Modulation of Hippocampal NCAM Polysialylation and Spatial Memory Consolidation by Fear Conditioning

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Background: *Cell adhesion molecule function is involved in hippocampal synaptic plasticity and associated with memory consolidation. At the infragranular zone of the dentate gyrus, neurons expressing the polysialylated form of the neural cell adhesion molecule (NCAM PSA) transiently increase their frequency 12 hours after training in different tasks.*

Methods: *Using immunohistochemical procedures, we investigated NCAM polysialylation following training in a contextual fear conditioning paradigm that employed increasing shock intensities to separately model stressful and traumatic experiences in adult male Wistar rats.*

Results: *Fear conditioning with a stressful .4-mA stimulus resulted in an increased frequency of dentate polysialylated neurons, the magnitude of which was indistinguishable from that observed following water maze training. By contrast, training with a traumatic 1-mA stimulus resulted in a significant decrease in the frequency of polysialylated neurons at the 12 hours posttraining time. Whereas sequential training in the water maze paradigm followed by fear conditioning resulted in potentiated consolidation of spatial information when conditioning involved a .4-mA stimulus, amnesia for spatial learning occurred when conditioning was performed with a 1-mA stimulus.*

Conclusions: *These results suggest traumatic fear conditioning suppresses NCAM-PSA–mediated plasticity and the concomitant inability to store the trace of recently acquired information.* Biol Psychiatry 2003;54: 599–607 *© 2003 Society of Biological Psychiatry*

Key Words: Hippocampus, contextual fear conditioning, rat, emotional memory, traumatic memory, cell adhesion molecules

Introduction

The hippocampus, along with associated cortical structures in the temporal lobe, has been implicated in holding and processing information destined for consolidation as long-term memory within the neocortex (Alvarez and Squire 1994); however, the influence of the amygdala over hippocampal function is essential in registering the emotive content of information and appropriately modulating its storage (Cahill 1997; McGaugh 2000; Schafe et al 2001; Kim and Diamond 2002). Excessive activity of this normally adaptive system may underlie aberrations in memory formation observed in situations of extreme emotional stress. Such aberrations present in two distinct, and possibly related, forms of memory: recall of vivid and overwhelming pathopsychological memory such as that seen in posttraumatic stress disorder (PTSD) (Charney et al 1995; Cahill 1997) and trauma-induced amnesia (Krystal et al 1995; Brewin and Andrews 1998; Richter-Levin 1998). Understanding the influence of excessive stress on hippocampal memory processing systems may be a key factor in elucidating the molecular mechanisms of trauma-induced memory disorders.

Considerable evidence now suggests consolidation of long-term declarative memory to involve change in hippocampal neuronal connectivity pattern (Andersen and Soleng 1998; Bailey et al 2000; Martin et al 2000). As part of a molecular cascade underlying such connectivity change, cell adhesion molecules (CAMs) are proposed to play a key role in a number of critical synaptic functions, including cell-cell adhesion and the activation of intracellular signal transduction cascades (Murase and Schuman 1999; Benson et al 2000). In particular, the neural CAM (NCAM) is required for the establishment of enduring memories, as antibodies to this molecule, injected during the posttraining period, interfere with subsequent retrieval of previously acquired information (Doyle et al 1992; Scholey et al 1993; Roullet et al 1997). Moreover, NCAM has been implicated in the maintenance of long-term potentiation (LTP), a cellular model of learning and

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memory (Lüthi et al 1994; Rønn et al 1995; Muller et al 1996), and in the consolidation of fear conditioning (Merino et al 2000b).

In addition, NCAM can exhibit a posttranslational modification that is also implicated in the neural mechanisms involved in long-term memory consolidation. This consists of the addition of extended chains of α2,8-linked polysialic acid (PSA) homopolymers, a modification that has been shown to be required for activity-dependent synaptic remodeling (Nothias et al 1997; Hoyk et al 2001). In particular, PSA is believed to decrease NCAM homophilic and heterophilic interactions (Sadoul et al 1983; Rougon 1993), a mechanism proposed to be important for the final elimination and/or selection of synapses transiently overproduced during the formation of the memory trace (Murphy and Regan 1998). Consistent with this view is that removal of PSA with endoneuraminidase N has been found to interfere with the induction and maintenance of hippocampal LTP and to produce spatial learning deficits in the Morris water maze paradigm (Becker et al 1996; Muller et al 1996). Moreover, the frequency of NCAM polysialylated hippocampal neurons at the dentate infragranular border transiently increases at 10 to 12 hours following acquisition of either spatial or conditioned avoidance tasks (Doyle et al 1992; Fox et al 1995; Murphy et al 1996). These frequency changes are learning specific, as they are not observed in animals rendered amnesic with either scopolamine or propofol (Doyle and Regan 1993; O'Gorman et al 1998). In addition, NCAM polysialylation in the hippocampus has been shown to be modulated by stress hormones (i.e., glucocorticoids) (Rodriguez et al 1998).

The aim of this study was to investigate NCAM polysialylation mediated synaptic reorganization in the dentate gyrus following a stressful learning task that models traumatic experience. For this purpose, we employed the hippocampal-dependent contextual fear conditioning paradigm (Kim and Fanselow 1992; Phillips and LeDoux 1992). In this task, rats developed characteristic immobility, or "freezing" response, when re-exposed to the context in which they had previously experienced brief, inescapable shocks. Three experimental situations were included involving weak (.2 mA), moderate (.4 mA), or high (1 mA) shock intensities at training, as the shock intensity at which rats are trained shows a positive correlation with both the extent and duration of conditioned fear and posttraining corticosterone levels (Cordero et al 1998). Moreover, animals trained at the high-shock intensity (1 mA) seem to exhibit some of the characteristics seen with posttraumatic stress disorder (PTSD) in humans (Yehuda and Antelman 1993; Cordero et al 2002). Part of this work has previously been published in abstract form (Merino et al 2000b).

Methods and Materials

Animals

Male Wistar rats (Harlan Iberica, Spain), weighing 150 to 175 g on arrival, were housed in groups of three per cage, under controlled conditions of temperature (22 ± 2°C) and light (12:12 light-dark cycle; lights on at 7:00 AM), and had free access to food and water in a colony room. At age 11 weeks, they were handled daily for 3 days for habituation to the experimental manipulation. Experiments started following the third day of handling, when rats were postnatal day 80. Behavioral experiments were always conducted between 9:30 AM and 2:30 PM, except for those experiments including a retrieval test at 12 hours posttraining, in which testing was carried out between 8:30 PM and 12:00 AM. Animal care procedures were conducted in accordance with the guidelines set by the European Community Council Directives (86/609/EEC).

Contextual Fear Conditioning

Training and testing took place in a rodent observation cage (30 × 37 × 25 cm) that was positioned inside a sound-attenuating chamber. The side walls of the observation cage were constructed of stainless steel, and the back walls and doors were constructed of clear Plexiglas. The floor consisted of 20 steel rods through which a scrambled shock from a LETICA I.C. shock generator (Model LI100–26, Spain) could be delivered. Each observation cage was cleaned with a 1% acetic acid solution before and after each session. The sound-attenuating chambers were illuminated with a 20-W white light bulb. Ventilation fans provided background noise at 68 dB.

On the training day, each rat was transported from the colony room to the laboratory (situated in an adjacent room) and placed into the conditioning chamber. After 3 minutes, the rats received three 1-second shocks (unconditioned stimuli) of varying intensity (.2, .4, or 1 mA) depending on the experimental group to which they had been assigned. The intershock interval was 60 seconds, and the rats were removed from the conditioning chambers 30 seconds after presentation of the final shock and returned to their home cages. Thus, a conditioning session lasted approximately 330 seconds. An additional group received the same experimental manipulation but did not receive any shock. In experiments designed to assess learning-induced modulation of NCAM PSA in dentate gyrus, testing for contextual fear conditioning was performed 12 hours after conditioning to assess for the retention of the behavioral response under study. At testing, rats were placed back into the same chamber as used in conditioning, in the absence of shock, for an 8-minute context test. In addition, a further experiment involving the same experimental groups was performed in which the rats were sacrificed immediately after the conditioning session to assess their corticosterone levels after exposure to the different training conditions. The reason to study hormone levels at this time point is that corticosterone is normally elevated up to 60 to 90 minutes after exposure to stress, and therefore, it is during the immediate posttraining period when it is relevant to study the possible differential activation of this steroid in relation to the various training conditions.

A video camera was used to record the behavior of rats both during training and testing. Subsequently, the time spent by each rat, either freezing or active, was scored blind assisted by a computer program (Ethovision 1.9, Noldus IT, The Netherlands). *Freezing* was defined as behavioral immobility except for movement needed for respiration. Behavior was evaluated in each experimental session. At training, behavioral scores were carried out for the 3-minute period before shock (preshock period) and for the 2.5-minute period starting immediately after presentation of the first shock (postshock period). Scores for each of these periods were analyzed separately. At the testing session, behavior was scored during the 8-minute re-exposure to the training context. In experiments involving immunohistochemical analyses, 9 to 10 minutes after the start of the testing session, the rats were decapitated.

Water Maze Learning

The Morris water maze was a black circular pool (2-m diameter, 45 cm high) filled with water (30-cm depth) at 25°C. The pool was divided into four quadrants of equal size. An invisible escape platform (11-cm diameter) was placed in the middle of one of the quadrants (1.5 cm below the water surface) equidistant from the side wall and middle of the pool. The behavior of the animal (latency, distance, and swim speed) was monitored by a video camera mounted in the ceiling above the center of the pool and a computerized tracking system.

Four different starting positions were equally spaced around the perimeter of the pool. The first training session always consisted of either four or eight training trials, which were started from one of the four start positions in the same random sequence for each rat. A trial began by placing the rat into the water facing the wall of the pool at one of the starting points. If the rat failed to escape within 120 seconds, it was guided to the platform by the experimenter. Once the rat reached the platform, it was allowed to stay there for 30 seconds and then placed in a holding cage for an intertrial period of 30 seconds. After the last trial, the rats were dried off by placing them in a waiting cage for 30 minutes in a room heated to 30°C. In this cage, there was always at least one companion rat to avoid any isolation stress. Subsequently, they were returned to their home cages.

Two experiments in this study included training rats in the water maze paradigm. Each of them involved a different number of training trials to accomplish the specific aim addressed in each experiment. The first experiment was designed to check whether the experimental conditions involved in the current study led to a comparable activation of dentate polysialylated granule neuron frequency at 12 hours posttraining, as has been described previously (Murphy et al 1996). For this purpose, rats were trained for eight consecutive trials (as described above) to ensure a high acquisition level in a single training session, and at 12 hours posttraining, they were tested for their retention of spatial orientation by giving them a 60-second free swim trial without a platform (probe test). Following the probe trial, they were decapitated.

The second experiment was designed to assess the possible influence of training in the fear conditioning paradigm on the processing of information recently acquired in the water maze task. In this experiment, we aimed to train rats to intermediate learning levels; therefore, they were submitted to a water maze training session of four trials and then dried for an 8-minute period. Immediately afterward, they were exposed to a contextual fear conditioning session, as described above, with shock intensity of either 0 mA, .4 mA, or 1 mA. Forty-eight hours later, the rats were tested in the water maze by first giving them a 60-second probe test, a free swim without platform with data analyzed separately for the first and last 30 seconds. Immediately afterward, four consecutive training trials (relearning) were conducted with the submerged platform placed in the same location as in the first training session. Therefore, in addition to the probe test, we also ran a relearning session, in which the first and last two trials were averaged for statistical analysis. On the following day, the rats were tested for their retention of fear conditioning by an 8-minute re-exposure to the conditioning chamber, as described above.

Determination of Plasma Corticosterone Levels

One experiment was performed to assess plasma corticosterone levels elicited by contextual fear conditioning at the different shock intensities, with rats being decapitated 9 to 10 minutes after the start of the testing session. Trunk blood was collected and the samples were centrifuged (3000 rpm for 20 minutes at 4°C). Plasma was stored at −35°C. Corticosterone was measured using a radioimmunoassay (RIA) kit according to the instructions supplied by the manufacturer (Coat-A-Count, Diagnostics Products Corporation, Los Angeles, CA). The intra-assay variability of the RIA ranged between 3.1% and 4.5%. Its sensitivity (minimal detectable dose) was approximately 5.7 ng/mL.

Quantification of Dentate Hippocampal Polysialylated Neurons

Immediately after decapitation, the brain was removed and immediately frozen in a dry ice cooled *n*-hexane. Subsequently, the brains were coded and stored at −80°C until required for further processing. PSA immunocytochemistry was used to detect hippocampal polysialylated neurons using techniques described previously (Fox et al 1995). In brief, cryostat-cut axial sections of 12 μm were fixed in 70% ethanol (vol/vol H_2O) and incubated overnight with anti-PSA ascitic fluid (generous gift of Professor G. Rougon) (Rougon et al 1986) diluted 1:500 with phosphate buffered saline (PBS). The sections were exposed for 3 hours to fluorescein-conjugated goat antimouse immunoglobulin M (IgM) (Calbiochem, Nottingham, UK) diluted 1:100 PBS and mounted in Citifluor (Agar, Stansted, UK), a fluorescence-enhancing medium. Nuclei were fluorescently counterstained by a brief (60 seconds) exposure to propidium iodide (40 ng/mL PBS) (Sigma Chemical, Gillingham, UK) to facilitate counting.

The total number of PSA-immunopositive neurons at the granule cell layer and hilus border was counted in 7 alternate 12-μm sections commencing −5.6 mm from bregma (Paxinos and Watson 1986), thereby precluding double counting of the 5- to 10-μm perikarya. Cell counts were divided by the total area of the granule cell layer and multiplied by the average granular cell layer area, which was .15 ± .01 mm^2 at this level. The mean ±

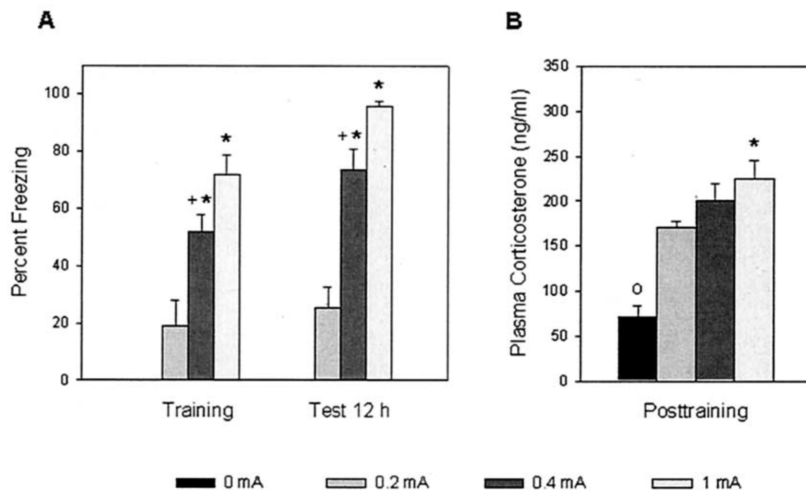

Figure 1. Percentage of time spent freezing at training (postshock period) and testing (12 hours posttraining) **(A)** and posttraining plasma corticosterone levels **(B)** following contextual fear conditioning using stimuli of increasing shock intensity. Results are the mean ± SEM from 9 to 10 animals per group. * $p < .05$ versus .2 mA group; + $p < .05$ versus 1 mA group; $^{\circ}p < .05$ versus each of the shocked groups.

SEM value was calculated, and the results were expressed as numbers of PSA-positive cells per unit area. Area measurements were performed using a Quantimet 500 Image Analysis System (Leica, Ashbourne, Ireland). Immunofluorescence was specific, as it was eliminated by omission of either the primary or secondary antibody; by preabsorbing anti-PSA with colominic acid (1 mg/mL), which contains α2,8-linked homopolymers of sialic acid; or by prior incubation of the sections with .3% endoneuraminidase-N (vol/vol PBS) (Fox et al 1995; Murphy et al 1996).

Statistics

The behavioral data regarding the time each rat spent freezing were transformed to a percentage freezing per session. All results were expressed as mean ± SEM and analyzed by analyses of variance (ANOVA) or Student *t* tests, as appropriate. Differences between shock intensities were further evaluated for significance with post hoc Tukey and Student *t* tests when appropriate. In all cases, $p < .05$ was accepted to indicate significant difference.

Results

Effects of Contextual Fear Conditioning on Freezing Values and Plasma Corticosterone

Rats develop a characteristic immobility, or a "freezing" response, when re-exposed to the context in which they have previously experienced brief, inescapable foot shock. Moreover, the extent and duration of this conditioned fear is positively correlated with shock intensity. As a consequence, the fear conditioning paradigm employed in these studies used a range of shock intensities to reproduce stressful (.4 mA) and traumatic (1 mA) experiences. No freezing was observed in the preshock period in any of the experimental groups employed (data not shown). In Figure 1A, it is clear that increasing shock intensity significantly intensifies the freezing response both during the training session (postshock period) and at the 12 hours posttraining test, as judged by ANOVA analysis [Training: $F(2,26) = 39.47$, $p < .0001$; Test 12 hours: $F(2,26) = 34.25$, $p < .0001$]. Moreover, post hoc Tukey analyses indicated a significantly different freezing response between the groups receiving shock intensities of .4 mA and 1 mA ($p < .05$).

Fear conditioning has also been shown to increase corticosterone levels (Cordero et al 1998). In an additional experiment, posttraining plasma corticosterone levels also showed a significant correspondence with increasing shock intensity, as judged by ANOVA analysis [$F(3,35) = 23.01$, $p < .0001$] (Figure 1B). All experimental groups showed higher corticosterone levels than the 0 mA control group (all $p < .05$). In addition, the 1 mA group displayed higher hormone levels than the .2 mA group ($p < .03$).

Effects of Contextual Fear Conditioning on the Frequency of PSA-Immunoreactive Neurons in the Hippocampal Dentate Gyrus

PSA immunohistochemical analysis revealed a distinct population of polysialylated neurons at the infragranular zone of the hippocampal dentate gyrus (Figure 2). Immunoreactivity was predominantly located on the cell surface and associated dendrites that extended through the granule cell layer and into the molecular layer, as has been described previously (Fox et al 1995). In addition, the mossy fibers of these presumptive granule cells were immunopositive.

The discrete staining pattern of these immunopositive dentate neurons facilitated quantitative evaluation of their frequency at 12 hours following contextual fear conditioning, a period when learning-associated increases in their prevalence have previously been observed following training in avoidance conditioning and spatial learning paradigms (Fox et al 1995; Murphy et al 1996). Analysis of variance of the data obtained subsequent to fear condition-

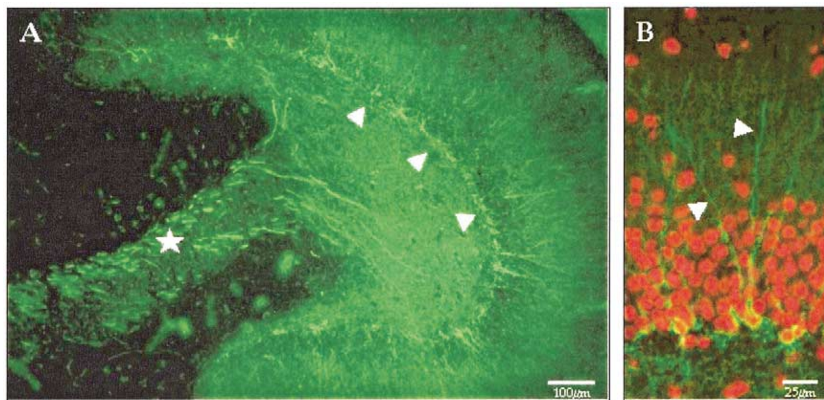

Figure 2. Photomicrographs of dentate polysialylated neurons in the hippocampal formation. **(A)** Intense labeling of the polysialylated neurons at the infragranular zone of the hippocampal dentate gyrus (arrowheads) and mossy fiber bundle (star). **(B)** A higher magnification of the dentate polysialylated neurons. In this panel, the green fluorescing cells are clearly distinguished from those stained with propidium iodide (red), and their dendrites (arrowheads) can be observed to extend into the molecular layer.

ing revealed a significant group effect [$F(3,35) = 6.81$, $p < .001$]. Post hoc Tukey analyses demonstrated values from the .4 mA group to be significantly higher than those displayed by all other groups ($p < .05$; Figure 3). By contrast, the 1 mA group showed a significant reduction in frequency of polysialylated cells as compared to the other groups ($p < .05$). Polysialylated cell frequency in the group receiving a 2-mA shock did not differ from that of the control group (0 mA). No regional differences in PSA-positive cell expression were noted following exposure to increasing shock intensities.

Given the unexpected decrease in polysialylated cell frequency following fear conditioning with a 1-mA shock intensity, the possibility of interlaboratory variation was

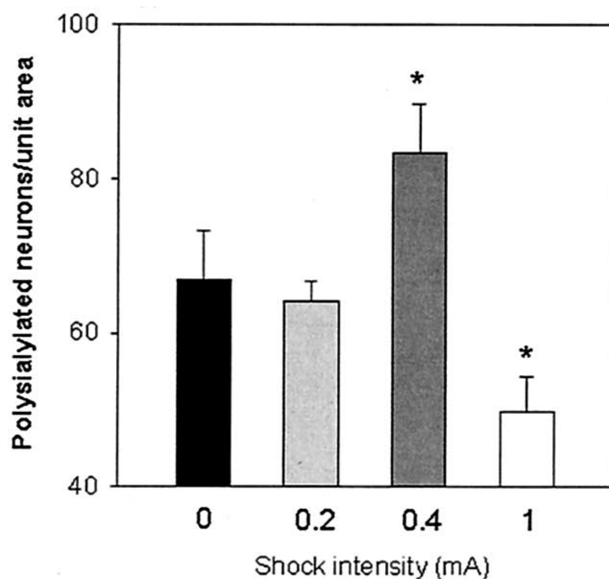

Figure 3. Quantitative illustration of dentate polysialylated cell frequency 12 hours after training rats in the contextual fear conditioning task with stimuli of increasing intensity. Data represent mean ± SEM ($n = 9$ to 10 per group). * $p < .05$ versus 0 mA control group.

investigated. For this purpose, animals were trained in the water maze paradigm for eight consecutive trials and sacrificed at 12 hours following the last trial, and the expected learning-induced modulation of polysialylated dentate cell frequency was determined by immunohistochemical procedures. Rats acquired the water maze task as judged by the progressive decrease in their latencies to find the platform (Figure 4A). Moreover, a probe test given 12 hours later demonstrated that a significant amount of searching time was restricted to the target quadrant (34 ± 2.1% time spent in target quadrant vs. 20 ± 1.9% time in opposite quadrant). As expected from previously published work (Murphy et al 1996), rats trained in the water maze task showed a significant increase in the number of polysialylated neurons when compared with levels from control rats ($df = 19$, $t = 2.76$, $p < .013$; Figure 4B). Moreover, the frequency of polysialylated neurons observed at 12 hours following water maze training (89.67 ± 5.27 cells/unit area) was indistin-

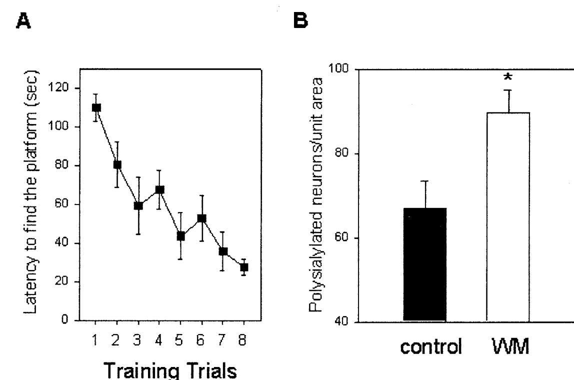

Figure 4. Influence of water maze training on frequency of dentate polysialylated neurons at 12 hours posttraining. **(A)** Demonstrates latency decrease with increasing trials to find platform. **(B)** 12 hours posttraining frequency increase in polysialylated neurons. Data represent mean ± SEM ($n = 10$ to 11 per group) * $p < .05$ versus control group. WM, watermaze.

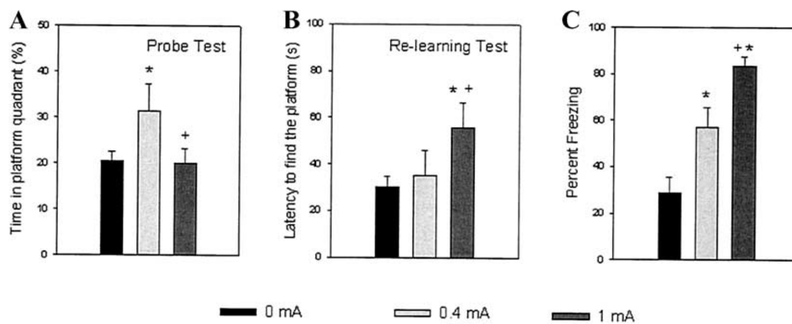

Figure 5. Influence of sequential training in water maze followed by fear conditioning on task consolidation. **(A)** Illustrates water maze probe trial latencies, and **(B)** illustrates water maze relearning (average of trials 1 and 2) at 48 hours following sequential training in water maze and fear conditioning paradigms. **(C)** Recall of the fear conditioning paradigm at 72 hours following sequential training in water maze and fear conditioning paradigms. All values are the mean ± SEM (n = 7 to 9 per group). * $p < .05$ versus 0 mA control group; + $p < .05$ versus .4 mA group.

guishable from that observed previously with this spatial task (86.68 ± 3.96 cells/unit area) (Murphy et al 1996) and also that observed in the .4 mA fear conditioning group of the present study (83.4 ± 2.25 cells/unit area).

Effects of Contextual Fear Conditioning on Consolidation of a Recently Acquired Water Maze Learning

Suppression of the learning-induced polysialylated neuronal frequency increase by traumatic (1 mA), as opposed to the increase induced by stressful (.4 mA), fear conditioning suggested that traumatic experiences may be encoded by a mechanism that suppresses the transient frequency increase of polysialylated neurons in the 12 hours posttraining period. As a corollary, learning paradigms requiring a PSA response may not be consolidated effectively when accompanied by a traumatic fear conditioning. To explore this possibility further, we first trained animals in the water maze paradigm and immediately followed this task with either stressful or traumatic fear conditioning paradigms.

For this purpose, rats were trained in the water maze for four consecutive trials, and 8 minutes later they were exposed to a contextual fear conditioning session using either the .4-mA or 1-mA shock intensities. Recall of spatial orientation in the water maze was tested 48 hours later by a probe trial, and immediately thereafter, the animals' ability to relearn the spatial task was tested by exposing them to four additional training trials with the platform in the same location as at initial training. As can be seen in Figure 5A, rats trained in a water maze paradigm followed by a stressful (.4 mA) fear conditioning paradigm showed facilitated retention of platform location, as indicated by the increased time spent in the appropriate (platform) quadrant during the transfer test (30 first seconds: .4 mA vs. both control and 1 mA groups; $p < .05$) at 48 hours posttraining. By contrast, when maze training was followed by a traumatic (1 mA) fear conditioning paradigm, the animals exhibited no recall of platform location. Their percent time in the platform quadrant was identical to that of the control group (0 mA), which would be expected to have little recall of platform position after a single training session of four trials.

Moreover, when the platform was again placed in the same location as at initial training, animals that had received either no shock or one of a .4 mA intensity in the fear conditioning paradigm performed equally well in relearning the task, as observed when averaged the first two relearning trials (Figure 5B). Animals that had received the 1-mA shock, however, exhibited significantly greater escape latency times (1 mA group vs. both 0 mA and .4 mA groups; $p < .05$), implying they had virtually no recall of the task; however, no group differences were observed in performance when evaluating the relearning trials 3 and 4 (data not shown).

In addition, the same rats were tested for their retention of the contextual fear conditioning information 24 hours after the water maze relearning task (i.e., 72 hours after the initial conditioning session). As shown in Figure 5C, freezing values of .4 mA and 1 mA trained rats were significantly higher than those displayed by the 0 mA control group ($p < .05$), and values from each shocked group also significantly differed from each other ($p < .05$). Thus, acquisition of the fear conditioning paradigm was unaffected by prior training in the water maze paradigm.

Discussion

In this study, we have shown that the regulation of NCAM polysialylation in the hippocampal dentate gyrus, 12 hours after training rats in the contextual fear conditioning task, to be dependent on the shock intensity at which the animals were trained. No changes in polysialylation were found in the hippocampus of rats trained at the low-shock

intensity (.2 mA), a situation that does not induce marked freezing; however, the marked freezing response observed in the group trained with a moderate-shock intensity (.4 mA) was associated with a significant posttraining frequency increase of dentate polysialylated neurons. This finding provides further support for hippocampal involvement in contextual fear conditioning, a belief that has recently been challenged (Anagnostaras et al 2001). Moreover, contextual fear conditioning using a stimulus of moderate intensity produces a polysialylation response that is indistinguishable from that which occurs in the same posttraining period following water maze training (Murphy et al 1996). Interestingly, the activation of PSA is not seen in passive animals following either passive avoidance or water maze learning (Fox et al 1995; Murphy et al 1996), and removal of PSA with endoneuraminidase-N has been found to impair spatial learning (Becker et al 1996). Therefore, these observations also support the idea that NCAM-mediated neuroplastic processes, together with many other molecular events that contribute to synaptic plasticity, serve as a general remodeling mechanism facilitating the synaptic reorganization that underlies memory formation (Murphy and Regan 1998).

However, an exception to this rule was found with animals trained with a more extreme conditioning stimulus (1 mA), where a 12 hours posttraining decrease in the frequency of dentate polysialylated neurons was observed, despite the acquisition of a more robust and longer-lasting freezing response (Cordero et al 1998). These contrasting modulations of dentate polysialylated cell frequency in response to fear conditioning with stimuli of increasing intensity may reveal a distinct consolidation mechanism that is unique to the nature of memory induced by traumatic experiences (Yehuda and Antelman 1993; Charney et al 1995; Krystal et al 1995). Since previous evidence indicates that NCAM protein expression decreased in the hippocampus 12 hours after contextual fear conditioning regardless of the shock intensity used at training (Merino et al 2000a), we cannot exclude that the reduced polysialylation observed in the 1 mA trained group might be, at least in part, secondary to a down-regulation of NCAM levels.

Water maze training followed immediately by contextual fear conditioning with a stimulus of .4 mA resulted in the effective consolidation of both tasks and an enhanced retention of the former task, as suggested by the increased persistence to swim in the platform quadrant during the probe test. The facilitation of spatial memory induced by .4 mA fear conditioning might be related to previous findings indicating that increasing corticosterone levels after water maze training can potentiate the consolidation of the recently acquired spatial information (Sandi et al 1997), a phenomenon which has also been shown with other learning paradigms (Sandi and Rose 1994; Cordero and Sandi 1998).

These observations suggest that whereas fear conditioning at the high-shock intensity might be considered a model for trauma, training at the moderate-shock intensity might lead to a qualitatively different experience. The complete immobility developed by 1 mA trained rats may reflect suppression of a searching strategy to escape the fear-associated environment. This reasoning is supported by the fact that training at the moderate-shock intensity (.4 mA) induces an increase in NCAM polysialylation similar to that found in other stressful learning tasks (such as water maze or passive avoidance) in which rats learn to develop an adaptive behavioral response.

Exposure to traumatic events is known to cause a complex pattern of memory alterations (Charney et al 1995; Krystal et al 1995) that, among other manifestations, include 1) a potentiation of memory for contextual elements that may account for the subsequent triggering of symptoms (including behavioral inhibition); and 2) retrograde amnesia or impaired retrieval of information that was processed just before the trauma (Schacter et al 1996; Diamond et al 1999; Ehlers and Clark 2000). Neurobiological hypotheses developed to account for memory alterations typically observed in PTSD have repeatedly proposed that traumatic memories are stored through a unique mechanism that determines their strength and resistance to erasure characteristics (Cahill 1997). The most parsimonious explanation is that the information related to the traumatic fear conditioning is processed by a mechanism(s) other than those used for normal memory consolidation. Our findings suggest this mechanism(s) seems to involve suppression of polysialylated cell frequency at the 12 hours posttraining period, an effect that would result in decreased plasticity and the concomitant inability to modify the trace in relation to previously stored memories.

Polysialylation of NCAM appears to promote remodeling of synaptic membranes by interfering with the adhesion properties of other adhesion molecules (Nothias et al 1997). Learning-induced PSA activation has been suggested to play a role in synapse formation but also in the final elimination and/or selection of the relevant synapses from those that might have been transiently overproduced during training (Doyle et al 1992; Murphy and Regan 1998). By extension, the decrease in dentate polysialylation found in this study following fear conditioning with 1.0-mA shock intensity might prevent synapse relaxation/selection process in favor of a rapid synapse fixation. Evidence for this proposal is observed in these studies, as water maze learning is suppressed followed traumatic fear conditioning. Moreover, the traumatic event itself would be very strongly encoded within the context of the occur-

rence, which is in agreement with the potentiation of memory for contextual elements described for traumatic memories in humans (Schacter et al 1996; Ehlers and Clark 2000).

In conclusion, these studies provide strong evidence for a hippocampal, stress-sensitive memory encoding system that responds specifically to situations involving divergent stress levels. This mechanism potentially underlies the memory abnormalities that are observed following traumatic events. Exposure to traumatic events is known to cause a complex pattern of memory alterations. Among other manifestations, these include a potentiation of memory for contextual elements that account for subsequent behavioral inhibition and amnesia of information processed at the time of the trauma (Schacter et al 1996). Our results show that fear conditioning with high-shock intensity can lead to these two memory phenomena.

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