Learning under stress: The inverted-U-shape function revisited

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Although the relationship between stress intensity and memory function is generally believed to follow an inverted-U-shaped curve, strikingly this phenomenon has not been demonstrated under the same experimental conditions. We investigated this phenomenon for rats’ performance in a hippocampus-dependent learning task, the radial arm water maze (RAWM). Variations in stress intensity were induced using different water temperatures (25°C, 19°C, and 16°C), which elicited increased plasma corticosterone levels. During spatial training over three consecutive days, an inverted-U shape was found, with animals trained at 19°C making fewer errors than animals trained at either higher (16°C) or lower (25°C) stress conditions. Interestingly, this function was already observed by the last trial of day 1 and maintained on the first day trial of day 2. A long-term recall probe test administered under equal temperature conditions (20°C) revealed differences in performance according to the animals’ former training conditions; i.e., platform searching for rats trained at 25°C was less accurate than for rats trained at either 16°C or 19°C. In reversal learning, groups trained at both 19°C and 25°C showed better performance than the 16°C group. We also found an interaction between anxiety and exploration traits on how individuals were affected by stressors during spatial learning. In summary, our findings confirm, for the first time, the existence of an inverted-U-shape memory function according to stressor intensity during the early learning and memory phases in a hippocampus-dependent task, and indicate the existence of individual differences related to personality-like profiles for performance at either high or low stress conditions.

[Supplemental material is available online at http://www.learnmem.org.]

Physiological stress responses are used by organisms to adapt to changing, demanding circumstances, so these responses are of enormous adaptive value (Lightman 2008). However, survival success depends not only on the immediate ability to respond to threat, but also on the integration of previously acquired knowledge and skills into effective strategies to facilitate coping with similar demands in the future. This view provides an evolutionary explanation for stress effects on learning and memory processes.

Understanding the nature of stress—memory interactions has attracted significant attention in recent years. Surprisingly, despite much investigation, it is still not known how stress severity affects memory function. It is generally believed that the relationship between stress intensity and memory function follows an inverted-U-shaped curve, with memory increasing with stress to an optimal point, above or below which memory decreases. However, this stress–memory relationship seems to not apply to classical (Pavlovian) conditioning processes (for review, see Sandi and Pinelo-Nava 2007). Rather, current evidence supports a linear relationship between stressor intensity and the strength of the fear-conditioned memory formed, with an asymptotic waveform for high-to-very-high stress intensities (Fanselow and Bolles 1979; Shors and Servatius 1997; Beylin and Shors 1998; Cordero et al. 1998; Radulovic et al. 1998; Anagnostaras et al. 2000; Merino et al. 2000; Laxmi et al. 2003).

The inverted-U shape function was originally proposed by Yerkes and Dodson (1908) to explain the relationship between stimulus strength and the rapidity of habit formation for “difficult” discrimination learning tasks in mice. In their experimental conditions, as with those of Broadhurst (1957), “easy” tasks followed a linear relationship, as discussed above for classical conditioning. Hence, the so-called Yerkes-Dodson law implies that cognitive performance in difficult tasks is best when an individual is under optimal stress; performance would be impaired under conditions above or below optimal stress levels (Yerkes and Dodson 1908; Broadbert 1965; Mendl 1999). Despite the great popularity of the inverted-U curve, or the Yerkes-Dodson law, to describe the relationship between stress and performance (Diamond 2005), the validity of the law has been criticized due to significant methodological problems in the study performed by Yerkes and Dodson (1908) and their data being judged insufficient to substantiate conclusions, among other reasons (Brown 1965; Baumler and Lienert 1993; Baumlner 1994; Teigen 1994; Hancock and Ganey 2003; Diamond et al. 2007). In 1957, Broadhurst provided further evidence for the inverted-U-shape function using more refined methods and a visual discrimination task similar to that used by Yerkes and Dodson (1908). In Broadhurst’s experiments, variations in stress levels were achieved by exposing rats to different lengths of air deprivation just before the start of each trial. Therefore, stress was applied within the learning context, but did not originate from elements related to the cognitive task, so the stress could be considered “extrinsic” to the learning task. In the field of animal learning, it is surprising to note that not a single report has described an inverted-U-shape function for the relationship between “intrinsic” stress (i.e., induced by elements related to the cognitive task) and learning under the same experimental conditions (Morris 2006; Sandi and Pinelo-Nava 2007). For example, recent proposals of an inverted-U-shaped function during spatial learning in rodents are based...
on independent, composite observations from different experimental settings and laboratories examining different parts (ascending or descending) of the function (Mendl 1999; Morris 2006; Park et al. 2006; Sandi and Pinelo-Nava 2007).

Here we aimed to evaluate, for the first time, the validity of the inverted-U-shape function to account for the impact of variations in intrinsic stressor intensity on memory processes in a spatial learning task. We used the radial six-arm water maze (RAWM), in which animals learn to find a hidden escape platform located at the end of one of the arms with the help of extramaze visual cues. This task was chosen because it was previously shown to be both hippocampus dependent and sensitive to modulation by stress (Diamond et al. 1999). To evaluate the effect of stressor intensity on task learning, we trained rats at different water temperatures, which produce different plasma corticosterone levels, and explored the rats’ performance throughout each memory phase (learning acquisition, long-term memory retention, and reversal learning).

Furthermore, in line with the pioneering work by Eysenck (1955), who questioned the role of personality in stress-influenced performance during learning tasks, as well as our own work relating anxiety-like trait with differences in spatial learning abilities (Herrero et al. 2006) and behavioral and neurobiological vulnerability to stress (Jakobsson et al. 2008; Sandi et al. 2008; Luksy et al. 2009), we set a second goal of capturing individual differences in the relationship between intrinsic stress and learning based on rat’s personality traits.

Results

Plasma corticosterone levels induced by swimming at different water temperatures

Our first step was to select three water temperatures that represent a gradation of physical stressor intensities when rats are placed in a pool without concomitant spatial learning. Based on pilot and previous experiments (Sandi et al. 1997; Akirav et al. 2001, 2004), 16°C, 19°C, and 25°C were chosen, and plasma levels of the stress hormone corticosterone were evaluated after submitting animals to one cued training session in the RAWM (i.e., cued platform) at one of the three water temperatures (n = 6 rats/temperature; Fig. 1A). A one-way ANOVA indicated an effect of temperature (F(2,15) = 5.29, P < 0.05), with corticosterone levels from animals trained at 16°C being significantly higher than levels from animals trained at 25°C (P < 0.05), and levels from animals trained at 19°C falling between the other two levels. A linear regression analysis confirmed the existence of a significant negative relationship between water temperature level and corticosterone level. Across the training sessions, ANOVA also revealed an effect of water temperature (F(2,119) = 12.38, P < 0.0001). This effect was further confirmed when performance across the three training days was averaged (F(2,119) = 12.38, P < 0.0001; Fig. 2B). Interestingly, post-hoc analyses indicated the existence of an inverted-U-shaped function, with animals trained at 19°C making less errors to find the platform than those trained at either 16°C (P < 0.0001) or 25°C (P < 0.0001). No difference in performance was found between rats trained at either 16°C or 25°C water temperature. A repeated-measures ANOVA on data represented in Figure 2A yielded no interaction between water temperature and training day (F(4,228) = 0.74, n.s.). Separate ANOVAs for each training day indicated that on each training day, rats trained at 19°C outperformed those trained at either 16°C or 25°C (P < 0.01). To examine possible differences in motor performance that may have been caused by the different water temperatures, the swimming speed of the different groups was compared. No significant differences were found between swimming speeds of groups trained at different temperatures (F(2,119) = 0.12, n.s.; Supplemental Fig. S2). It is important to note that the different patterns of performance observed at the different water temperatures were already observed on the first training day, but not on the first training trial, in which no significant differences were found among the different groups (Supplemental Fig. S3). However, on the last trial (Trial 4) of day 1, animals trained at 19°C performed significantly better than animals trained at either 16°C or 25°C (Supplemental Fig. S3).

Evaluation of motivational factors in animals trained at different water temperatures

A critical issue was whether the differences in path length observed in rats trained at different water temperatures were due to differential motivation to escape from the water (i.e., to find the platform) or to genuine differences in spatial learning capabilities. To address this issue, a similar experiment was carried out on a new set of animals (n = 9; temperature group), although here the platform was visible (not submerged) and cued. A repeated-measures ANOVA indicated an effect of training days (F(2,48) = 5, P < 0.05), confirming that rats progressively reached the platform after shorter navigation distances. In contrast, there was no effect for water temperature (F(2,24) = 1.5, n.s.) and no interaction between the two factors (F(4,48) = 0.37, n.s.; Fig. 2D). Similar findings were obtained when data from the three training days were averaged, yielding no differences in the number of errors to find a cued platform among groups of rats trained at different temperatures (F(2,24) = 1.5, n.s.; Fig. 2E).

Learning in the RAWM at different water temperatures

Rats were trained in the spatial version of the RAWM under the three selected water temperatures (16°C, n = 35; 19°C, n = 50; or 25°C, n = 37) over three consecutive days (Fig. 2A). A repeated-measures ANOVA on the arm entry errors revealed an effect of training days (F(2,28) = 26.78, P < 0.0001), confirming that animals progressively learned the spatial location of the platform across the training sessions. ANOVA also revealed an effect of water temperature (F(2,119) = 12.38, P < 0.0001). This effect was further confirmed when performance across the three training days was averaged (F(2,119) = 12.38, P < 0.0001; Fig. 2B). Interestingly, post-hoc analyses indicated the existence of an inverted-U-shaped function, with animals trained at 19°C making less errors to find the platform than those trained at either 16°C (P < 0.0001) or 25°C (P < 0.0001). No difference in performance was found between rats trained at either 16°C or 25°C water temperature. A repeated-measures ANOVA on data represented in Figure 2A yielded no interaction between water temperature and training day (F(4,228) = 0.74, n.s.). Separate ANOVAs for each training day indicated that on each training day, rats trained at 19°C outperformed those trained at either 16°C or 25°C (P < 0.01). To examine possible differences in motor performance that may have been caused by the different water temperatures, the swimming speed of the different groups was compared. No significant differences were found between swimming speeds of groups trained at different temperatures (F(2,119) = 0.12, n.s.; Supplemental Fig. S2). It is important to note that the different patterns of performance observed at the different water temperatures were already observed on the first training day, but not on the first training trial, in which no significant differences were found among the different groups (Supplemental Fig. S3). However, on the last trial (Trial 4) of day 1, animals trained at 19°C performed significantly better than animals trained at either 16°C or 25°C (Supplemental Fig. S3).
Figure 2. Effect of water temperature on performance, as indicated by the number of arm entry errors, in the RAWM during spatial learning (A–C) or cued training (D–F). (A) Spatial learning: When compared with the 19°C group, rats trained at either 16°C or 25°C showed delayed acquisition (**), $P < 0.01$ vs. 19°C for 16°C and 25°C in each training day. (B) Average error values collapsed for performance across the three training days. (***) $P < 0.001$ vs. 16°C and 25°C. (C) Number of errors incurred on the first training trial of day 2. (*) $P < 0.05$ vs. 16°C and 25°C. (D) Cued training: No differences were found in reaching the cued platform for groups trained at different water temperatures. (E) Average arm entry errors made to reach the platform collapsed over the three cued training days reveals no differences among groups. (F) No differences were found in performance on the first trial of the second cued training day. Data are the mean ± SEM.

Evaluation of long-term retention

Long-term retention was tested both during and after the initial spatial training by applying different testing opportunities. First, we analyzed data from the first trial of the second training day, because this trial can be considered the earliest long-term memory test (Fig. 2C) (note that this was not a probe trial, but a regular training trial). An ANOVA indicated an effect of water temperature ($F_{(2,119)} = 3.3$, $P < 0.05$), and post-hoc analyses confirmed that the data followed a U-shape function, with animals trained at 19°C making a significantly lower number of arm entry errors to find the platform than those trained at either 25°C or 16°C ($P < 0.05$). When the same analysis was performed on data from the cued platform version, no differences in performance were found among animals trained at different temperatures on the first trial of day 2 ($F_{(2,24)} = 0.65$, n.s.; Fig. 2F).

On day 8 (i.e., 5 d after the last training session), rats were administered a probe test in which the platform was removed and the water temperature was kept equal (20°C ± 0.5°C) for all groups. Different parameters were analyzed for the duration of the probe trial (60 sec) and for the first and second half of the test trial. No significant differences were found in the arm entry errors to reach the virtual platform among animals that had been trained at the different water temperatures ($F_{(2,119)} = 2.06$, $P = 0.13$; Supplemental Fig. S4). However, an interesting finding arose when animals’ strategies (i.e., whether they spent more time in the error arms, the target arm, or the center of the pool) were evaluated in a time-dependent fashion (i.e., the first and last 30 sec of the probe trial analyzed separately; Fig. 3). An ANOVA on the amount of time spent in the target arm over the first 30 sec indicated a significant difference among the groups trained at 19°C, showing performance significantly superior to animals trained at 25°C during the first 30 sec.

The impact of increasing psychogenic stress: Reversal learning

After a further training day (day 9; no differences in performance were found among the groups; data not shown), on which animals were retrained as on days 1–3, all groups were submitted to a reversal learning session on day 10 using water temperatures that matched temperatures from previous training days (16°C, 19°C, or 25°C) (Fig. 4). An ANOVA on the arm entry errors from trials 2–4 (the first trial was novel to all animals and not indicative of learning processes, so it was excluded from analyses) indicated a significant effect of temperature ($F_{(2,119)} = 3.55$, $P < 0.05$), and post-hoc analyses indicated that animals trained at 16°C made significantly more errors to reach the platform than those trained at either 19°C ($P = 0.05$) or 25°C ($P < 0.05$).

Individual differences and the inverted-U shape

One of the goals set for this study was to evaluate whether animals showing different behavioral profiles differ in how stress affects their performance during learning and memory tests. Data from principal component analyses performed on tests administered for behavioral characterization were used to classify animals into dichotomized variables for each behavioral trait (i.e., locomotion, anxiety, and exploration) (see Materials and Methods for details on the principal component analyses performed and the extracted factors, and Supplemental Tables S1–S3). Thus, animals were classified according to whether their score

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was above or below the mean for each factor into groups of low (LL) or high (HL) locomotion, low (LA) or high (HA) anxiety, and low (LE) or high (HE) exploration. Mean comparisons on the scores for each variable confirmed that the dichotomized groups representing high and low scores for each trait differed significantly (all Student t-tests; P < 0.01; Supplemental Fig. S5). RAWM performance of these groups was then compared using parametric analyses on the learning and memory data.

A factorial ANOVA, with temperature and the three behavioral traits extracted from the principal component analyses as the factors, performed on data from the first three RAWM training days, revealed a lack of significant interaction (n.s.). There was also no effect of each personality factor (n.s.). Further factorial ANOVAs performed on combinations of two behavioral traits and water temperature as factors did not yield statistical significance, except when anxiety and exploration were combined (Fig. S2, 110) = 4.64, P < 0.05). Next, simple main effect analyses were performed to evaluate the impact of water temperature on RAWM learning for each behavioral profile resulting from the dichotomized groups of anxiety and exploration (HA-HE, LA-LE; Fig. 5). These analyses confirmed a U-shape relationship for the HA-HE profile, with animals trained at 19°C performing significantly better than those trained at 16°C (P < 0.05) or 25°C (P < 0.01). Among both the LA-HE and the HA-LE profiles, the group trained at 16°C performed significantly worse than the 19°C group (P < 0.01), whereas performance at 25°C did not differ significantly from either of the other groups (n.s.). Interestingly, a different pattern was observed for the LA-LE profile; animals trained at 25°C performed significantly worse than those trained at 19°C (P < 0.01) or 16°C (P < 0.05). Furthermore, an analysis of performance at each water temperature for the different personality groups revealed an effect of personality profiles at 25°C, with superior performance being observed for the HA-LE and LA-HE groups relative to the HA-HE and LA-LE groups (Supplemental Fig. S6).

**Discussion**

We report here, for the first time, the existence of an inverted-U-shape relationship between intrinsic stress intensity and performance in a hippocampus-dependent learning task, the RAWM. Various stress intensities were achieved using water temperatures of 25°C, 19°C, and 16°C to elicit increasing plasma corticosterone levels. By submitting rats to different training and testing protocols, we confirmed the existence of an inverted-U-shape function for performance at training; animals trained at 19°C made less errors to find the platform than animals trained at either higher (16°C) or lower (25°C) stress conditions. However, a long-term memory (probe) test performed 1 wk after training under equal temperature conditions (20°C ± 0.5°C) revealed a different performance pattern (Fig. 3). Although the groups did not significantly differ in the number of errors made before reaching the virtual platform, analysis of the behavioral profile displayed during the test revealed that rats trained at 25°C were less accurate in platform searching than rats trained at either 16°C or 19°C, while no difference in searching was found for the latter two groups. When a cognitive challenge was subsequently introduced by changing the platform location (reversal learning), the groups trained at both 19°C and 25°C showed better performance than rats trained at 16°C, suggesting that cognitive difficulty affects the cognitive impact of "physical" stress. Furthermore, we presented evidence supporting the view that stress does not affect spatial learning and memory uniformly in all individuals. Rather, performance at either the high- or the low-stress levels is differentially affected in individuals with different personality-like profiles.

Previous studies using the Morris water maze (Morris 1984) showed that rats trained at 19°C perform better than rats trained at 25°C, and corticosterone levels after the first training session were higher in rats trained at the colder water temperature (Sandi et al. 1997; Akirav et al. 2004). A similar association among water temperature, learning and memory rate, and post-training corticosterone levels was also recently described in mice (Conboy and Sandi 2010). Selden et al. (1990) presented evidence for impaired training at lower water temperatures (12°C) in rats, and they implicated corticosterone-cortical noradrenergic projections in the impairing effects of high stress on spatial learning. Here, we confirm the ascending portion of the U-shaped curve for the RAWM acquisition phase at 25°C (low stress) and 19°C (optimal stress) water temperature. Moreover, we show evidence supporting the existence of the descending portion of the U-shaped curve in animals trained at 16°C (high stress, or physical conditions leading to highest corticosterone levels) under otherwise identical experimental conditions. Importantly, differences in performance between animals trained at different temperatures seem to be related to the spatial learning nature of the RAWM task, previously reported to be hippocampus dependent (Diamond et al. 1999), since no differences were found when animals were trained in the nonhippocampus-dependent cued platform version. These results are in agreement with pioneering observations by Wever at 19°.
(1932), who observed no differences in latency to escape from a nonspatial water task in rats trained at temperatures ranging from 10°C to 25°C. Therefore, we show evidence for the existence of an inverted-U-shape function between stressor intensity and performance, specifically for spatial learning and in the memory test administered 24 h after the first training session.

While this curvilinear function was not captured in earlier work using intrinsic stress approaches, studies involving manipulations of the noradrenergic (Introini-Collison et al. 1994) and glucocorticoid (Lupien and McEwen 1997; Conrad 2005; Joëls 2006) systems have successfully substantiated the inverted-U-shape relationship between increasing glucocorticoid levels/function and both learning and synaptic plasticity. Glucocorticoids are adrenal hormones released into the bloodstream that, due to their lipophilic nature, can enter the brain, where they can influence brain function and cognition through genomic and nongenomic effects (de Kloet et al. 1999, 2005). Glucocorticoid receptors [GR] (mineralocorticoid receptors [MR]) are expressed in different brain areas, including regions that are central to learning and memory formation (e.g., hippocampus, amygdala, and prefrontal cortex) (Sandi 1998; de Kloet et al. 1999). In chicks (Sandi and Rose 1997), ground squirrels (Mateo 2008), rats (Roozenendaal et al. 1999; Okada et al. 2004), and humans (Andræano and Cahill 2006), either very low or high glucocorticoid levels were reported to be associated with poor performance in a variety of learning and memory tasks (Park et al. 2006). Similarly, the magnitude of hippocampal primed burst potentiation (i.e., a physiological type of synaptic plasticity) was shown to follow an inverted-U function relative to serum corticosterone levels (manipulated through adrenalectomy and different corticosterone concentrations delivered through subcutaneous pellets) (Diamond et al. 1992). In agreement with this finding, opposite effects were described for activation of MR and GR in hippocampal long-term potentiation (Pavlides et al. 1995, 1996) and in a spatial learning task in rats (Conrad et al. 1997), with activation of the MR exerting facilitatory and GR exerting inhibitory effects. However, despite this congruent evidence supporting a U-shape relationship between glucocorticoids and cognitive function, the full story is likely more complex than presented here. For example, cognitive effects depend on many factors, such as surrounding context (de Kloet et al. 1999; Joëls et al. 2006), memory phase (Roozenendaal 2003), sex (Conrad et al. 2004; Andræano and Cahill 2006), estrus cycle in females (Andræano et al. 2008), previous experience, and emotional state (de Quervain 2008; de Quervain et al. 2009).

A key question arising from the observed differences in performance of learning and memory tasks when animals were trained under different stress levels is whether these differences translate into differences in the strength of the long-term memory developed. To address this question, all groups were administered a probe test under equal temperature conditions. Strikingly, despite the inferior performance shown during training by the 16°C-trained group (i.e., the high-stress group), their behavioral pattern during the long-term probe test was very similar to that of the 19°C-trained group, which performed optimally during training. However, the group that was trained at 25°C (i.e., the low-stress group) showed during the first 30 sec the most erratic search patterns of the three groups, being the group that spent the most time in the error arms and the least time in the target arm. These data suggest that the 16°C-trained rats formed a stronger memory for the platform location than the 25°C-trained rats. The contribution of state-dependent mechanisms in this latter group cannot be discarded, since this group was the only one that was tested in the probe trial at a lower temperature than in previous sessions, and this difference might have produced an additional stress contributing to the impairing effect during this testing session. These findings also suggest that the deficits observed during the training phase in the 16°C group were probably due not only to impaired learning but also to impaired
performance, particularly toward the final phase of training. This possibility would agree with a proposal by de Kloet et al. (1999) and [ööl et al. (2006), both of whom suggested that when individuals are confronted with high stress levels, their strategy switches from an information-processing mode to a more opportune response that is adapted to the actual condition. More specifically, animals tested at a lower temperature in the probe trial may have changed strategies to conserve energy at the expense of navigation. Although this interpretation is plausible, we were surprised to find no differences in the speed at which the different water temperature groups swam and, hence, find no evidence for a change in a metabolic-related behavioral strategy. Furthermore, we did not find differences among groups in body temperature following training at different temperatures. Moreover, the fact that no differences were observed in the cued platform version of the task further suggests that the training deficits found in the 16°C and 25°C groups were, at least in part, related to the spatial orientation learning nature of the task and not to nonspecific effects related, for example, to swimming ability, hypothermia, or tracking down the relevant cues. Importantly, these results also strongly support the current view that stress (and glucocorticoids) facilitates memory consolidation (Oitzl and de Kloet 1992; Sandi and Rose 1994; Sandi 1998; Roozendaal et al. 1999, 2008; Sandi and Pinelo-Nava 2007; de Quervain et al. 2009).

We also found that in a subsequent training session using temperatures matching initial training, all groups achieved similar performance levels, suggesting that the observed inverted-U-shape relationship might be related to the initial stages of learning acquisition. With overtraining, initial differences due to variations in stress level seem to disappear. Then, when a new cognitive challenge was introduced (i.e., change of platform location in the reversal learning session), the 16°C group became impaired not only relative to the 19°C group, but also relative to the 25°C group, and performance of the 25°C group resembled the 19°C optimally performing group. This result is in agreement with studies testing the prediction from the Yerkes-Dodson law that the optimal stress or arousal state decreases with increasing task difficulty (Mendl 1999). Therefore, the increase in task difficulty produced by the platform change would have extended the level of optimal stress for this type of learning from 19°C to 25°C (Hancock and Ganey 2003).

Finally, we examined whether all individuals equally displayed the U-shape effects that we observed during training and the 24-h memory test. Previously, we reported that certain behavioral traits, such as anxiety, render subjects more sensitive to the behavioral and neurobiological effects of stress (Jakobsson et al. 2006; Sandi et al. 2008; Lukys et al. 2009) and influence spatial learning abilities (Herrero et al. 2006). Here, we considered whether more than one personality trait could contribute to differential performance under stress. To do so, we first extracted personality traits by applying principal component analyses to a series of behavioral tests for spontaneous behavior of rats. While the factor “locomotion” did not contribute to defining individuals with different responsiveness, the combination of the factors “anxiety” and “exploration” resulted in a meaningful interaction, yielding four personality-like profiles (HA-HE, HA-LE, LA-HE, and LA-LE). Animals falling into each of these different profiles showed different patterns of “learning under stress.” Highly anxious and highly explorative animals (HA-HE) were the only animals whose learning under different stress levels exhibited the U-shape function. Interestingly, among the remaining three profiles, two (HA-LE and LA-LE) showed optimal performance in the low-stress condition (25°C water) and impaired performance in the high-stress condition (16°C water), but one profile (LA-LE) showed the opposite pattern (i.e., optimal performance in the high-stress condition and impaired performance in the low-stress condition). These opposite response patterns to the different stress levels explain why an inverted-U shape is observed at the population level. In addition, these findings raise many interesting questions and, given the lack of similar studies in humans, raise the interest of addressing similar questions in humans. One interesting implication to extract from this study is that different personality types may be differentially affected in their cognitive functioning under varying stress levels. Therefore, the precise shape of the inverted-U-shape curve may vary for different personality types from a narrow bell revealing that performance is maximal only within a limited range of stimulus intensities (as observed in the HA-HE group) to curves that show maximal performance at either low (LA-HE, HA-LE) or high (LA-LE) stress levels (Supplemental Fig. S7). Interestingly, a physiological treatment that results in decreased anxiety to novelty (Vataeva et al. 2001) was found to improve learning in the water maze at a temperature of 16°C–17°C, whereas performance was impaired at 23°C–24°C (Vataeva et al. 2005). Accordingly, our study supports the conclusion that stress effects on hippocampus-dependent learning tasks vary for different personality profiles. Furthermore, our findings provide an attractive behavioral model to characterize the neurobiological mechanisms involved in the differential impact of stress levels in cognitive performance as well as the intrinsic interactions among personality, stress, and cognitive processes.

Materials and Methods

Subjects

Adult male Wistar rats (Charles River Laboratories, Lyon, France), weighing 200–225 g at the beginning of the experiments, were housed in groups of three per cage. They were maintained under light (12 h light/dark cycle; lights on at 7:00 am) and temperature (22°C ± 2°C)-controlled conditions. Food and water were available ad libitum. Animal care procedures were approved through a license issued by the Cantonal Veterinary Authorities (Vaud, Switzerland).

General procedure

All experiments were conducted between 9:00 and 14:00 h. Approximately 2 wk after arrival, each rat was handled for 3 d, 2 min per day, just before the behavioral characterization started. The behavioral characterization included, first testing in the elevated plus maze, and 4 d afterward testing in the open field and novel object reactivity test. One week afterward, animals were distributed into three groups that were balanced for behavioral traits and body weights, and each group was submitted to training in the RAWM at a different water temperature (16°C, 19°C, or 25°C). Water-maze training was performed using either a cued-platform version or a spatial learning version. In all behavioral tests, the behavior of each rat was monitored using a video camera located on the ceiling, and movements of the rats were automatically registered and analyzed with a computerized tracking system (Ethovision 3.1.16, Noldus IT).

Behavioral characterization

Elevated plus maze (EPM)

The first behavioral test was the elevated plus-maze (Herrero et al. 2006), which is widely used to evaluate animals’ anxiety-related behaviors. The elevated plus maze consists of two opposing open arms (45 × 10 cm) and two closed arms (45 × 10 × 50 cm) that extend from a central platform (10 × 10 cm), elevated 65 cm above the floor. The rats were placed individually on the central platform, always facing the same enclosed arm, and were allowed to freely explore the maze for 5 min. Different parameters were evaluated with the video tracking system: total distance moved (centimeters), distance moved (centimeters), and time spent (seconds) in the open and closed arms, and number of times
the animal entered each type of arm. The floor of the apparatus was washed after each testing with 1% acetic acid solution to remove odors left by previous subjects.

**Open field (OF) and novel object reactivity (NOR) tests**

Animals’ behavior was also assessed in the open field test (OF), which involves placing the animals in a circular open arena (100-cm diameter, 32-cm high). For analysis, the floor was divided into three virtual concentric parts, with a center zone in the middle of the arena (20-cm diameter), an interior zone (60-cm diameter), and an exterior zone made up of the remaining area along the sidewalls. At the start of the test, animals were placed in the center of the arena, and their behavior monitored for 10 min using a video camera mounted on the ceiling above the center of the arena. Different parameters were evaluated with the video tracking system: distance moved (centimeter) and time spent (seconds) in each zone.

Immediately after the open field test, rats were submitted to the novel object reactivity (NOR) test. For this purpose, a small, white plastic bottle (3 × 1.5 × 5 cm) was placed into the center of the open field while the rat was inside. Rats were then given 5 min to freely explore the novel object. Different parameters were evaluated with the video tracking system: time spent (seconds) in the center and the periphery of the compartment, number and latency of entries to the center, total distance moved (centimeters) in the center and in the whole compartment. The time spent exploring (touching) the novel object and the freezing time were recorded manually from the video recordings (Jakobsson et al. 2008).

**RAWM**

The apparatus used for testing spatial memory was a round black Plexiglas tank that was filled with clear water. The tank had a diameter of 170 cm and a height of 45 cm. Within the tank were Plexiglas walls that extended from the floor to a height of 43 cm and had a length of 60 cm. The walls were positioned to produce six swim paths radiating out of an open central area. A black metal platform (11-cm diameter) positioned 1.5 cm below the surface of the water was located at the end of one of the swim paths (arms), and the platform edge was ~8 cm from the tank wall. When the rats swam to the end of this arm (referred to as the “target” arm) they could climb onto the platform to get out of the water. The tank was located in the middle of a well-lit testing room. Visual cues were attached to the walls adjacent to the tank. All animals from each home cage were tested under the same water temperature conditions. They were taken individually from the adjacent housing room and directly tested in the water maze.

The following parameters were evaluated with the video tracking system: latency (seconds) to find the platform, distance (centimeters) traveled to find the platform, and swim speed. The number of arm entry errors was determined according to the criteria established by Diamond et al. (1999) as the number of arm entries that did not result in the rat finding the escape platform. An arm entry was defined as a rat having all four paws extended out of the center area into an arm. An error was committed if a rat entered an arm that did not contain the platform or if a rat entered the correct arm but did not find the platform. Since we observed a very high correlation for the parameters “distance” to find the platform and “arm entry errors” (r = 0.92, p < 0.001) and confirmed that the data for “distance” and “latency” gave similar results to analyses on “arm entry errors,” we only present the data corresponding to the “arm entry errors” parameter.

Animals were trained in either a cued or a spatial version of the RAWM. In the cued version, the platform is elevated slightly over the surface of the water and signaled with a 10-cm flag. This version is not sensitive to hippocampal lesions, so it is considered a hippocampus-independent task. Performance in the spatial version (in which the escape platform is hidden) is sensitive to hippocampal damage, so it is considered a hippocampus-dependent task. In each of these tasks, three different groups of animals were trained at different water temperatures that were selected with the goal of representing different stressor intensities (i.e., low, moderate, high stress). The temperatures of 19°C and 25°C were previously shown to be appropriate temperatures to elicit different stress levels in rats. The temperature of 16°C was added to enhance stressor intensity. Two independent experiments were performed with the cued version paradigm, one designed to evaluate plasma corticosterone levels immediately after the first training session at the different water temperatures (n = 6/group), and a second one to evaluate motivational factors and performance over consecutive days (n = 9/group). As to the spatial version protocol, a higher number of animals per group than in conventional studies was required to perform analyses, taking into account the combination of personality traits (final number of animals per group: 16°C, n = 35; 19°C, n = 50; 25°C, n = 37). A total of five replication experiments were performed, three of them including all water temperatures (n = 8–9/group for each replication) and two of them involving two water temperature conditions (19°C in both cases and either 16°C or 25°C in each of the replications; n = 12/group for each replication). In each replication experiment, all water-temperature groups were included and sequentially tested on the same days in a single RAWM. The order at which the different groups were run on each training day was counterbalanced both for different training days within each replication experiment and for each of the training days across the different replication experiments following a semirandom schedule. Every day, water temperature was easily changed by either adding hot water (available through the tap water) or ice obtained from an ice-making machine located in the animal facilities.

The acquisition phase of the spatial task consisted of a block of four trials per day run on each of three consecutive days. Four different starting arms were equally chosen around the perimeter of the pool. On each day, all four start positions were used once in a random sequence that was held constant for all rats. A trial began by placing the rat into the water facing the center of the pool at one of the starting points. If the animal failed to escape within 90 sec, it was manually guided to the platform. The animal was allowed to remain on the platform for 15 sec and was then placed into a holding cage under a warming lamp for 30 sec until the start of the next trial. After a rest period, on day 8 rats received a 60-sec free-swim period, during which the platform was removed from the maze (probe trial). At the end of the probe trial, the platform was reinserted into the pool and rats remained on it for 15 sec. On day 9, rats were retrained with another four-trial training session under the same conditions as described above. One day later (day 10), a reversal learning session was conducted, in which the platform position was changed to a different arm. Similar to the first 3 of the experiment, the reversal session included four trials. After the last trial on each day, the rats were carefully dried with a towel and placed in the heated waiting cage for 10 min. Rats were then returned to their home cages.

**Corticosterone analysis**

Trunk blood was collected by decapitation 45 min after the beginning of a 1-d cued training session. The experiment was performed between 10 am and 1 pm. Samples were centrifuged (4000 rpm for 20 min at 4°C), and the serum was extracted and stored at −20°C. Corticosterone levels were assayed by ELISA (Assay Design) according to the manufacturer’s instructions.

**Statistical analyses**

Results are expressed as mean ± SEM. The SPSS 13.0 statistical package was used for the statistical analyses.

**Parametric statistics**

Mean comparisons were carried out with either one-way or factorial ANOVAs; simple main effects analyses or post-hoc comparisons were made with LSD tests when appropriate. Normality and homogeneity of variance was tested, and adjusted statistics
were used if required. Unless otherwise indicated, analyses of behavioral parameters excluded the first training trial (when no learning has as yet taken place).

Principal component analyses (PCA)
PCAs were applied to characterize animals according to their behaviors from the EPM, OF, and NOR tests. For the factorial analysis, which was twofold, the number of extracted factors was not predefined. Rather, PCAs were applied separately to a range of extracted parameters from the EPM (Supplemental Table 1) and the OF-NOR tests (Supplemental Table 2). Then, an overall principal component analysis was performed on the extracted factors, which revealed three factors that were termed “locomotion,” “anxiety,” and “exploration” according to the parameters that defined them (Supplemental Table 3). A continuous, interval scale score was calculated for each factor using principal components as the extraction method and varimax rotation with Kaiser normalization rotation. Then, individual factor scores were calculated for each subject through the relative weight and orientation (eigen values) of the parameters for each factor. Scores were generated using a Z distribution, where the value 0 corresponds to the mean, and values are expressed in terms of standard deviations. Animals were matched for their scores in the different factors and classified into the different experimental groups to yield groups with similar personality traits. In addition, data from the factorial analyses were used to investigate the modulatory effect of factor score differences on the learning measures investigated. For this study, animals were classified into groups such that scores laid either above or below the mean for each of the factors.

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