been shown to be a valuable means of detecting independent behavioural dimensions with a common underlying feature.[44–46]

For the study, we used B6D2F1 mice since this strain offers at least 2 advantages over the widely used non-inbred strains (i.e., Swiss or OF1). Genetic factors are thought to play a key role in the interindividual variability in stress responses.[6,9,27] These B6D2F1 mice result from the cross-breeding between C57BL/6J and DBA/2 inbred strains, which have been shown to be contrasted in various behavioural and biological measures of stress responsiveness.[47,48] Therefore, the impact of genetic influences in B6D2F1 mice will be correspondingly reduced by mixing the alleles that may be involved in the vulnerability or the resistance to stressors. In addition, all the subjects are genetically identical, effectively eliminating genetic variability.

## Methods

All procedures described in this study followed the ethical guidelines developed by the French Ministry of Agriculture.

### *Animals*

One hundred and thirty-five, 3-month-old female B6D2F1 mice (Iffa-Credo, Lyon, France) were used. Female mice were chosen since it has been shown previously in rats that females are more vulnerable to stressors than males.[49] The mice were brought into the laboratory 1 month before the start of the experiment. On arrival, the animals were housed in groups of 5 in the animal research facility and maintained under standard laboratory conditions: 12 hour light/dark cycle (lights on at 0730), temperature 22 ± 2 °C, and food and water *ad libitum*. At the start of the experiment, stressed animals were housed in a separate room and had no contact with the control animals. Except as described below, stressed animals were housed singly under the same standard laboratory conditions.

### *General procedure*

The total duration of the experiment was 8 weeks. At the beginning of the experiment, the animals (aged 4 months) were divided into 2 groups. The experimental group ($n$ = 67) was placed in a separate room and subjected to the stress regimen. The stress regimen was administered for a total of 8 weeks. The control group ($n$ = 68) was left undisturbed in the animal research facility. Each group (stressed and control) was then further divided into 8 subgroups. At the end of the first week, 1 subgroup of stressed mice ($n$ = 8) and 1 subgroup of control mice ($n$ = 8) were observed in the behavioural task. The same procedure was applied after the end of each week for the duration of the experimental period. With the exception of the subgroup observed after week 4, which comprised 12 animals, all other subgroups comprised 8 animals. All mice were used only once. Behavioural testing was carried out on Mondays between 1000 and 1300.

### *Stress regimen*

Our procedure was based solely on environmental and social stressors. Isolated animals were subjected to a sequential application of a variety of mild stressors including: repeated periods of cage tilt (30°); confinement to small cages (11 × 8 × 8 cm); 2 2-hour periods of paired housing; 1 overnight period of difficult access to food (without a reduction in the daily food ration); 1 period of continuous overnight illumination; or 1 overnight period in a soiled cage (50 mL water in 1000 mL of sawdust bedding). Animals were also placed on a reversed light/dark cycle from Friday evening to Monday morning. In the paired housing condition, animals were always housed in the same pairs, but the location alternated between the home cages of each member of the pair. These stressors were scheduled over a 1-week period and repeated throughout the 8-week experiment. Except for the weekend, animals were subjected to a single 1-hour period of morning stress, a single 2-hour period of afternoon stress and an overnight stress (1800 to 0900), with a minimum interval of 2 hours between each stress-inducing period. We refer to this procedure as the CUMS procedure; this is a purely descriptive term and makes no assumptions about its effects.

### *Decision-making task*

#### The model

Our task was derived from a Spontaneous Alternation (SA) test. The SA test is used to measure the strong tendency of rodents to explore a new maze arm after the animal has visited a different maze arm.[50–55] During trial 1, an animal turns right or left in a T-maze, and during trial 2 the animal will either choose the same arm or the alternate arm. This choice situation can be viewed as a decision-making situation. Since the 2 arms of the T-maze are identical in their physical characteristics, the "alternation" response is considered the "correct choice." This choice is thought to be a reflection of mnesic abilities; therefore, the influence of attention on the choice of arm cannot be discriminated from memory.[56] In addition, the task does not involve any emotional component, since all the arms of the maze are equally "safe."

To examine the DM process in mice, we considerably modified the SA experimental procedure and the physical characteristics of 1 arm of the T-maze to make it "insecure." These modifications were designed so that the exploratory drive (that which makes the animal enter the non-explored arm on the second trial) competes with a characteristic of the situation, that is, the perceived lower security in the non-visited arm. The animal was first subjected to a forced-choice trial (reference trial) where it had to explore the secure arm only. This trial was followed by a free-choice trial (test trial). To accomplish the task, the animal had to choose between exploring the new but "insecure" arm (alternation response) or returning to the previously visited but "secure" arm (avoidance response). The choice was indicated by entry into a goal box at the end of each arm. Under these conditions, the physical characteristics of the new arm in the test trial test the emotional reactivity of the animals and will tend to enhance attentiveness to the task.[57] It was hoped this would also examine the influence of attentional abilities on choice behaviour.

In our task, the insecurity of the new arm in the test trial, together with the animal's past experience with the maze were used to generate a conflict in each arm: 1 arm is attractive by its novelty but repulsive by its insecurity, whereas the other arm is attractive by its security but repulsive because it has already been visited. Therefore, in our task neither choice can be qualified as being the correct choice. The choice made by the control group will therefore be considered as "normal" and the choice made by the stressed mice will be considered inadequate in comparison with the control group.

#### Apparatus

Behavioural testing was performed in a modified T-maze as described above. The apparatus (Fig. 1) consisted of 3 arms of equal dimensions (41.5 cm long and 6 cm wide, made of grey Plexiglas) standing on a table, 1 m above the floor. The central path and the right arm were secure arms, the walls of which were made of transparent Plexiglas (6 cm high) with a roof. The left arm was considered to be potentially insecure; it was suspended in the open above the floor, with several openings through the walls and with no roof. The walls of the left goal box was made of transparent Plexiglas (6 cm high) and it had a roof. The starting box (7.5 × 6 × 6 cm) at the beginning of the central path, and both goal boxes (7.5 × 6 × 6 cm) at the end of the left and the right arms could be closed with sliding doors.

The arrangement and size of the openings in the insecure arm were based on results of previous experiments with outbred mice (unpublished data). With the standard SA test (i.e., maze with 2 closed arms), more than 90% of the mice displayed alternation. In the modified SA test (i.e., a closed arm and an elevated open arm), none of the mice went as far as the goal box in the elevated open arm. The insecure arm was therefore designed so that 25% of the animals entered the goal box of this arm on the second trial.

### Experimental procedure

The behavioural paradigm consisted of 2 trials separated by a 10-second interval. During the first trial (reference trial), the mouse was forced to explore the secure arm, since access to the other arm was blocked by a door. To begin this forced reference trial, the animal was placed in the starting box and after 10 seconds the door to the central path was opened. The trial was considered to be complete when the mouse had all 4 legs inside the goal box, at this point the door was closed. No time limits were imposed. After a 5-second confinement in the goal box, the animal was removed and placed in the starting box for the second trial (test trial). Ten seconds later the door was opened. The animal then had free access to both arms. The trial was considered to be complete when the mouse had all 4 legs in either of the goal boxes, or after 5 minutes had elapsed.

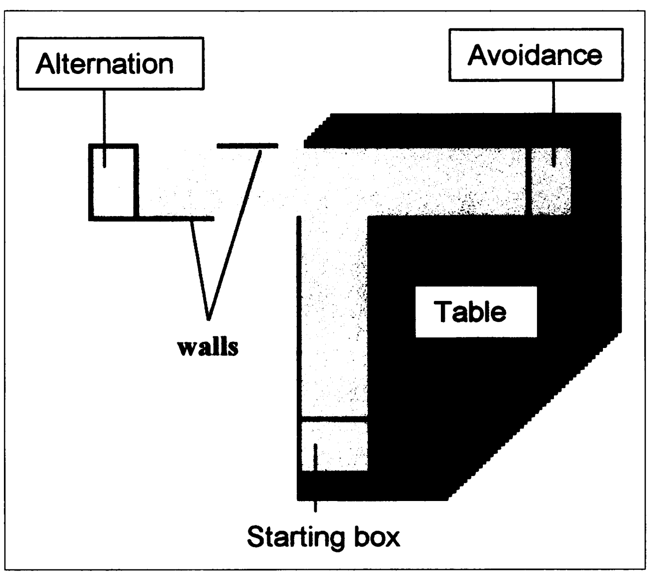


**Fig 1: Diagram of the decision-making apparatus used in this study.**

### Variables

The behavioural testing was videotaped and subsequently analysed. The overall behavioural pattern performed in the 2 trials was observed. In the test trial, we considered that a "choice" was made when the animal had reached a goal box before the time had elapsed, or that "no choice" was made if the mouse had not reached a goal box after 300 seconds. The chosen option was noted (alternation, avoidance or no choice). For the statistical analysis, we constructed an ordinal variable, awarding a value of 2 to the alternation response, a value of 1 to the avoidance response, and a value of 0 to the no choice response. In both trials, the time that elapsed between opening the starting-box door and closing the goal box door (time to solve the task [TST]) was recorded with a maximum of 300 seconds for the test trial. The time taken to go between the branch point of the arms and the goal box (last entry), was also recorded (time of final response [TFR]). The exploratory behaviour (exploratory movements [EM]) preceding entry into the goal box was observed. All the exploratory movements during the trial (entries into the arms, reentering the start box, and in the test trial, movement of the head forward to look through the openings in the left arm) were noted. In the free exploration test, EM were assumed to reflect the process of information gathering related to knowledge of the content and spatial layout of the apparatus.[35,57,58] Therefore, the number of EM were considered to provide an estimate of the amount of information gathered. As the duration of the trial was not fixed, we defined an index for the level of exploratory activity (exploratory activity index [EAI]) as the number of these movements per minute.

#### *Statistical analysis*

The statistical analyses were carried out using SAS software (SAS Institute Inc, 1990).

The choices made by stressed and control animals were compared using Fisher's exact test (2-tailed).

Post hoc stepwise discriminant analysis was used to analyse the data from all animals in order to determine if there were differences between the "no choice" subgroup of control animals, the "choice" subgroup of control animals and the stressed animals. It was then used to analyse data from stressed and control "choice" animals to determine whether animals choosing either the alternation or the avoidance response differed.

For the analysis of the stress effect, we first compared the overall behavioural pattern of groups of mice in each trial, using a multivariate analysis of variance (MANOVA) involving the following dependent variables: TST, TFR, EM and EAI. Factors included: stress, week and the stress × week interaction. For this analysis we excluded the "no choice" subgroup from the control animals for the the following reasons: first, the no choice response was only observed in the control animals; second, this response was defined by a time limit for the TST in the test trial, and the TFR in the test trial could not be recorded in these subjects, and; third, these animals exhibited a profile of exploration clearly different from other control animals but also from stressed mice.

Since no significant effects of week, or stress × week interaction were found by MANOVA in either trial, we regrouped the data from all weeks for subsequent analysis. Each variable was analysed by a repeated mixed analysis of heterogeneous variance (SAS proc mixed), with stress being a factor for all analyses, with a separate experimental variance for each level of stress factor. Results were expressed as least square means and standard errors (SE). Partial comparisons were made on these means using Student's *t* test. Values of $p < 0.05$ were considered significant for all the analyses.

Finally, bearing in mind the multidimensional nature of DM behaviour, all variables (except response) were submitted to a principal component analysis with varimax rotation. This statistical tool was used to determine if distinct behavioural dimensions were measured, and the way the different variables relate to each other. Only components with eigen values greater than 1 were retained. Based on the factors' loading of each variable, mean factor scores were calculated for each group, allowing graphic representation of the 2 groups for each component. Each component was then submitted to a 2-way ANOVA.

## Results

### *Responses*

The distribution of the 3 possible responses was significantly different between the stressed and control animals (Fig. 2; $\chi^2 = 12.42$, $p < 0.0001$). The choices made by the control animals were distributed over 3 possibilities (no choice, alternation or avoidance), whereas those made by the stressed animals were distributed over 2 possibilities (alternation or avoidance). In both the stressed and control animals, the avoidance response was the most frequent choice (61.7% in control group versus 67.2% in stressed group). We observed a higher frequency of the alternation response in the stressed animals (32.8% in stressed group v. 22.1% in control group). Of control animals, 16.2% did not choose a behavioural option, but this possibility was not observed in the stressed animals. In the stressed animals, the choice was found to depend on the duration of CMS exposure. During the first 3 weeks, 95% of the stressed animals chose the avoidance response, whereas after 4 weeks random choices were observed (52.3% for the alternation response v. 47.7% for the avoidance response). Both distributions were statistically different from the control animals ($\chi^2 = 9.59$, $p = 0.01$ for weeks 1 to 3 and $\chi^2 = 12.42$, $p = 0.0004$ for weeks 4 to 8).

### *Discriminant analysis*

This analysis was conducted to determine which linear

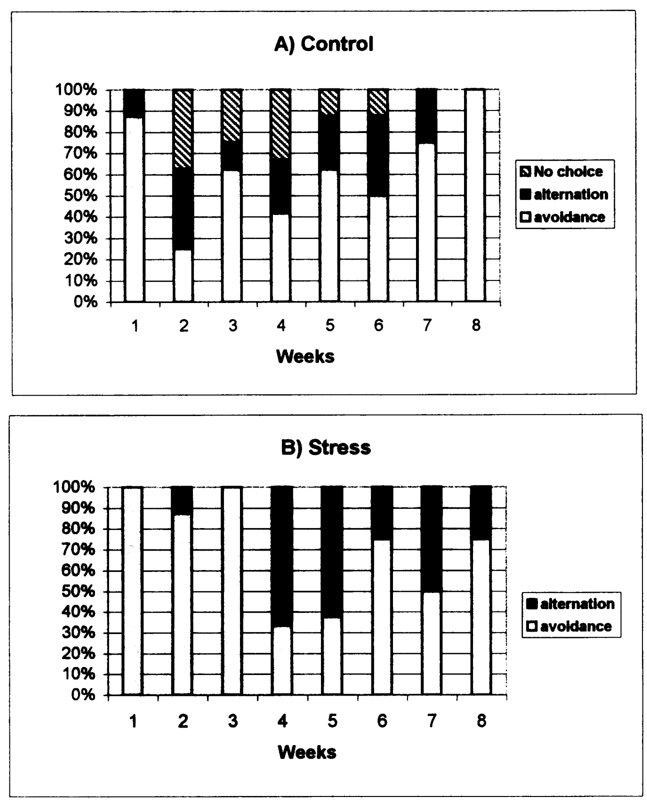


**Fig. 2: Influence of chronc ultramild stress on control A) or stressed B) animals. Results are displayed as the percentage of animals that displayed each behavioural option, for each week of the study ($\chi^2 = 12.42$, $p < 0.0001$).**

combination of variables discriminated the 3 groups (i.e., "no choice" group, control group making any choice, and stressed group). The results of the stepwise discriminant analysis showed that all variables of the task discriminated the 3 groups (Table 1). In control animals, the "no choice" and "choice" groups differed in the following variables: EAI ($r^2 = 0.23$) in test trial, TFR ($r^2 = 0.15$), EM ($r^2 = 0.11$), and TST ($r^2 = 0.11$) in the reference trial, accounting for 52% of the variance. The "choice" control group and the stressed group differed in the following variables: EAI ($r^2 = 0.22$) and EM ($r^2 = 0.16$) in the test trial; EAI ($r^2 = 0.12$) and TFR ($r^2 = 0.03$) in the reference trial, accounting for 53% of the variance. Finally, the "no choice" control group and the stressed group differed in the following variables: EAI ($r^2 = 0.42$) in the test trial, TFR ($r^2 = 0.26$) in the reference trial, EM in the reference trial ($r^2 = 0.08$) and in the test trial ($r^2 = 0.06$), TST in the reference trial ($r^2 = 0.08$) and EAI in the reference trial ($r^2 = 0.06$), accounting for 96% of the variance. From these results we considered the "no choice" group as an extreme group of control animals. Control animals making any choice were, therefore, considered as a reference group for the analysis of the stress effect since they represented 84% of the control population.

In the "choice" control group, none of the variables of the task discriminated between the subjects that chose either response. In addition, in the stressed group, TFR in the test trial was the only variable that differentiated subjects that chose the "alternation" or the "avoidance" responses. In stressed animals, TFR was significantly longer in subjects chosing the "alternation" response, but remained different from that measured in control "choice" animals (data not shown). Since the response variable was the only 1 affected by the duration of CUMS exposure, the stressed mice were considered as a homogeneous group.

### *Effect of stress on the behaviour preceding the choice*

Behavioural scores are listed in Table 1. The TST was lower in the stressed group, regardless of trial ($F_{1,71} = 15.75$, $p < 0.0002$ for reference trial and $F_{1,71} = 33.02$, $p < 0.0001$ for test trial). TFR was reduced in the stressed animals in both trials ($F_{1,71} = 26.15$, $p < 0.0001$ for reference trial and $F_{1,71} = 28.69$, $p < 0.0001$ for test trial). Stressed and control animals did not differ with respect to the number of EM in the reference trial. In the test trial, stressed animals made significantly fewer EM $F_{1,71} = 14.89$, $p < 0.0002$). Stressed animals also exhibited higher EAI scores in both trials ($F_{1,71} = 15.51$, $p < 0.0002$ for reference trial and $F_{1,71} = 23.48$, $p < 0.0001$ for test trial).

### *Principal component analysis*

Three components with eigen values higher than 1 emerged from the principal component analysis based on the correlation matrix of all open field variables. These 3 components explained 71.6% of the total variability. Since the different components were orthogonal to each other, it is generally assumed that they reflect distinct behavioural dimensions. The factor loadings after rotation for each variable, which represent the correlation between the variable and each component, are

**Table 1: Effects of chronic ultramild stress procedure on behaviour preceding choice in the decision-making task**

| | Behaviour | | |
|---|---|---|---|
| | | Choice | |
| Variables | No choice mean (standard error) | Unstressed animals mean (standard error) | Stressed animals mean (standard error) |
| Time to solve the task 1, s | 144.3 (21.9)‡§ | 62.8 (7.9) | 24.8 (2.6)¶ |
| Time to solve the task 2, s | 300.0 (0)‡§ | 85.4 (10.4) | 26.0 (2.8)** |
| Time for final response 1, s | 10.6 (1.0)‡§ | 6.6 (0.4) | 4.6 (0.2)** |
| Time for final response 2, s | 0 | 7.8 (0.6) | 5.2 (0.2)** |
| Exploratory movements* 1 | 4.5 (0.6)‡§ | 2.2 (0.2) | 1.8 (0.2)†† |
| Exploratory movements* 2 | 12.4 (2.3)§ | 10. 4 (1.0) | 6.4 (0.6)¶ |
| Exploratory activity index† 1 | 3.1 (0.5)§ | 2.7 (0.2) | 4.5 (0.3)¶ |
| Exploratory activity index† 2 | 2.5 (1.5)‡§ | 10.1 (0.8) | 16.1 (0.8)** |

1 = reference trial; 2 = test trial.
*No. of events
†No. of exploratory movements per min
‡Statistically different from control mice
§Statistically different from stressed mice
¶$p < 0.01$ compared with unstressed subjects
**$p < 0.0001$ compared with unstressed subjects
††Not significant

shown in Table 2. In accordance with the nature of the variables loading together on the same component, we could propose a behavioural interpretation for this component. To facilitate this interpretation, only the more significant loadings (higher than 0.3) are presented.

Component 1, which explained 24.1% of the total variability, comprised variables sharing in common the rapidity of information processing in both trials. Positive loadings come from EAI at both trials. Since the number of EM in a novel environment reflects a process of information gathering on the situation,[57,58] the number of EM per minute (EAI) is a reflection of information processing. Conversely, variables with negative loadings come from TST in the 2 trials, which reflect the time spent in solving a problem presented in 2 situations differing in the number of environmental stimuli, and from TFR in the 2 trials, which reflects the duration of 1 isolated EM, and therefore of the processing of 1 information. The positive side of component 1 was assumed to reflect faster information processing.

Component 2, which explained 23.8% of the total variability, comprised TST in the 2 trials, EM in the reference trial and EAI in the test trial. Overall, these variables indicated the level of hesitation and are sensitive to the choice between 2 opposite behavioural reactions. In a situation like the reference trial (novel, simple and secure environment), the TST to reach the goal box and the amount of previous EM are variables proposed to reflect hesitation (i.e., an antagonism between curiosity or interest toward novelty [neophilia] and an apprehension of novelty [neophobia]).[59] TST in the test trial is also related to decisional processes, since it reflects the duration of choice between the 2 behavioural options, each being associated with opposite values (new *but* safe arm v. familiar *but* unsafe arm). EAI in the test trial (with negative loading) may also be viewed in this way, as the faster the processing of information, the less the subject will hesitate. Positive mean factor scores would thus represent a higher level of hesitation.

Component 3 (explaining 23.7% of the total variability), comprised 3 variables of the test trial: TST, EM and TFR, all which are positively loaded to component 3, which may reflect evaluative processes. The number of EM could be viewed as an approximation of the amount of information gathered on the situation.[57,58] Principal component analyses performed on the elevated plus maze indicate that behavioural parameters, such as entries in closed and open arms, and head movements, reflect risk assessment and situation evaluation.[60] The positive side of the axis would thus represent a high level of evaluation.

The results of the ANOVA performed on these components evidenced significant effects of stress on speed of information processing ($F_{1,120} = 32.59$, $p < 0.0001$), hesitation ($F_{1,120} = 4.74$, $p = 0.03$) and evaluation ($F_{1,120} = 16.64$, $p < 0.0001$). No significant response and stress × response interaction effects were found. As shown in Fig. 3, the stressed mice processed information faster, were less hesitant and tended to evaluate the situation less.

## Discussion

The objective of the present study was to determine the effects of a CUMS procedure, applied over an 8-week period, on cognitive-behavioural function in B6D2F1 female mice. Our data show that DM is strongly affect-

**Table 2: Principal component analysis of 8 variables from the decision-making task. Factor loadings produced by varimax rotation are shown for each component (only the more significant [i.e., > 0.30] are presented)**

| Variable | Component 1 Rapidity of information processing | Component 2 Hesitation | Component 3 Evaluation |
|---|---|---|---|
| Time to solve the task 1 | −0.48 | 0.76 | |
| Time to solve the task 2 | −0.39 | 0.33 | 0.72 |
| Time for final response 1 | −0.63 | | |
| Time for final response 2 | | | 0.65 |
| Exploratory movements 1 | | 0.94 | |
| Exploratory movements 2 | | | 0.90 |
| Exploratory activity index 1 | 0.91 | | |
| Exploratory activity index 2 | 0.47 | −0.49 | |
| Variability, % | 24.1 | 23.8 | 23.7 |

1 = reference trial, 2 = test trial.

ed by CUMS, irrespective of the distribution of the possible responses and the behaviour preceding the choice response.

Our first finding was that stressed and control animals differed in the choices they made. The choices made by the control animals were distributed across the 3 possibilities, whereas, only 2 of the 3 possibilities were observed in the stressed group. In the control group, the avoidance response was the most frequent choice, observed in about two-thirds of the animals, the alternation response was observed in about one-quarter of the animals, whereas some animals (16%) did not reach any of the goal boxes. In the stressed group, the most noteworthy result was the absence of the no choice response. Another striking result observed in the stressed mice was an increased frequency in the avoidance response during the first 3 weeks of CUMS exposure, and an opposite tendency after 4 weeks, with an increased frequency in the alternation response. This variable was the only one affected by the duration of CUMS exposure.

Our data also demonstrated that the behaviour preceding the choice involved 3 independent behavioural dimensions: speed of information processing, hesitation and evaluation of the choice situation. Stressed and unstressed "choice" animals could be discriminated on the basis of these 3 dimensions; whereas in both groups animals choosing either response are not clearly different. For methodological reasons, "no choice" animals could not be included in the principal component analysis. Inclusion of this group resulted in a component comprising only TST and TFR in the test trial, which could only be explained by a time limit for these 2 variables. However, the results of the discriminant

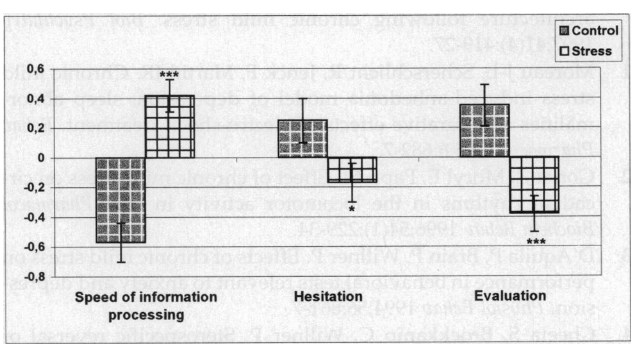


**Fig. 3: Mean factor scores of control and stressed animals making any choice. Results are least square means (with standard error bars). *$p$ < 0.05; **$p$ < 0.001; ***$p$ < 0.0001 compared with control mice.**

analysis revealed that "no choice" animals had a unique behavioural profile, clearly different from control "choice " animals but also from stressed animals. On the basis of the components emerging from the principal component analysis, the no choice response with longer TST at both trials, longer TFR in the reference trials, fewer EM in the reference trial and a lower level of exploratory activity in the test trial, could be considered primarily as indicating more hesitation. However, they could also be considered as a slower processing of environmental information, and to a lesser extent a longer evaluation of the situation than the control "choice" animals. Thus, the greater difficulty in making a choice in the "no choice" animals may be due to a higher level of anxiety or apprehension of the new environment than to a lack of exploratory motivation, since the animals spent more time on the first trial and investigated more thoroughly this new and secure environment,[59] but also since they displayed a high level of evaluation of the choice situation.

Conversely, stressed mice exhibited a faster processing of environmental information, associated with a lower level of evaluation of the choice situation. These mice tended to be less hesitant than the control "choice" mice. The contrast was more pronounced when stressed mice were compared to "no choice" mice. It should be noted that the level of hesitation was less affected by stress than the other dimensions; for example, EM, the variable with a hesitation component, was unaffected by stress.

The more likely explanation for the effect of CUMS on decision-making behaviour would be a deficit in attentiveness. This may stem from a lack of sustained attention or more distractibility and, therefore, deficits in selective attention. In fact, stressed mice displayed a higher level of exploratory activity in both trials. Hyperactivity observed in some free exploration tests is thought to reflect a deficit in habituation (i.e., to a lack of inhibition of irrelevant information about the environment).[35,57] In accordance with this hypothesis, we have demonstrated in another study[61] that CUMS, irrespective of its duration, induces an increased in general (locomotor and exploratory) activity in an open field, without affecting emotional reactivity. Overall, the results suggest that the facilitation of stressed mice in solving the problem presented was secondary to a higher level of distractability.

This assumption is in line with the results of several studies in rats, showing that in various tests of attention, such as the 5-choice serial reaction time task,[62] the 3-choice visual discrimination task,[63] and the sustained attention task,[64] distractable animals display a pattern of disturbances quite similar to that observed in our stressed mice. Abnormal distraction is characterized by a disinhibition of response, reflected by an increased total number of responses (correct or not) and premature anticipatory responses, an overall reduction in choice accuracy and an increased number of errors of various kinds.[62-66] This pattern corresponds to an alteration in the speed of decisional processes as a result of deficits in inhibitory processes.

This distribution of the chosen response with time is an intriguing result; in stressed mice, we noted an increased frequency for choice of the safe and familiar arm (95.5%) during the first 3 weeks of the experiment, followed by an increased frequency of choice of the novel "insecure" arm (47.3%). The initial phase, which can be viewed as an improved performance may correspond to an adaptation to the chronic stress. The second phase, with a random response, may correspond to a phase of behavioural disruption and an onset of stress-induced pathology. It would be of interest to monitor the HPA axis during the 8 weeks of CUMS to find out whether, after a phase of cognition facilitation, chronicity of stress generates cognitive dysfunction, these phenomena being related to HPA axis functioning.[67] In non-pathologic conditions, activation of the HPA axis facilitates stress adaptation, whereas prolonged hypersecretion of corticosterone is associated with stress-related disorders including various cognitive dysfunctions.

NB: An automated version of this test is under development and pending patent.

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