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Contextual and Serial Discriminations: A New Learning Paradigm to Assess Simultaneously the Effects of Acute Stress on Retrieval of Flexible or Stable Information in Mice

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The present study was aimed at simultaneously determining on the same subject, the effects of stress on retrieval of flexible (contextual or temporal) or stable (spatial) information. Three behavioral paradigms carried out in a four-hole board were designed as follows: (1) Simple Discrimination (SD), in which mice learned a single discrimination; (2) Contextual and Serial Discriminations (CSD), in which mice learned two successive discriminations on two different internal contexts; (3) Spatial Serial Discriminations (SSD), in which mice learned two successive discriminations on an identical internal context. The stressor (three inescapable electric footshocks) was delivered 5 min before retention, occurring 5 min or 24 h after acquisition. Results showed that this stressor increased plasmatic corticosterone levels and fear reactivity in an elevated-plus-maze, as compared with nonstressed mice. The stressor reversed the normal pattern of retrieval observed in nonstressed controls in the CSD task, this effect being context dependent, as it was not observed in the SSD task. Overall, our study shows that stress affected the retrieval of flexible and old information, but spared the retrieval of stable or recent ones. Therefore, these behavioral paradigms allow us to study simultaneously, on the same animal, the effects of stress on distinct forms of memory retrieval.

Extensive evidence indicates that stress or pharmacologically induced modifications of the emotional state can influence cognitive function (Kirschbaum et al. 1996; Lupien and McEwen 1997; Belanoff et al. 2001; Wolf et al. 2001). It has been demonstrated that the stress-induced release of glucocorticoids is one of the main factors responsible for these effects. However, glucocorticoids have differential effects depending on the memory phase or the memory system concerned in animal and human subjects (Roosendaal 2002). Glucocorticoids enhance the consolidation of new memories for emotionally arousing experiences (Kovacs et al. 1977; Buchanan and Lovallo 2001), whereas glucocorticoids impair retrieval of long-term spatial memory in rats (De Quervain et al. 1998) and free recall of verbal material in humans (De Quervain et al. 2000; Wolf et al. 2001). Interestingly, even though the modification of the affective state can act on different memory and neurobiological systems (Packard and Cahill 2001), it has been shown that working and declarative memory retrieval are particularly sensitive to glucocorticoids' administration (Lupien et al. 1999; De Quervain et al. 2003).

Animal studies have focused mainly on the effects of stress on acquisition, consolidation, and long-term storage of newly acquired information (Lupien and McEwen 1997). Only a few studies have been carried out in animals to determine the effects of stress on retrieval processes (De Quervain et al. 1998; Bats et al.

2001). More specifically, De Quervain et al. (1998) have shown that stress and glucocorticoids impaired retention performance in a water-maze spatial task, as a function of the time interval elapsed between the delivery of the stressor and retention testing. Moreover, it has been shown that the memory load (difficulty or memory demand of the task) is a critical factor in observing the detrimental effects of stress on retrieval processes, not only in animals (Diamond et al. 1999), but also in humans (De Quervain et al. 2003). However, most of the studies showing an interaction between stress and retrieval processes in animals are based on a simple spatial discrimination task, which involved the memory of stable information rather than of flexible ones. Therefore, our aim in the present study was to (1) develop an original behavioral paradigm allowing us to study simultaneously the retrieval of flexible information (i.e., contextually and temporally distinct information) and of invariant ones (spatial information), and (2) to vary the difficulty or memory demand of the task.

The acute stress used in the present study consisted of three successive inescapable electric footshocks delivered 5 min before retention testing. In the first step, we described the physiological consequences of stress on the activation of the hypothalamo-pituitary-adrenal axis (HPA axis) by measuring the stress-induced release of corticosterone using Radioimmunoassay (RIA). Then, we characterized the emotional consequences of such a stress by measuring emotional reactivity linked to the electric footshocks delivered in the elevated-plus-maze. Once having shown that the acute stress had a significant physiological and emotional stressful value, we studied the effects of acute stress on memory retrieval in three different memory paradigms. These three para-

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digms, varying by the memory load, were based on the search of a food reward in a four-hole-board apparatus and involved spatial, contextual, and serial order information processing. In the first memory paradigm (Simple Discrimination task, SD), food-deprived mice learned to locate a rewarded hole in one of the four holes of the board. Five minutes or twenty-four hours later, animals were submitted to acute stress, and then, 5 min later, to retention testing. In the second memory paradigm (Contextual and Serial Discriminations, CSD), mice learned two successive spatial discriminations involving two different rewarded holes (1 and 2). These serial discriminations were separated by a 2-min interval, and differed from each other by the internal context of the hole-board (namely the color of the floor). Five minutes or twenty-four hours later, animals were submitted to acute stress, and then, 5 min later, retention testing either for 1 or 2 by placing the subject on the floor of the initial acquisition. This second paradigm allowed us to study the influence of the first trial on retention of the second one (proactive interference) and conversely (retroactive interference). In the third memory paradigm (Spatial and Serial Discriminations, SSD), mice learned two successive spatial discriminations involving two different rewarded holes (1 and 2). These serial discriminations were separated by a 2-min interval, but in contrast to the second paradigm, the internal context of the hole-board was the same for the two discriminations. Twenty-four hours later, the animals were submitted to acute stress and then, 5 min later, to retention testing.

RESULTS

Physiological Effect of Acute Stress on Glucocorticoids' (Corticosterone) Release

The aim of these experiments was (1) to study the HPA axis response amplitude to acute stress (electric footshocks), and (2) to study the time course of glucocorticoid secretion after the delivery of the electric footshocks.

HPA Axis Response Amplitude

In this experiment, we measured plasmatic corticosterone levels elicited by acute stress in stressed mice ($n = 6$) as compared with nonstressed mice ($n = 6$) and quiet control ($n = 5$).

ANOVA analysis showed a significant effect of stress ($F_{(2,14)} = 7.2$; $P = 0.007$). Precisely, stressed mice exhibited a higher level of plasmatic corticosterone ($21.14 \pm 6.3 \mu\text{g/dl}$) as compared with nonstressed ($4.02 \pm 0.4 \mu\text{g/dl}$; $P = 0.0064$) and quiet control groups ($2.56 \pm 0.7 \mu\text{g/dl}$; $P = 0.0051$). Nonstressed mice were not significantly different from quiet controls ($P = 0.7$; Fig. 1).

Time Course of Glucocorticoids' Secretion

In this experiment, we studied the time course of glucocorticoids' secretion after the acute stress by collecting blood samples at different delay intervals after footshock stimulation as follows: 10 min ($n = 10$), 30 min ($n = 7$), 60 min ($n = 7$), and 120 min ($n = 7$) after stress. Stressed animals were compared with a quiet control group ($n = 6$).

ANOVA analysis showed a significant effect of delays of blood taking ($F_{(4,32)} = 4.85$; $P = 0.004$). Precisely, there was a significant increase in plasma corticosterone 10 min ($10 \pm 1.2 \mu\text{g/dl}$; $P = 0.0007$) and 30 min ($7.9 \pm 1.6 \mu\text{g/dl}$; $P = 0.025$) after footshock stimulation as compared with the quiet control group ($3.6 \pm 1.2 \mu\text{g/dl}$). Corticosterone level was not different from basal level 60 min ($5.2 \pm 0.7 \mu\text{g/dl}$; $P = 0.36$) and 120 min ($4.9 \pm 1.1 \mu\text{g/dl}$; $P = 0.45$) after footshock stimulation. Furthermore, corticosterone level was higher 10 min after stress than 60 min ($P = 0.007$) and 120 min after stress ($P = 0.004$); there was no

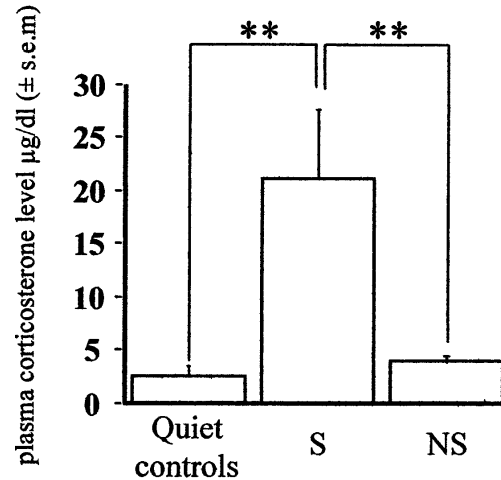


Figure 1 Effect of acute stress (three inescapable electric footshocks) on HPA (hypothalamo-pituitary-adrenal) axis response amplitude measured by radioimmunoassay (RIA) of plasma corticosterone level for Stressed mice (S), nonstressed mice (NS), and quiet controls. Acute stress induced a significant increase of plasma corticosterone level as compared with nonstressed mice and quiet controls (** $P < 0.01$).

significant difference in plasma corticosterone between 30, 60, and 120 min after stress ($P > 0.1$ in all comparisons; Fig. 2)

Effect of Acute Stress on Emotional Reactivity

This experiment was designed to measure the emotional reactivity induced by electric footshocks for stressed mice ($n = 7$) as compared with nonstressed mice ($n = 9$). To that end, subjects were placed for 5 min in the elevated plus maze 5 min after being stressed.

ANOVA showed that the electric footshocks had significant effect on the number of entries in open arms ($F_{(1,14)} = 5.95$; $P = 0.03$) and on the total number of entries in the four arms ($F_{(1,14)} = 6.8$; $P = 0.02$). Specifically, stressed mice visited the open arms of the maze less frequently (10.4 ± 1.1 entries) than non-

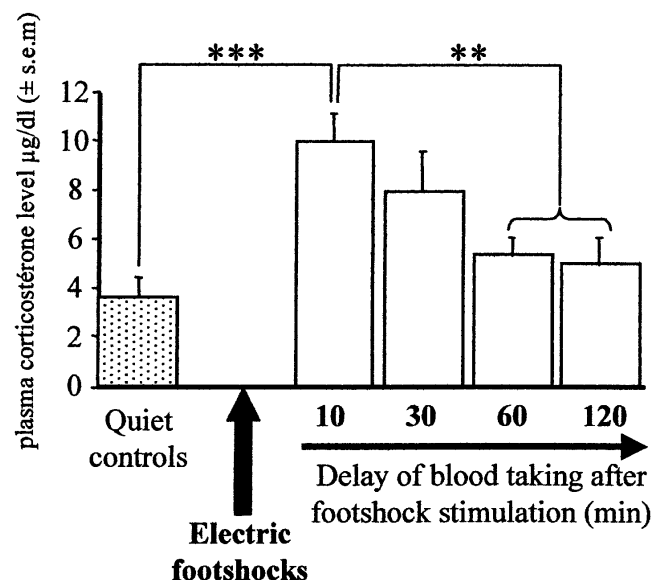


Figure 2 Time course of corticosterone release after acute stress. Corticosterone level of stressed mice reached a maximal value 10 min after footshock stimulation, and decreased progressively to get back to basal level between 60 and 120 min after stress (** $P < 0.01$; (***) $P < 0.001$).

stressed animals (13.9 ± 0.8 entries). Stress did not influence the number of entries in closed arms ($F_{(1,14)} = 1.5$; $P = 0.2$). So far, the reduction of number of visits in open arms had repercussions on the total number of visits in the four arms, which was weaker for stressed animals (21.4 ± 0.85) than for nonstressed animals (26.1 ± 1.4 ; $P = 0.02$; Fig. 3).

Effects of Acute Stress on Memory Retrieval

Experiment 1: Simple Discrimination

The aim of this experiment was to (1) determine whether mice were able to learn a one-trial simple discrimination in the four-hole board apparatus, (2) study the effects of the length of the retention intervals on memory retrieval, and (3) study the effects of acute stress delivery 5 min before testing on performance.

ANOVA analyses showed a significant effect of delay intervals (5 min vs. 24 h; $F_{(1,37)} = 12.9$; $P = 0.0009$). Specifically, mice exhibited higher percents of correct responses at the 5-min retention delay ($59.3 \pm 2.7\%$ of correct responses) as compared with the 24-h retention delay ($42.3 \pm 3.8\%$ of correct responses; $F_{(1,39)} = 13.6$; $P = 0.0007$). Stress had no effect on performance ($F > 1.0$), and the stress X retention delay interaction was not significant ($F < 1.0$). Finally, student-*t*-test showed that performances of all four groups were significantly above chance level (25%) (nonstressed/5-min retention delay: $t = 7.02$; $P < 0.0001$; Stressed/5-min retention delay: $t = 11.89$; $P < 0.0001$; nonstressed/24-h retention delay: $t = 2.61$; $P = 0.03$, and stressed/24-h retention delay: $t = 3.96$; $P = 0.003$). Furthermore, a global statistical analysis showed a significant effect of retention intervals on global exploration ($F_{(1,37)} = 12.95$; $P = 0.0009$), but stress did not affect global exploration ($F < 1.0$), and the interaction stress X retention intervals was not significant ($F < 1.0$). Precisely, mice submitted to the 5-min retention delay significantly explored less than mice submitted to the 24-h retention delay (48.7 ± 5 and 83.9 ± 8.4 total visits in the four holes, respectively; $F_{(1,39)} = 13.6$; $P = 0.0007$; Fig. 4).

Experiment 2: CSD Task

The aim of this experiment was to (1) study the effect of proactive and retroactive interference on the retrieval of two serial discriminations performed in a different internal (floor) context of

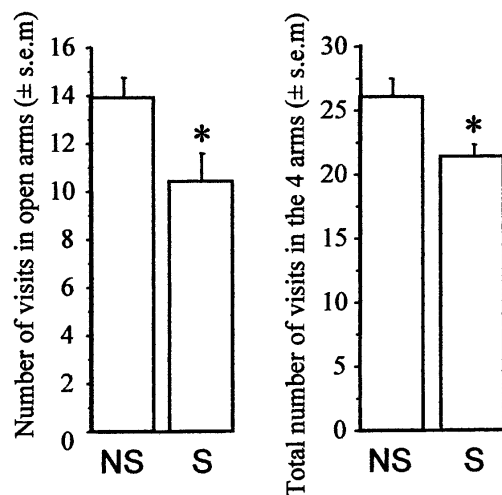


Figure 3 Study of emotional reactivity in the elevated-plus-maze for stressed mice (S) and nonstressed mice (NS). Acute stress had an anxiogenic like effect, as it significantly reduced the number of visits in open arms and in the four arms as compared with nonstressed animals (* $P < 0.05$).

5 min retention interval 24 h retention interval

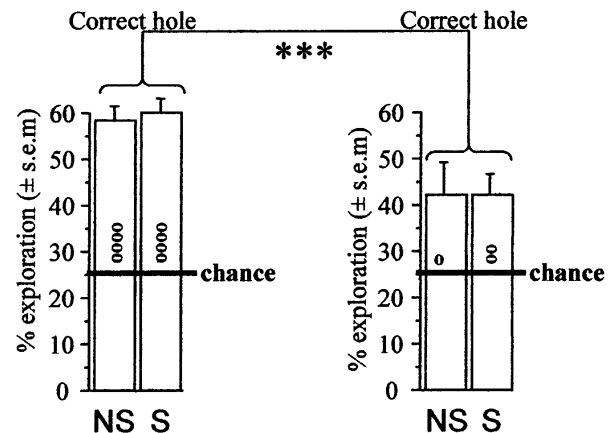


Figure 4 Results of Simple Discrimination task (SD) for the 5-min (left) and 24-h (right) retention intervals. Results are expressed in percent exploration in the correct hole (previously baited hole) for the stressed mice (S) and nonstressed mice (NS). Chance level was 25% (one baited hole over four). Performances were significantly above chance level for the two retention intervals (oooo) $P < 0.0001$; (oo) $P < 0.01$; (o) $P < 0.05$ as compared with chance level. Performances were better for the 5-min than for the 24-h retention interval (***) $P < 0.001$. Acute stress had no effect on performances whatever the retention interval.

the board, (2) study the effects of retention intervals on performance, and (3) to study the effects of acute stress delivery 5 min before testing on the retrieval of these two spatially, contextually, and temporally separated discriminations.

Five-Minute Retention Interval

As shown in Figure 5, neither the type of discrimination (1 vs. 2; $F < 1.0$) nor stress (stressed vs. nonstressed; $F < 1.0$) significantly affected the exploration rates of the previously rewarded hole, and the interaction discrimination X stress was not significant ($F < 1.0$). Moreover, performances were significantly above chance (25%) whether mice were stressed or not and whatever the discrimination tested (1 vs. 2) ($P < 0.05$ for each group). Furthermore, statistical analysis showed that neither the type of discrimination (1 vs. 2; $F < 1.0$), nor stress (stressed vs. nonstressed; $F < 1.0$) significantly affected global exploration (total number of head dips in the four holes).

An ANOVA analysis performed on data from Experiment 1 (SD) and experiment 2 (CSD) for the 5-min retention delay showed a significant effect of interference (one discrimination vs. two successive discriminations; $F_{(1,56)} = 13.7$; $P = 0.0005$), but stress did not affect performance ($F < 1.0$), and the interference X stress interaction was not significant ($F < 1.0$). In other words, discrimination was more accurate in animals submitted to only one discrimination ($59.3 \pm 2.7\%$ of correct responses) than in animals submitted to two successive discriminations ($43.0 \pm 1.8\%$ of correct responses), whether the animals were shocked or nonshocked.

Twenty-Four Hour Retention Interval

Analysis of Correct Responses

Correct responses consisted of exploration into the hole baited on a specific floor context. As shown in Figure 5, neither the type of discrimination (1 vs. 2; $F < 1.0$) nor stress (stressed vs. nonstressed; $F_{(1,45)} = 1.09$; $P = 0.3$) significantly affected performances. However, the discrimination X stress interaction was significant ($F_{(1,45)} = 13.4$; $P = 0.0007$). Specifically, nonstressed animals performed significantly better for discrimination 1

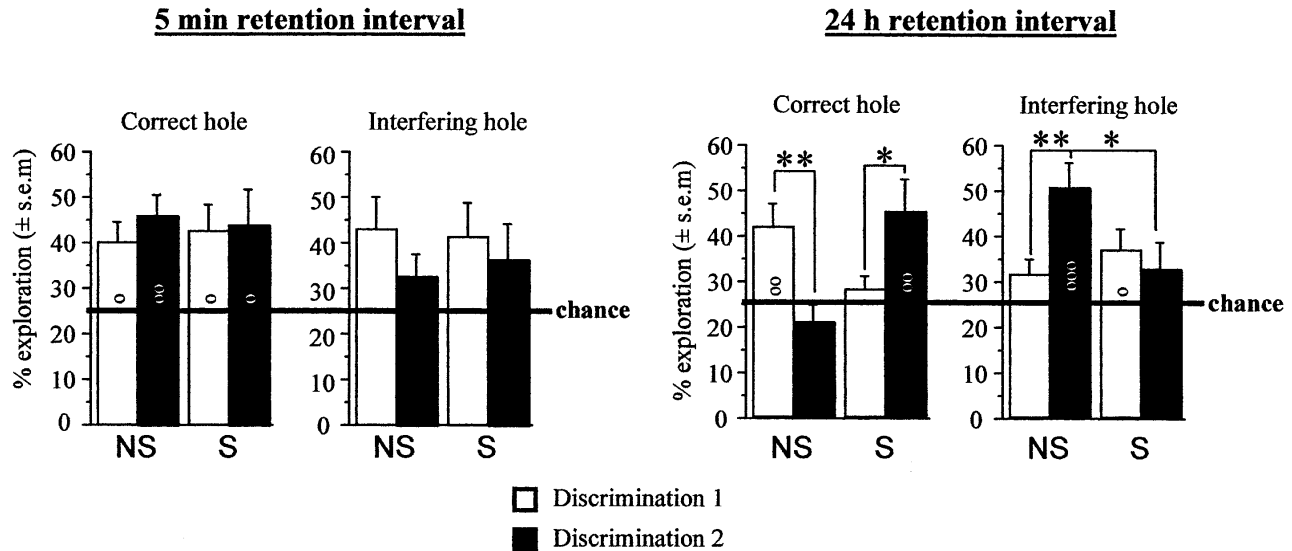


Figure 5 Results of Contextual Serial Discriminations task (CSD) for the 5-min (left) and 24-h (right) retention intervals. Results are expressed in percent exploration in the correct hole (previously baited hole in the same internal context) and in the interfering hole (previously baited hole in the other internal context) for the stressed mice (S) and nonstressed mice (NS). Acute stress modulated performances for the 24-h retention interval (but not for the 5-min retention interval) by producing an inversion of the serial order spontaneously expressed by nonstressed mice. This modulation depended on variations of proactive and retroactive interference (ooo) $P < 0.001$; (oo) $P < 0.01$; (o) $P < 0.05$ as compared with chance level, and (**) $P < 0.01$; (*) $P < 0.05$.

($41.5 \pm 5.2\%$ responses in correct hole) than for discrimination 2 ($20.8 \pm 3.7\%$ responses in correct hole; $F_{(1,23)} = 10.1$; $P = 0.004$). The inverse pattern of exploration was observed in stressed animals that performed significantly worse for discrimination 1 ($28.0 \pm 3\%$ responses in correct hole) than for discrimination 2 ($45.1 \pm 6.9\%$ responses in correct hole; $F_{(1,22)} = 4.5$; $P = 0.04$). Moreover, electric footshocks significantly reduced performance for the discrimination 1 (stressed vs. nonstressed: $F_{(1,22)} = 4.5$; $P = 0.04$), whereas it significantly improved performance for the discrimination 2 (stressed vs. nonstressed: $F_{(1,23)} = 9.1$; $P = 0.006$). Student-*t*-test showed that only stressed animals submitted to discrimination 2, and nonstressed animals submitted to discrimination 1 performed significantly above chance level (25%) ($t = 2.9$; $P = 0.01$ and $t = 3.15$; $P = 0.008$, respectively). In contrast, stressed animals submitted to discrimination 1 and nonstressed animals submitted to discrimination 2 performed at chance ($t = 0.9$; $P = 0.3$ and $t = -1.14$; $P = 0.3$, respectively). A sequential analysis performed by ANOVA with repeated measures showed that all subjects exhibited significantly more correct responses ($80 \pm 4.3\%$) during the last 3 min of retention testing than during the first 3 min ($F_{(1,48)} = 4.2$; $P = 0.05$). Furthermore, statistical analysis showed that neither the type of discrimination (1 vs. 2; $F < 1.0$) nor stress (stressed vs. nonstressed; $F < 1.0$) significantly affected global exploration.

An ANOVA analysis performed on data from Experiment 1 (SD) and Experiment 2 (CSD) for the 24-h retention delay showed that neither interference (one discrimination vs. two successive discriminations; $F_{(2,62)} = 1.6$; $P = 0.2$) nor stress ($F < 1.0$) significantly affected performance. However, the interference X stress interaction was highly significant ($F_{(2,62)} = 7.1$; $P = 0.0015$). Specifically, among nonstressed mice, animals submitted to discrimination 2 of the CSD task were impaired ($20.8 \pm 3.7\%$ of correct responses) as compared with animals submitted to the one-discrimination situation of Experiment 1 (SD) ($42.1 \pm 6.7\%$ of correct responses; $P = 0.008$). In contrast, animals submitted to discrimination 1 of the CSD task ($41.5 \pm 5.2\%$ of correct responses) performed as well as animals submitted to the one-discrimination situation of Experiment 1 ($P = 0.9$). Among

stressed animals, mice submitted to discrimination 1 of the CSD task ($28.0 \pm 3.0\%$ of correct responses) showed an impairment close to statistical significance as compared with animals submitted to the one-discrimination situation ($42.3 \pm 4.4\%$ of correct responses; $P = 0.06$). In contrast, animals submitted to discrimination 2 of the CSD task ($45.1 \pm 6.9\%$ of correct responses) performed similarly to animals submitted to the one-discrimination (SD) situation ($P = 0.7$).

Analysis of Errors

Errors consisted of exploration into the two holes that were never baited during the acquisition phase. ANOVA analyses showed that neither the type of discrimination (1 vs. 2; $F_{(1,45)} = 1.6$; $P = 0.2$) nor stress (stressed vs. nonstressed; $F < 1.0$) significantly affected percent of responses in the two nonbaited holes. Discrimination X stress interaction was not significant ($F_{(1,45)} = 2.7$; $P = 0.1$). Moreover, student-*t*-test showed that percent of errors was significantly under chance level for all four groups (nonstressed animals submitted to discrimination 1, $t = -5.56$; $P = 0.0001$; nonstressed animals submitted to discrimination 2, $t = -4.31$; $P = 0.0012$; stressed animals submitted to discrimination 1, $t = -2.27$; $P = 0.02$; and stressed animals submitted to discrimination 2, $t = -7.77$; $P < 0.0001$).

Analysis of Interfering Responses

During retrieval of discrimination 1, interfering responses consisted of exploration into the hole baited on discrimination 2, and inversely. ANOVA analyses showed that neither the type of discrimination (1 vs. 2; $F_{(1,45)} = 2.25$; $P = 0.14$) nor stress (stressed vs. nonstressed; $F_{(1,45)} = 1.6$; $P = 0.2$) significantly affected percent of responses in the interfering hole. On the other hand, discrimination X stress interaction was significant ($F_{(1,45)} = 5.4$; $P = 0.02$). Specifically, nonstressed animals submitted to discrimination 2 exhibited significantly more interfering responses than animals submitted to discrimination 1 (respectively, $50.3 \pm 3.3\%$ and $31.4 \pm 5.6\%$ responses in interfering hole; $F_{(1,23)} = 8.7$; $P = 0.007$). For the stressed group, there was no difference between animals submitted to discrimination 1 and ani-

mals submitted to discrimination 2 ($F < 1.0$). Moreover, stressed animals submitted to discrimination 2 exhibited a percent of interfering response lower than nonstressed animals submitted to discrimination 2 ($32.6 \pm 5.7\%$ and $50.3 \pm 5.6\%$; $F_{(1,23)} = 4.9$; $P = 0.04$), whereas there was no significant difference between stressed and nonstressed animals submitted to discrimination 1 ($F < 1.0$). Student-*t*-test showed that only stressed animals submitted to discrimination 1 and nonstressed animals submitted to discrimination 2 exhibited a rate of interfering responses significantly above chance ($t = 2.5$; $P = 0.03$ and $t = 4.5$; $P = 0.0009$, respectively). In contrast, stressed animals submitted to discrimination 2 and nonstressed animals submitted to discrimination 1 exhibited a number of interfering responses at chance level ($t = 1.3$; $P = 0.2$ and $t = 1.9$; $P = 0.09$, respectively).

Finally, a correlation analysis showed that percentage of interfering responses was negatively correlated to the percentage of correct responses for the four groups ($r = 0.67$; $P < 0.0001$).

Experiment 3: SSD Task

This experiment was designed to evaluate the effects of context change (floor) between the two discriminations in the CSD task. To that end, mice were submitted to the same experimental conditions as described for Experiment 2 (SCSD), but the same floor context was used for the two successive discriminations. Mice were tested only after a 24-h retention delay.

An ANOVA performed on percentage of exploration in the first baited hole (D1) and the second baited hole (D2) showed a significant serial effect (first baited hole vs. second baited hole; $F_{(1,17)} = 9.2$; $P = 0.0075$) as well as a significant effect of stress (stressed vs. nonstressed mice; $F_{(1,17)} = 5.4$; $P = 0.03$). Specifically, electric footshocks improved retrieval of discrimination 1 ($64.0 \pm 8.9\%$ of responses in first baited hole) as compared with nonstressed animals ($40.2 \pm 6.5\%$ of responses in first baited hole; $F_{(1,17)} = 5.8$; $P = 0.03$). In contrast, the electric footshocks had no effect on retrieval of discrimination 2 ($25.7 \pm 6.9\%$ of responses in second baited hole) as compared with the nonstressed situation ($20.3 \pm 7.1\%$ of responses in second baited hole; $F < 1.0$). Furthermore, a student-*t*-test showed that nonstressed animals as well as stressed animals performed significantly above chance level for discrimination 1 ($P = 0.04$ and $P = 0.03$, respectively) but responded at chance for discrimination 2 ($P = 0.9$ and $P = 0.5$, respectively). Global exploration was not affected by stress ($F < 1.0$; Fig. 6).

DISCUSSION

Results of Experiment 1 (SD) showed that mice were able to learn and to remember the spatial location of one baited hole of the four holes on the board in only one training session, whatever the retention interval considered. However, performances were higher at the 5-min retention interval than at the 24-h interval. This decay of performances as a function of the length of the retention interval represents the time-dependent forgetting of the discrimination. Furthermore, results showed that the acute stress delivered 5 min before retention testing had no effect on performances, whatever the retention interval. These results are congruent with those of De Quervain et al. (1998), who found no effect of a stressor given 2 min before long-term retrieval in a water-maze spatial task.

One could surmise that the relative simplicity of the task could prevent observance of an eventual effect of stress on retrieval (see Diamond 1999). To test this hypothesis, a second information (to be remembered) was added (Experiment 2, CSD), in order to increase the memory load of the task. In this way, interference between information was increased. Furthermore, as compared with the SD task, the CSD task involved some flexible features (processing of spatially, contextually, and temporally

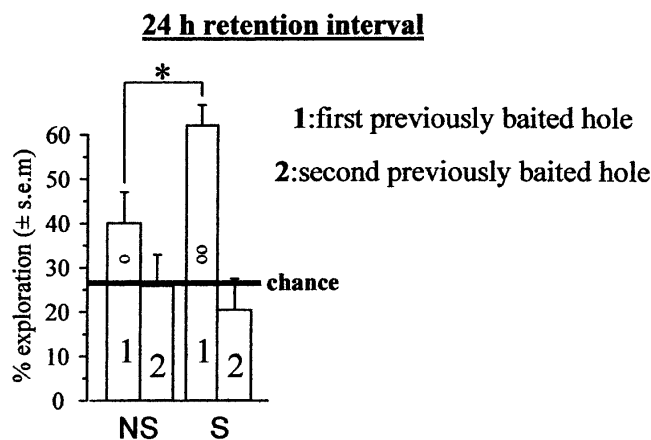


Figure 6 Results of Spatial and Serial Discriminations task (SSD) for the 24-h retention interval. Results are expressed in percent exploration in the first hole (1: first previously baited hole) and in the second hole (2: second previously baited hole) for the stressed mice (S) and nonstressed mice (NS). Acute stress did not produce an inversion of serial order when the two successive discriminations took place in the same internal context. (oo) $P < 0.03$; (o) $P < 0.04$ as compared with chance level, and (*) $P < 0.05$.

separated information). Data showed that performances during retention testing were higher when animals had learned only one discrimination, than when they had learned two successive discriminations, whatever the retention interval. This decay of performances in the CSD task represents the deleterious effects of proactive or retroactive interference as compared with the situation in which animals learned only one discrimination.

CSD results drawn from the 24-h retention interval showed that stress delivery 5 min before retention testing affected performances. This result conflicts with those of De Quervain et al. (1998) showing no effect of a stressor given 2 min before long-term retrieval in a water-maze spatial task. The contradiction between the present findings and those of De Quervain may be due to the complexity of the CSD task. The CSD task involved two spatially, contextually, and temporally separated information, whereas in the water-maze spatial task, animals had to remember a single spatial location. In support of this idea, we found no effect of the stressor given 5 min before retention testing in Experiment 1 (SD), in which animals had to remember a single spatial location.

In the CSD task, nonstressed animals exhibited higher discrimination rates for discrimination 1 than for discrimination 2. In other words, mice spontaneously expressed a specific serial order. On the other hand, we showed that the effects of stress depended on the serial order of the discrimination, as stress improved retrieval of discrimination 2, but impaired retrieval of discrimination 1. Thus, stress produced an inversion of the normal retrieval pattern as compared with nonstressed subjects. Such an effect of stress indicated that the second discrimination was correctly acquired and memorized, but was not spontaneously expressed in nonstressed mice, as they performed at chance for discrimination 2. Stress allowed mice to express the more recently acquired discrimination. In other words, the two successively acquired information compete during retrieval, and stress acts on this reciprocal interaction. To explain the reversal effect of stress in the CSD task, we suggest that acute stress could activate some cerebral structures involved in the contextual cues processing, such as the hippocampus (De Quervain et al. 2003; Roozendaal et al. 2003), so that the contextual cues would act as a reminder at the time of retrieval in stressed subjects. The contextual change is a key factor sustaining the stress-induced effect

on retrieval. Comparison between results of Experiment 3 (SSD task) and Experiment 2 (CSD task) showed that nonstressed animals exhibited the same pattern of performances (i.e., higher performances for discrimination 1 than for discrimination 2) either in the SSD or in CSD tasks. These data indicate that nonstressed mice spontaneously expressed a specific serial order that did not depend on contextual cues. In contrast, the stress-induced reversal effect in the CSD task depended on contextual cues, as this stress-induced inversion was not observed in the SSD task (Experiment 3). Moreover, this context-dependent effect of stress on retrieval appeared at the 24-h retention interval but not at the 5-min retention interval, suggesting that the processing of contextual cues was not immediate, but needed some consolidation time.

Interestingly, in CSD task, correct-response rate was always negatively correlated to the rate of interfering responses. In other words, the fall of performance is specifically due to proactive or retroactive interference between information rather than to a pure forgetting. In contrast, the rate of errors (visits of nonbaited holes) was always below chance level and was not modified by stress. These data indicated that the retrieval of spatial information in the CSD task (i.e., memory of spatial locations of the two previously baited holes and avoidance of spatial locations of the two nonbaited holes) was not sensitive to acute stress. Thus, stress acts on contextual rather than on spatial retrieval. According to the upper definition, spatial information in the CSD task could be considered as stable and invariant between the two successive discriminations. This result appears to conflict with those of De Quervain et al. (1998) and Roozendaal et al. (2003) showing that stress and glucocorticoids impaired spatial memory retrieval. However, these discrepancies could be due to methodological differences. Firstly, in the CSD task, mice were exposed for 12 min to the spatial environment within a single learning session, whereas in Roozendaal et al. (2003) and De Quervain et al. (1998) studies, rats were submitted to a more distributed spatial learning. One could suggest that the memory of spatial information is more robust in the CSD task, and as a consequence, less sensitive to stress. Secondly, the water maze is a task involving a moderately stressful component, whereas the CSD task is based on the search for a food reward. The opposite valence of the reinforcer in the two tasks could explain the discrepancies between the studies.

The study of emotional reactivity showed that, in our behavioral conditions, stress produced an anxiety-like reactivity in the elevated-plus-maze as compared with nonstressed subjects. This finding is congruent with data drawn from the corticosterone radioimmunoassay showing that stress produced an increase of corticosterone level. This HPA activation was specific to footshock stimulation, as nonshocked animals did not exhibit such activation and had the same corticosterone level as subjects of the quiet control group. Furthermore, results showed that corticosterone level of stressed mice reached a maximal value 10 min after the footshock stimulation, which decreased progressively to basal level between 60 and 120 min after stress. Taken together, these data indicated that the stressor used in our study produced a significant physiological response (activation of the HPA axis) and a significant emotional behavioral response (increased anxiety-like reactivity in the elevated plus maze), which rendered it relevant to study the effects of stress on memory retrieval.

Our study does not show a conclusion to a functional link between the variation of corticosterone levels and the memory effects of stress. Nevertheless, it is important to observe that 80% of correct responses in CSD task occurred during the last 3 min of testing, when corticosterone reached the maximal value. The synchronization between the peak of corticosterone and stress

effect on retrieval suggests that the glucocorticoid release elicited by electric footshocks could be, at least in part, responsible for the modulation of recall. Even though extensive evidence has shown that ACTH and other stress-related compounds also affect memory retrieval (Izquierdo and Pereira 1989; Borde et al. 1997, 1998; Borde and Beracochea 1999; Vianna et al. 2000), several arguments suggest a causal role of glucocorticoids in the stress-induced modulation of memory retrieval. First, glucocorticoids can exert their effects either by long genomic mechanisms involving glucocorticoid and mineralocorticoid receptors (Douma et al. 1998) or by more rapid (between a few seconds to several minutes) nongenomic mechanisms (Borski et al. 1991; Rose et al. 1993; Breuner et al. 1998; De Quervain et al. 1998). Second, a recent study carried out by our team showed that the increase of the delay between acute stress delivery and retention testing (2 h instead of 5 min; i.e., so far, memory retrieval took place when plasmatic corticosterone got back to basal level) totally abolished the stress-induced retrieval modulation in the CSD task (A. Celerier, C. Pierard, A. Sarrieau, and D. Beracochea 2003, unpubl.). Finally, another study carried out in our laboratory indicated that the suppression of corticosterone synthesis with metyrapone given before the stress delivery impaired the stress-induced modulation of retrieval in the CSD task (Celerier 2002; A. Celerier, F. Chauveau, C. Pierard, and D. Beracochea, in prep.).

Taken together, these data suggest that corticosterone could be involved in the stress-induced modulation of the retrieval pattern, even though the relationships between stress-induced glucocorticoids' release and cognitive performance are complex (Roozendaal et al. 1996).

Conclusion

Our aim was to design a behavioral paradigm allowing us to observe a modulation of retrieval by acute stress in a task involving the processing of both flexible or stable information. Taken together, data showed that electric footshocks used as acute stress had an anxiogenic-like effect in the elevated-plus maze and produced a significant physiological activation of the HPA axis. Behavioral studies indicated that this acute stress can specifically affect memory retrieval in the CSD task, as it was delivered just before retention testing. Furthermore, stress affected the retrieval of flexible, variant (contextual cues and serial order) and old (24-h retention interval) information but not retrieval of stable, invariant (spatial), and recent (5-min retention interval) ones. Finally, our study showed that the effect of stress, namely the inversion of the spontaneously expressed serial order, was dependent on contextual cues and was exerted by a modulation of interference. Overall, the CSD task allowed us to simultaneously study, on the same animal, memory of the temporal (serial order), contextual (underlined by stress effect), and spatial (spatial localization of baited holes) components of distinct past events. Thus, our study shows that the effects of stress on retrieval processes affected much more flexible forms of memory, while sparing more stable ones.

MATERIALS AND METHODS

Subjects

The subjects were 217 naive male mice of the BALB/c inbred strain obtained from IFFA Credo; 147 were randomly assigned to a group for the various behavioral experiments (see Table 1), 54 were submitted to corticosterone radioimmunoassay, and 16 were used to study emotional reactivity in the elevated-plus-maze. Mice were 6 mo old at the time of experiment, and weighed between 28 and 32 g. They were housed individually with continuous access to water on a 12-h light-dark cycle in a temperature-controlled and ventilated room. All subjects were maintained at 85%–90% of their ad libitum body weight

Table 1. Experimental Groups

	Retention interval	Stress (shocks)	Discrimination	Group size
Experiment 1 (SD) (simple discrimination)	5 min	Nonstressed	—	n = 11
	5 min	Stressed	—	n = 11
	24 h	Nonstressed	—	n = 9
	24 h	Stressed	—	n = 10
Experiment 2 (CSD) 2 successive discriminations 2 different contexts	5 min	Nonstressed	1	n = 10
	5 min	Nonstressed	2	n = 10
	5 min	Stressed	1	n = 8
	5 min	Stressed	2	n = 10
	24 h	Nonstressed	1	n = 13
	24 h	Nonstressed	2	n = 12
	24 h	Stressed	1	n = 11
	24 h	Stressed	2	n = 13
Experiment 3 (SSD) 2 successive discriminations 2 same contexts	24 h	Nonstressed	—	n = 10
	24 h	Stressed	—	n = 9

Composition of the different experimental groups: retention intervals (5 min or 24 h), stress conditions (stressed or nonstressed) and type of discrimination for CSD task (1) first acquired discrimination, or (2) second acquired discrimination during retention testing.

throughout the study. All test procedures were conducted during the light phase of the cycle between 8.00 a.m. and 12.00 a.m., and the groups were mixed throughout the experiments. During the food deprivation phase, mice were handled daily to habituate them to the experimenter.

Acute Stress

Stress treatment was carried out in a stress chamber placed in room C. The stress chamber (20 cm × 15 cm × 15 cm) was enclosed with Plexiglas walls, one transparent, and the three others painted brown. The floor of the conditioning chamber consisted of 35 stainless steel rods (3 mm in diameter), spaced 5 mm apart and wired to a shock generator for the delivery of the three successive footshocks (0.9 mA; 2 sec). Mice were placed in the stress chamber for 1 min, and received three successive electric footshocks after 10, 30, and 50 sec. The stress chamber was cleaned with 95% ethanol, then with water, after each mouse.

Physiological Effect of Acute Stress on Glucocorticoids' (Corticosterone) Release

Principle

Endocrinological study using circulating corticosterone radioimmuno-assay (RIA) was used to characterize (1) the HPA axis response amplitude to acute stress, and (2) the time course of glucocorticoids' secretion after the delivery of electric footshocks.

Procedure

HPA Axis Response Amplitude

Mice were placed for 1 min in the stress chamber described above. Stressed mice received three successive inescapable electric footshocks as reported earlier, and nonstressed mice were placed in the same condition, except that they did not receive any footshock. Subjects were decapitated 10 min after acute stress, and trunk blood was collected for RIA. Animals were divided into three groups as follows: stressed mice ($n = 6$), nonstressed mice ($n = 6$), and a quiet control group ($n = 5$) consisting of nonshocked mice directly from the animal room, which allowed measuring of the basal corticosterone level.

Time Course of Glucocorticoids' Release

Mice were placed for 1 min in the stress chamber described above and received three successive inescapable electric footshocks as reported earlier. Then, animals were decapitated and trunk blood was collected for radioimmunoassay at different delays after foot-

shock stimulation, that is, 10 min ($n = 10$), 30 min ($n = 7$), 60 min ($n = 7$), and 120 min ($n = 7$) after stress. A quiet control group ($n = 6$) consisted of nonshocked mice directly from the animal room and allowed us to measure the basal corticosterone level without stress.

RIA

Trunk blood was collected in heparinized tubes and stored on ice. After centrifugation at 3000 r.p.m. for 10 min, the supernatant was stored at -80°C until assay. Corticosterone samples were assayed in duplicate by Radioimmunoassay using a commercial kit (ICN Biomedicals).

Effect of Acute Stress on Emotional Reactivity

Apparatus

The effect of acute stress on emotional reactivity was studied in the elevated-plus-maze. The elevated-

plus-maze was a cross-shaped maze in gray Plexiglas, with two opposing open arms (30 cm × 7 cm) and two opposing closed arms with walls (30 cm × 7 cm × 17 cm). The maze was elevated to a height of 55 cm and placed in the center of a well-lit room (100 lux).

Procedure

Five minutes after being placed in the stress chamber, stressed ($n = 7$) and nonstressed mice ($n = 9$) were tested in the elevated-plus-maze to evaluate emotional reactivity. Mice were placed individually in the center of the maze (7 cm × 7 cm) in a PVC tube and allowed 5 min of free exploration. During this test period, mice behavior was recorded on video tape, and the observer measured time spent in the open arms, time spent in the closed arms, time spent in the center of the maze, number of entries into the open arms, number of entries into the closed arms, and the total number of entries into the four arms. An entry was defined as all four paws in the arm. The maze was cleaned with 95% ethanol, then with water, after each mouse was tested.

Effect of Acute Stress on Memory Retrieval

Apparatus

The four-hole board apparatus (45 cm × 45 cm × 30 cm) was enclosed with grey Plexiglas walls. The floor of the hole board was interchangeable (white and rough; black and smooth; gray and smooth). On the floor, four holes opening on a food cup (3 cm diameter × 2.5 cm in depth) were located 6 cm away from the sidewalls. Photocells placed in each hole allowed us to measure the following: the number of head dips in each hole (parameter 1); the duration of head dips in each hole (parameter 2); the total number of head dips in the four holes (parameter 3); the total duration of head dips in the four holes (parameter 4). Parameters 1, 2, 3, and 4 allowed calculus of parameters 5 and 6. These parameters were recorded by a computer that calculates the following: percent time (parameter 5): (parameter 2/parameter 4) × 100; percent visits (parameter 6): (parameter 1/parameter 3) × 100. Parameters 3 and 4 were considered to measure global exploration. As these two parameters were correlated ($r > 0.92$; $P < 0.0001$ in all comparisons), we only mentioned parameter 3 in our analysis. Memory of each discrimination was assessed by parameters 5 and 6. As these two parameters were correlated ($r > 0.92$; $P < 0.0001$ in all comparisons) we only mentioned parameter 6 in our analysis.

The four-hole board apparatus was placed in a room that provided a 60-dB background noise and a 20-lux light. The ap-

paratus was cleaned with 95% ethanol, then with water, before each mouse was trained or tested.

Procedure

The procedures of the three behavioral experiments are described in Figure 7.

Experiment 1: Simple Discrimination Task in the Four-Hole Board

Acquisition

Acquisition took place in room A. Mice were first placed in the center of the hole board in a PVC tube for 15 sec to allow a random start in the apparatus. Then, animals were allowed to explore the hole board for 6 min. Ten 20-mg pellets were available only in one hole of the four holes of the board. Location of the reinforcement varied between each subject. At the end of acquisition, mice returned to their home cage in the animal room. All the mice included in the statistical analyses finished eating the total allotted pellets in the time allowed. A few animals, who did not eat all pellets, were excluded from analyses.

Acute Stress

A total of 5 min or 24 h after acquisition, mice were placed for 1 min in the stress chamber described above placed in room C, and half of the mice (stressed mice) received three successive inescapable electric footshocks (0.9 mA; 2 sec). The other mice (non-

stressed) were placed in the same conditions, except that they did not receive any footshock. Animals were then placed in their home cage in room B for 5 min.

Retention Testing

Retention testing for simple discrimination took place in room A, 5 min after acute stress, and was carried out by measuring the exploration for each hole during 6 min, without any pellets in the apparatus. This procedure allowed us to measure performance (percent exploration in the correct hole defined as the previously baited hole) and errors (percent exploration in the three incorrect holes corresponding to the three previously non-baited holes) during retention testing.

Experiment 2 : Contextual and Serial Discrimination Task in the Four-Hole Board

Acquisition

In room A, mice were first placed in the center of the hole board in a PVC tube for 15 sec, and then learned the two successive spatial discriminations (1 and 2) for 6 min each. The two discriminations differed by the color of the floor, and were separated each by a 2-min time interval. During this delay interval, the mouse was placed in its home cage in room B. For discrimination 1, ten 20-mg pellets were available only in one hole of the four holes of the board. For discrimination 2, food pellets were located only in the symmetrical hole.

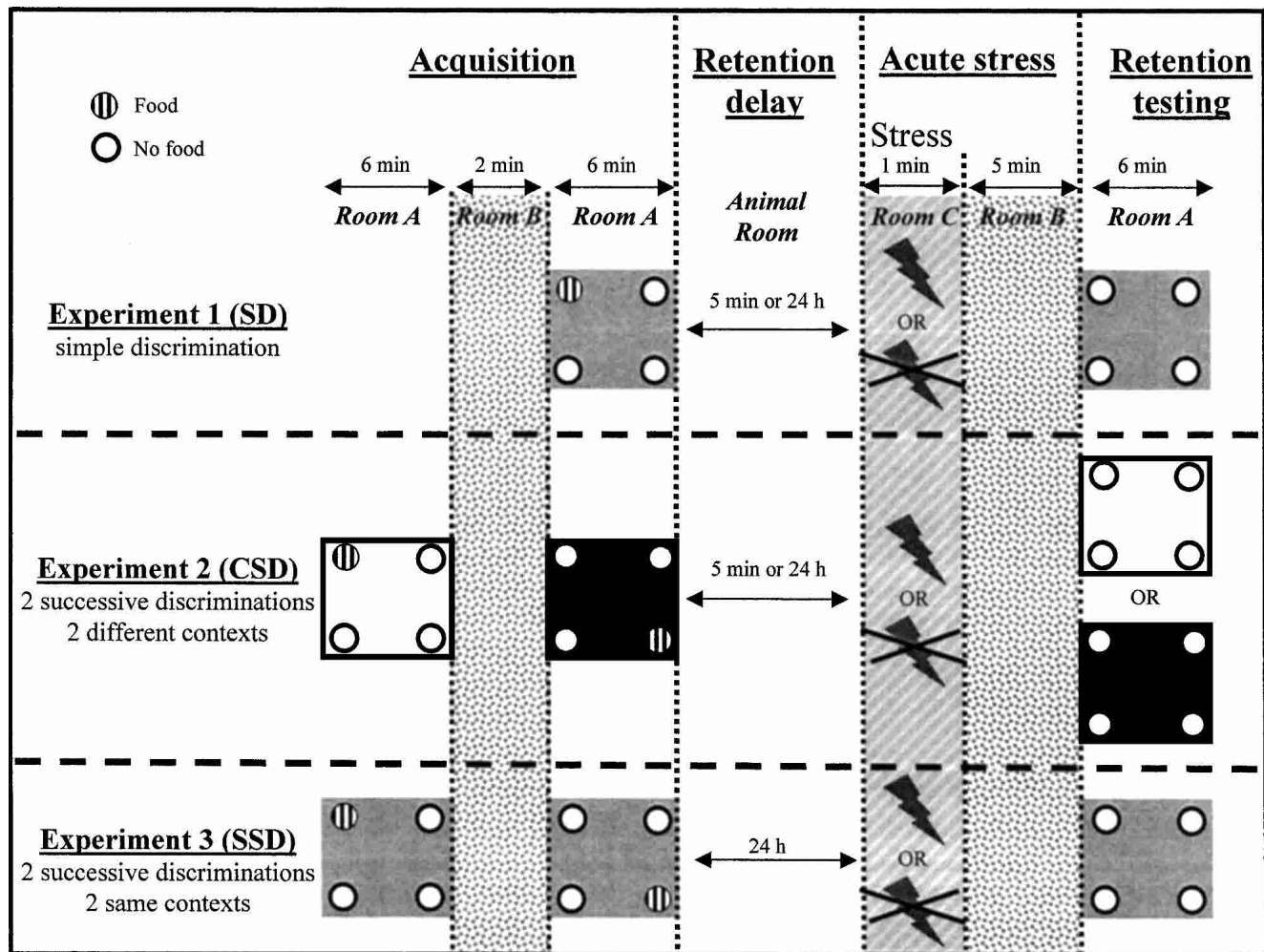


Figure 7 Behavioral procedures of the three experimental tasks; SD (top); CSD (middle), and SSD (bottom). Rooms A, B, and C were different.

Acute Stress

A total of 5 min or 24 h after these two acquisitions, mice were placed for 1 min in a stress chamber placed in room C, and half of the mice received three successive inescapable electric footshocks (0.9 mA; 2 sec). The other mice were placed in the same conditions, except that they did not receive any footshock.

Retention Testing

Retention testing (room A) either for discrimination 1 or for discrimination 2 was carried out on independent groups of mice 5 min after this acute stress phase, by measuring the exploration for each hole during 6 min without any pellets in the apparatus. This procedure allowed us to measure performance (percent exploration in the previously baited hole on the same floor-context), interfering responses (percent exploration in the previously baited hole on the other floor-context), and errors (percent exploration in the two holes not previously baited whatever the floor-context).

Experiment 3: Spatial and Serial Discrimination Task in the Four-Hole Board

Mice were submitted to the experimental conditions described for Experiment 2 (SCSD), but the two successive discriminations were learned on the same floor. Mice were tested only after a 24-h retention delay interval.

Ethical Statement

All experimental procedures were in accordance with official French Regulations for the care and use of laboratory animals.

Statistical Analysis

The data were analyzed using one-way or two-way factorial ANOVAs or ANOVA with repeated measures, followed, when adequate, by post hoc comparisons (Scheffe's test). Comparisons of performances with chance level were calculated with one sample Student-*t*-test (with hypothesized mean = chance level = 25%).

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