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CHAPTER 3

Amygdala modulation of memory-related processes in the hippocampus: potential relevance to PTSD

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Abstract: A key assumption in the study of stress-induced cognitive and neurobiological modifications is that alterations in hippocampal functioning after stress are due to an excessive activity exerted by the amygdala on the hippocampus. Research so far focused on stress-induced impairment of hippocampal plasticity and memory but an exposure to stress may simultaneously also result in strong emotional memories. In fact, under normal conditions emotionally charged events are better remembered compared with neutral ones. Results indicate that under these conditions there is an increase in activity within the amygdala that may lead to memory of a different quality. Studying the way emotionality activates the amygdala and the functional impact of this activation we found that the amygdala modulates memory-related processes in other brain areas, such as the hippocampus. However, this modulation is complex, involving both enhancing and suppressing effects, depending on the way the amygdala is activated and the hippocampal subregion examined. The current review summarizes our findings and attempts to put them in context with the impact of an exposure to a traumatic experience, in which there is a mixture of a strong memory of some aspects of the experience but impaired memory of other aspects of that experience. Toward that end, we have recently developed an animal model for the induction of predisposition to stress-related disorders, focusing on the consequences of exposure to stressors during juvenility on the ability to cope with stress in adulthood. Exposing juvenile-stressed rats to an additional stressful challenge in adulthood revealed their impairment to cope with stress and resulted in significant elevation of the amygdala. Interestingly, and similar to our electrophysiological findings, differential effects were observed between the impact of the emotional challenge on CA1 and dentate gyrus subregions of the hippocampus. Taken together, the results indicate that long-term alterations within the amygdala contribute to stress-related mnemonic symptoms and suggest that elucidating further these intra-amygdala alterations and their effects on modulating other brain regions is likely to be beneficial for the development of novel approaches to treat stress-related disorders.

Keywords: amygdala; animal-model; anxiety; hippocampus; juvenile-stress; LTP; PTSD; stress

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Amygdala modulation of synaptic plasticity in the hippocampus

The relationship between ''stress'' and ''memory'' is often conceived as one of ''stress impairs memory.'' Indeed numerous findings support this concept. For example, exposing rats to a cat impaired spatial working memory in a Morris Water Maze task (Diamond et al., 1999), which depends on the integrity of the hippocampus (Morris et al., 1982). This ''predator stress'' procedure also impaired the induction of long-term potentiation (LTP) — a synaptic model of memory — in the hippocampus (Mesches et al., 1999; Vouimba et al., 2006). Stress-induced impairment of hippocampal-dependent memory and LTP has been observed for various stress procedure including tail-shock (Foy et al., 1987; Diamond and Rose, 1994; Garcia et al., 1997), forced exposure to brightly lit room (Xu et al., 1997), and platform stress (Maroun and Richter-Levin, 2003).

Since LTP is a model for activity-dependent plasticity assumed to be related to the formation of memories, these findings suggest that stress impede hippocampal-dependent learning and memory-related processes by disrupting plasticity in the hippocampus.

We have hypothesized that exposure to stressors impairs hippocampal functioning via the activation of the basolateral amygdala (BLA).

Stress increase BLA activity and synaptic plasticity

Examining the involvement of the BLA in stress modulation of learning and memory processes, we first evaluated the effects of exposure to stressors on BLA activity. We found that BLA response to the entorhinal cortex (EC) stimulation (Yaniv et al., 2000, 2003) was increased following exposure to a platform stress (Kavushansky and Richter-Levin, 2006). Moreover, injecting corticosterone (CORT) yielded a similar dose-dependent effect (Kavushansky and Richter-Levin, 2006). In addition, we have shown that platform stress enhanced amygdala synaptic plasticity (Vouimba et al., 2004), a finding also reported by others in both humans and rodents (Schaefer et al., 2002; Correll et al., 2005; McGaugh, 2005). Together, these findings suggest that stressful experiences indeed increase activity in the BLA, enabling it to disrupt hippocampal functioning.

Bidirectional effects of ''stress'' on memory-related processes: the involvement of the BLA

In contrast to the prevailing ''stress impairs memory'' concept there are several observations that suggest that ''stress'' or ''emotionality'' do not always impair memory formation, but rather they can also enhance hippocampal LTP and memory (Sapolsky, 2003; Kavushansky et al., 2006) and in some cases may drastically enhance some aspects of memory formation, as in the case of traumatic memories which haunt patients suffering from post-traumatic stress disorder (PTSD) (Van der Kolk and Fisler, 1995; Bower and Sivers, 1998).

Furthermore, studies suggest that the amygdala may also mediate stress-related enhancement of hippocampal memory processes and LTP (Richter-Levin and Akirav, 2003; Kim et al., 2001; McGaugh, 2002). Thus, the BLA may play a key role in both the impairing and enhancing effects of stress on hippocampal functioning (Liang et al., 1994; Akirav and Richter-Levin, 1999a, b; Kim et al., 2001) through a differential activation. Supporting this stance, we have found that stress effects on the BLA are not uniform, but may depend on stress characteristics, such as intensity, valence, duration, and controllability. For instance, we have shown (Fig. 1) that rats trained under ''high-stress'' conditions (cold water, 19°C) learnt faster to find the hidden platform in the Morris Water Maze than rats trained under ''low-stress'' conditions (warm water, 25°C) (Akirav et al., 2001). Moreover, in comparison with naïve rats, only rats that were trained under ''high-stress'' conditions exhibited significant increased extracellular signal-regulated kinases (ERK2) phosphorylation, indicative of activating mitogen-activated protein kinase (MAPK) signaling cascades in the BLA. No significant activation was evident among rats trained under ''low-stress'' conditions, or rats that did not learn the task well under ''high-stress''conditions, nor among rats

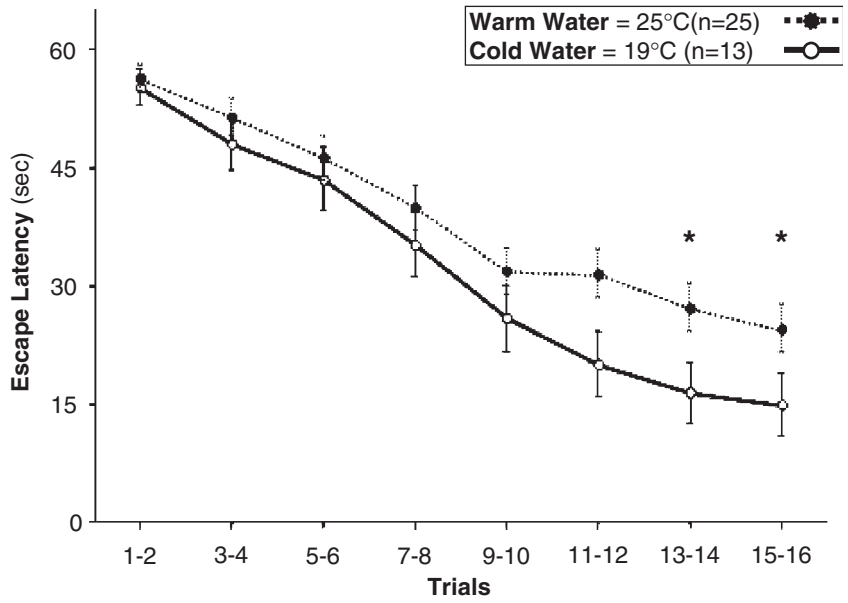

Fig. 1. Water temperature modulates stress levels and affects learning the Morris Water Maze. Rats trained under "high stress" conditions (cold water, 19°C) learnt faster to find the hidden platform in the Morris Water Maze than rats trained under "low stress" conditions (warm water, 25°C) (* = $p<0.05$).

that had no platform to learn to find under "high-stress" conditions (Akirav et al., 2001). In the cornu ammonis field 1 (CA1), training was accompanied by increased phosphorylation of ERK2 only in animals that have acquired the task (irrespective of whether they were trained in cold or warm water). Thus, it is likely that the activation of the amygdala (as seen by the activation of ERK2) following an emotionally charged hippocampal-dependent learning experience led to the better performance of the cold water trained rats in the spatial task (Akirav et al., 2001).

Further investigating the involvement of the BLA in modulating learning processes, we have shown that while an acute exposure to platform stress facilitated LTP in the BLA, a repeated exposure suppressed long-lasting LTP in the BLA (Vouimba et al., 2004). Such changes were associated with normal or enhanced LTP in the hippocampal dentate gyrus (DG) for acute stress exposure (Vouimba et al., 2004; Kavushansky et al., 2006) and impaired DG LTP for repeated stress (Vouimba et al., 2004).

Thus, alteration of hippocampal functioning consecutive to stressful experiences may involve differential changes in the BLA activity and/or synaptic plasticity.

BLA influences memory by tagging important information

Both human and animal studies indicate that emotionality-induced enhanced memory formation involves the activation of the amygdala, but how may the BLA influence memory consolidation-related processes in the hippocampus remains to be studied.

The hippocampus, being involved in the transformation of short- into long-term memories should be able to sort out the more significant from the less relevant aspects of an experience in order to transform only the former into long-term memory. One mechanism that could contribute to this selection is the emotional significance of the experience. Emotionally significant events are likely to be important to remember and their emotional load could mark them as important — a function that we have termed "Emotional Tagging" (Richter-Levin and Akirav, 2003).

According to this proposed ''Emotional Tagging'' mechanism the activation of the amygdala in emotionally arousing events marks the experience as important and aids in enhancing synaptic plasticity in other brain regions (Akirav and Richter-Levin, 2002; Richter-Levin and Akirav, 2003). We have also proposed a potential neural mechanism that may underlie ''Emotional Tagging.'' Long-term memory formation is considered to involve lasting alterations in synaptic efficacy, known as synaptic plasticity. Two factors were suggested as crucial for obtaining a synapse-specific long-term plasticity: (1) the successful activation of a synapse-specific, protein synthesis-independent tag (Frey and Morris, 1998) and (2) the activation of synapse-nonspecific protein synthesis (Matthies et al., 1990; Pittenger and Kandel, 1998). The activation of protein synthesis can then induce lasting plasticity only in those synapses marked by a tag. Interestingly and relevant to the ''Emotional Tagging'' hypothesis, Frey et al. (2001) demonstrated that the activation of the amygdala could transform ''transient'' (early-LTP) into ''long-lasting'' (late-LTP) plasticity. Thus, it seems reasonable to assume that the activation of the amygdala triggers neuromodulatory systems, which in turn reduce the threshold for the activation of the ''Synaptic Tag,'' facilitating the transformation of early- into late-phase memory (Richter-Levin and Akirav, 2003).

BLA modulates hippocampal LTP

To further test this ''Emotional Tagging'' hypothesis we examined whether BLA activation can affect memory-related processes (LTP induction) in the hippocampus.

Priming the BLA before the induction of LTP in the DG by stimulating the perforant path (PP) enhanced DG LTP (Akirav and Richter-Levin, 1999b, 2002; Vouimba and Richter-Levin, 2005). Furthermore, this effect was found to be mediated by CORT and norepinephrine (NE), when administered either systemically or directly in the BLA (Akirav and Richter-Levin, 2002; Vouimba et al., 2007). Similar effects of BLA priming on DG LTP were reported by Ikegaya et al. (1995) demonstrating that BLA activation reduces the threshold for the induction of DG LTP.

Differential outcome of ''stress'' or BLA activation within the hippocampus: CA1 vs. DG

Taken together, these findings suggested us that ''stress'' affects hippocampal functioning by activating the BLA. However, we noted that while BLA activation was found to enhance LTP in the DG, ''stress'' was found to attenuate the induction of LTP at least in the CA1 (Maroun and Richter-Levin, 2003).

One possibility that could explain this discrepancy could be that, this premise needs some adjustments, i.e., that both ''stress'' and BLA activation affect the hippocampus but each in a different manner, the former impairing while the latter enhancing memory-related processes. Alternatively, the different effects of ''stress'' and BLA activation may derive from differential effects of both on the CA1 vs. the DG subregions of the hippocampus (Fig. 2).

The latter proposal warrants examination since the majority of research on the effects of ''stress'' on hippocampal functioning focused on the CA1 (e.g., Foy et al., 1987; Diamond and Rose, 1994; Maroun and Richter-Levin, 2003), while research on the effects of BLA activation on the hippocampus functioning has focused mainly on the DG subregion (e.g., Ikegaya et al., 1995; Akirav and Richter-Levin, 1999a, b, 2002; Frey et al., 2003).

Indeed, both ''stress'' and BLA activation impaired CA1 LTP (Vouimba and Richter-Levin, 2005; Kavushansky et al., 2006; Vouimba et al., 2006). However, while DG LTP enhancement appear to depend on CORT or NE transmission in the BLA, the attenuation of the CA1 LTP appear to be independent of these stress hormones (Vouimba et al., 2007). A similar pattern of differential outcomes between these subregions of the hippocampus was found for ''stress.'' Exposure to uncontrollable swim stress enhanced DG LTP induction but impaired LTP in the CA1. Furthermore, these effects were moderated when controllable swim stress, presumed to represent lower

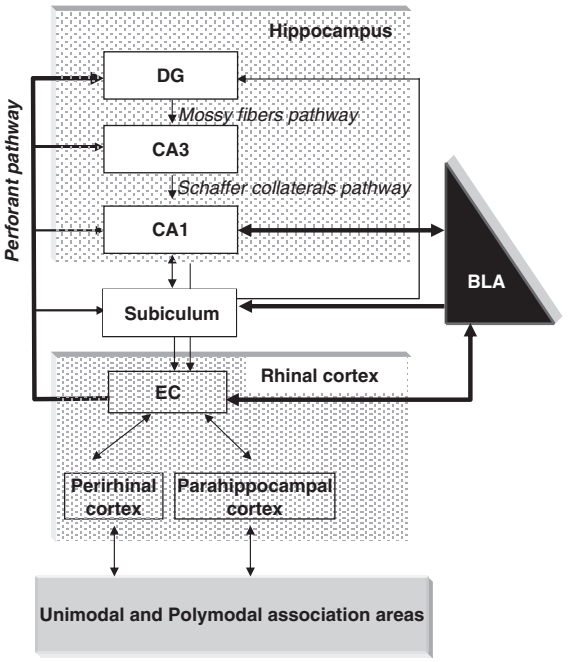

Fig. 2. A schematic diagram of hippocampal formation–amygdala connections. Unimodal and polymodal inputs from association areas reach the hippocampus (HPC) via the rhinal cortical regions (entorhinal, perirhinal, and parahippocampal cortices). Information flows from the entorhinal cortex (EC) to the HPC via the *perforant pathway*, the main afferent pathway to the HPC. Within the HPC, information coming into the dentate gyrus (DG) is processed and sent via the *mossy fiber pathway* to the CA3 subfield, which connects with the CA1 via the *Schaffer collateral pathway*. The CA1 sends significant output to the subiculum and both CA1 and subiculum project to the EC. The basolateral nucleus of the amygdala (BLA) sends monosynaptic projections to the CA1, the subiculum, and the EC. Given no monosynaptic interconnections between the BLA and DG, the BLA may modulate DG polysynaptically, i.e., via the EC or the subiculum.

stress levels, was applied (Kavushansky et al., 2006).

To summarize so far, both exposure to "stress" and BLA activation produced similar effects in the hippocampus, suppressing LTP in the CA1 while enhancing LTP induction in the DG. These results support the proposal that the effects of "stress" on hippocampal functioning are mediated to a large extent by the BLA differential modulation of hippocampal subregions. In support of this idea were the findings that exposure to "stress" activated the BLA and that lesion of the BLA suppressed the "stress" effects on CA1 LTP as well as on learning and memory (Akirav et al., 2001; Kim et al., 2001).

This novel realization that "stress" and amygdala activation exert a complex mixture of enhancing and suppressing effects on memory-related processes in different brain areas may suggest the following: under normal conditions emotionality may dictate the remembering of certain "important" (emotionally loaded) features of an event. However, if the same event is experienced under lower or higher levels of emotionality, altered memories may be formed, rendering certain features of the event as irrelevant while others as "unforgettable."

Post-traumatic stress disorder (PTSD)

Concomitant "stress" related impairment and enhancement of memories

Considering simultaneous opposite effects on memory processes brings to mind the PTSD, where the intense stress brought upon by the traumatic event confers a mixture of enhancing and suppressing effects on memory-related processes. On the one hand, as the diagnostic and statistical manual of mental disorders, 4th edition (DSM-IV) PTSD diagnosis requires, the "reexperiencing symptoms" like intrusive memories, recurrent dreams, flashbacks, and intense reactions in similar events (APA, 1994) indicate that there is enhancement of memories of certain features of the traumatic event. On the other hand, extensive research indicates that PTSD patients suffer also from impaired recall capacities that were related to altered hippocampal functioning (for review, see Nemeroff et al., 2006). We therefore speculated that the BLA response to stressful events, and its differential effects on the hippocampal subregions, may be of relevance to our understanding of the neurobiology of PTSD. We wanted to further examine this hypothesis but for that an animal model for PTSD was required. Unfortunately however, though some models were suggested, no

animal model has gained a widespread consensus as a valid and suitable model for PTSD.

Most attempts to develop such a model of PTSD dealt with the question of what kind of a stress protocol should be employed; some studies dealt with the question of when a stressor becomes traumatic. For example, Cordero et al. (2002) proposed that, an electric foot shock is stressful at the intensity of 0.5 mA but becomes traumatic at the intensity of 1.0 mA. Others proposed employing ethologically relevant stressors, which might represent a relevant traumatic event (Cohen et al., 2003, 2006; Woodson et al., 2003; Adamec et al., 2004, 2006; Cohen and Zohar, 2004).

A novel animal model for PTSD

We were concerned with a different aspect of the PTSD phenomenon — the question of why do some people develop PTSD following a traumatic event while others do not. Clearly, understanding what underlies the predisposition to develop PTSD would be instrumental to our understanding of the disorder. Furthermore, it would help producing "affected" animals that could then be utilized in promoting the research into the neurobiology of the disorder.

Among the suggested factors that might contribute to the vulnerability to develop PTSD is the exposure to stressful events early in life. Early-life stress (ELS) is considered a significant risk factor, predisposing people to mal-adaptively respond to traumatic events later in life. Reports on ELS are most prevalent among those individuals who develop PTSD following a traumatic event and significantly less prevalent among those who did not develop the disorder (Nemeroff et al., 2006). To mimic these conditions our model consists of an exposure to "stress" early in life and a subsequent exposure to stress in adulthood.

A unique feature of our model is the age of the early exposure to stress. While most ELS rodent models focus on the perinatal to pre-weaning periods and involve some form of maternal deprivation or separation (for review, see Sanchez et al., 2001), we focused on another ELS-sensitive period in the rat ontogeny, namely the juvenile stage (~28 days), the earlier phase of the adolescent/post-weaning to pre-pubertal period (Avital and Richter-Levin, 2005; Avital et al., 2006; Tsoory and Richter-Levin, 2006; Tsoory et al., 2007a, b). During the adolescent period (21–42 days), substantial maturational processes occur in the rat limbic system, including in the hippocampus and amygdala-based neurocircuits (for review, see Spear, 2000).

During the juvenile period the hypothalamic-pituitary-adrenal (HPA) axis response reaches its developmental asymptote (Vazquez, 1998); however this response lasts considerably longer than in adults (Vazquez, 1998; Romeo et al., 2004). Romeo et al. (2004) suggested that this slower shutoff of the HPA axis during juvenility may derive from less centrally mediated feedback from various underdeveloped forebrain limbic regions at this age. Indeed, exposure to stressors during juvenility was reported to produce more pronounced effects than exposure at earlier or later ages, affecting object exploration in adulthood (Einon and Morgan, 1977), fluid intake (McGivern et al., 1996), and adulthood social and nonsocial behaviors associated with disregulation of endogenous opioid system development (Van den Berg et al., 1999a–c, 2000). Adult rats chronically exposed to variable stressors throughout juvenility had an enhanced acoustic startle response similar to patients with PTSD (Maslova et al., 2002).

Exposure to acute stressors during juvenility produces increased vulnerability to stressful events in adulthood (60 days of age), resulting in an augmented response to adverse experiences. Adult rats that were exposed to stress both during juvenility and adulthood exhibited enhanced startle response and reduced exploration in a novel setting not only in comparison with naïve unexposed rats but also in comparison with rats that were exposed to stress only during juvenility and in comparison with those exposed to stress only in adulthood (Avital and Richter-Levin, 2005). A similar pattern of effects was also evident at the age of 90 days (Fig. 3).

We have proceeded to compare the effects of recurrent exposure to stress during juvenility and in adulthood, with recurrent exposure to stress in adulthood. Indeed, adult rats exposed to stressors

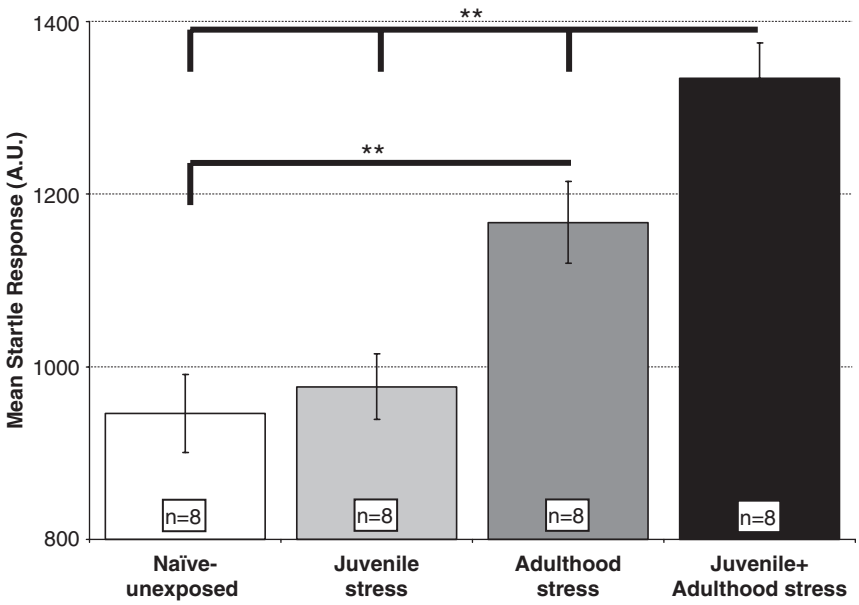

Fig. 3. The effect of exposure to stressors during juvenility, adulthood, or their recurrent combination on startle responses at the age of 90 days. Rats exposed to stress during juvenility and adulthood exhibited a significant higher startle response compared to all other groups. Rats exposed to stress only in adulthood had a significant increased startle response only in comparison with the naïve unexposed group ($^{**} = p < 0.01$).

during juvenility and adulthood exhibited increased startle responses significantly greater than those of rats that were exposed to the stressors twice in adulthood (Avital and Richter-Levin, 2005). Rats exposed to stress twice in adulthood exhibited startle responses that did not differ from those of rats exposed to stress only once in adulthood.

It is noteworthy, that further examination of the model indicated that the effects of exposure to juvenile and adulthood stress are long-term (Avital and Richter-Levin, 2005). Comparing the startle responses of 60- and 80-days-old rats exposed either to stress only during juvenility, only in adulthood (59 days of age), or that underwent recurrent exposure to stress during juvenility and in adulthood, revealed that the effect of exposure to stress in adulthood alone diminished over time whereas the effect of recurrent exposure to stress during juvenility and adulthood did not diminish over time (Fig. 4).

We have moved on to examine the effects of a short-term juvenile exposure to variable stressors on adulthood coping responses by using stressful challenges, namely, novel-setting exploration and two-way shuttle avoidance learning. We chose to utilize the two-way shuttle avoidance task since learning and performance in this task are dependent on the hippocampus (Schwegler et al., 1981; Becker et al., 1997) and the amygdala (Savonenko et al., 2003) and poor two-way shuttle avoidance performance was observed following both negligible and high doses of injected CORT (Kademian et al., 2005). Selective breeding based on "high/low-avoidance" performance was suggested to relate to differences in "emotional" factors (state/trait anxiety) that influenced performance (Brush, 2003). Indeed, exposure to stressors during juvenility significantly reduced adulthood exploratory behavior and impaired adulthood learning under stressful conditions. It reduced the rates of avoidance responses and increased the rates of escape failures while learning the two-way shuttle avoidance task (Tsoory et al., 2007a). Furthermore, the effects of exposure to stressors during juvenility (27–29 days) were found to be stronger than those of exposure to the same stress protocol during

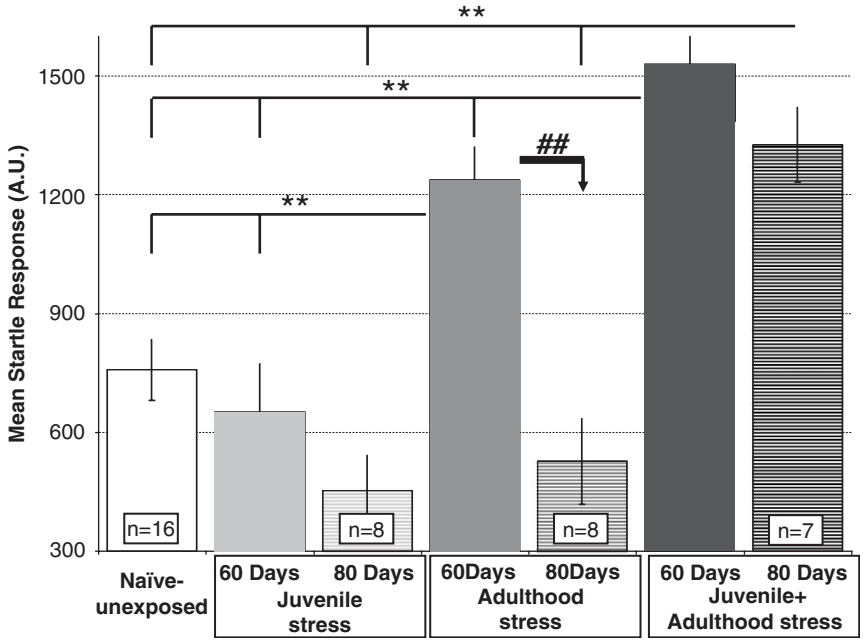

Fig. 4. The effect of exposure to stressors during juvenility, adulthood, or their recurrent combination on startle responses at the ages of 60 and 80 days. Recurrent exposure to stress (juvenility and adulthood) resulted in a significant higher startle response compared to all other groups at both 60 and 80 days ($^{**} = p < 0.01$). The effect of exposure to stress only in adulthood diminished over time ($^{\#\#} = p < 0.01$), while the effects of recurrent exposure did not.

mid-adolescence (33–35 days), indicating that within the post-weaning to pre-pubertal period the juvenile age (~28 days) is a stress-sensitive period (Tsoory and Richter-Levin, 2006).

Taken together, these results indicated "juvenility" (~28 days of age) as a stress-sensitive developmental period. Furthermore, the results strongly support the notion that "juvenile stress" may model the predisposing effect of ELS on stress responses later in life, which are related to PTSD (Appendix).

"Juvenile stress" affects neural cell adhesion molecules

Once a model was established, it could be utilized to study the neural consequences and correlates of an exposure to a trauma inducing experience. Among the candidate molecules to be studied in relation to pathological plasticity is the family of the neural cell adhesion molecules (NCAMs).

NCAMs are membrane-bound glycoproteins of the immunoglobulin superfamily of adhesion molecules, which mediate cell–cell interactions; by interacting with cytoskeletal components, they can activate specific intracellular signaling pathways (Cremer et al., 1997). The NCAM, polysialylated-NCAM (PSA-NCAM), and cell adhesion molecule L1 (CAM-L1) of this family play a pivotal role in neural development and regeneration, and are strongly implicated in synaptic plasticity and memory formation processes (Schachner, 1997; Kamiguchi, et al., 1998; Kiss et al., 2001; Welzl and Stork, 2003; Sandi, 2004; Gerrow and El-Husseini, 2006). The post-translational polysialylation of NCAM weakens its adhesive properties (Schachner, 1997) and thus it was suggested that PSA-NCAM acts as a plasticity promoter by decreasing overall cell adhesion, thereby allowing structural remodeling to occur (Rutishauser and Landmesser, 1996), whereas NCAM acts as a stability promoter (Ronn et al., 2000).

Alterations in the relative expression of PSA-NCAM to NCAM and in the expression of CAM-L1 were associated with development-related alterations (Edelman, 1984; Rutishauser and Jessell, 1988; Rutishauser, 1989; Kamiguchi, et al., 1998; Gerrow and El-Husseini, 2006). Alterations in the expression of these molecules were also found following learning, memory formation, and activity-dependent synaptic remodeling (Doyle et al., 1992a, b; Luthi et al., 1994; Ronn et al., 2000; Law et al., 2003; Welzl and Stork, 2003; Sandi, 2004).

A series of studies showed that chronic stress protocols known to produce cognitive and neural alterations (chronic restraint stress: 21 days $\times$ 6 h) markedly affected the expression of NCAMs, overall decreasing the expression of NCAM while increasing those of PSA-NCAM and CAM-L1 in the hippocampus and other brain areas (for review, see Sandi, 2004).

We have recently started to characterize the effects of exposure to stressors during juvenility on the expression levels of the NCAMs: NCAM, its polysialylated form PSA-NCAM, their expression ratio [PSA-NCAM/(NCAM + PSA-NCAM)], and CAM-L1 within the limbic system. Overall the results indicated that exposure to stressors during juvenility disrupts development-related alterations in the expression ratio of PSA-NCAM to NCAM in the BLA, CA1, DG, and EC (Tsoory et al., 2007b). It is noteworthy that differential effects were found between the CA1 and DG subregions of the hippocampus reminding us of the differences found between the effects of BLA activation on the DG and CA1 (Vouimba et al., 2007).

When the effects were examined soon after the "juvenile" stress exposure, 4 days following the last exposure to a stressor (at the age of 33 days) the expression ratio of PSA-NCAM to NCAM was increased among juvenile stressed rats in comparison with juvenile naïve rats in the CA1 but not in the DG (Fig. 5).

Similar differential effects were observed in adulthood for the expression of CAM-L1. Adult rats that were exposed to stress during juvenility exhibited increased levels of CAM-L1 expression in the CA1 compared with rats exposed to stress during juvenility and in adulthood. No such difference was found in the DG (Fig. 6).

Summary

Contrary to the prevailing concepts of "stress impairs memory-related processes" on the one hand and "stress promotes memory-related processes" on the other, our results indicate that an integrated view should be considered. The exposure to an emotionally or stressful experience modulates memory formation in a complex manner.

Some brain areas become more likely to process memories of certain aspects of the experience while memory formation in other brain areas may be suppressed. The result may not necessarily be "more" or "less" memory, but rather an "altered" memory. These alterations may relate to a range of features in the memory of the event, varying from differences with respect to which aspects of the experience are remembered, to how detailed the memory formed will be or how intense it will be, but also which brain areas will be recruited for its formation, maintenance, and recall.

In that respect, we were able to demonstrate that the amygdala, when activated, can concomitantly exert a mixture of plasticity-supporting and plasticity-suppressing influences. These are likely to contribute to the modification of the characteristics of the memory formed under emotional or stressful conditions.

It is easy to foresee that if amygdala functioning is altered due to a traumatic experience this would lead neither to the suppression of the traumatic event memory, nor to its enhancement, but rather to a complex mixture of both.

The "juvenile stress" protocol is suggested as an effective model for the induction of a predisposition and susceptibility to develop stress-related disorders in adulthood such as PTSD and post-traumatic depression.

The model can now be utilized to study how amygdala functioning is being modified following an exposure to a traumatic experience and how this affects the way the amygdala modulates memory-related processes in other brain regions.

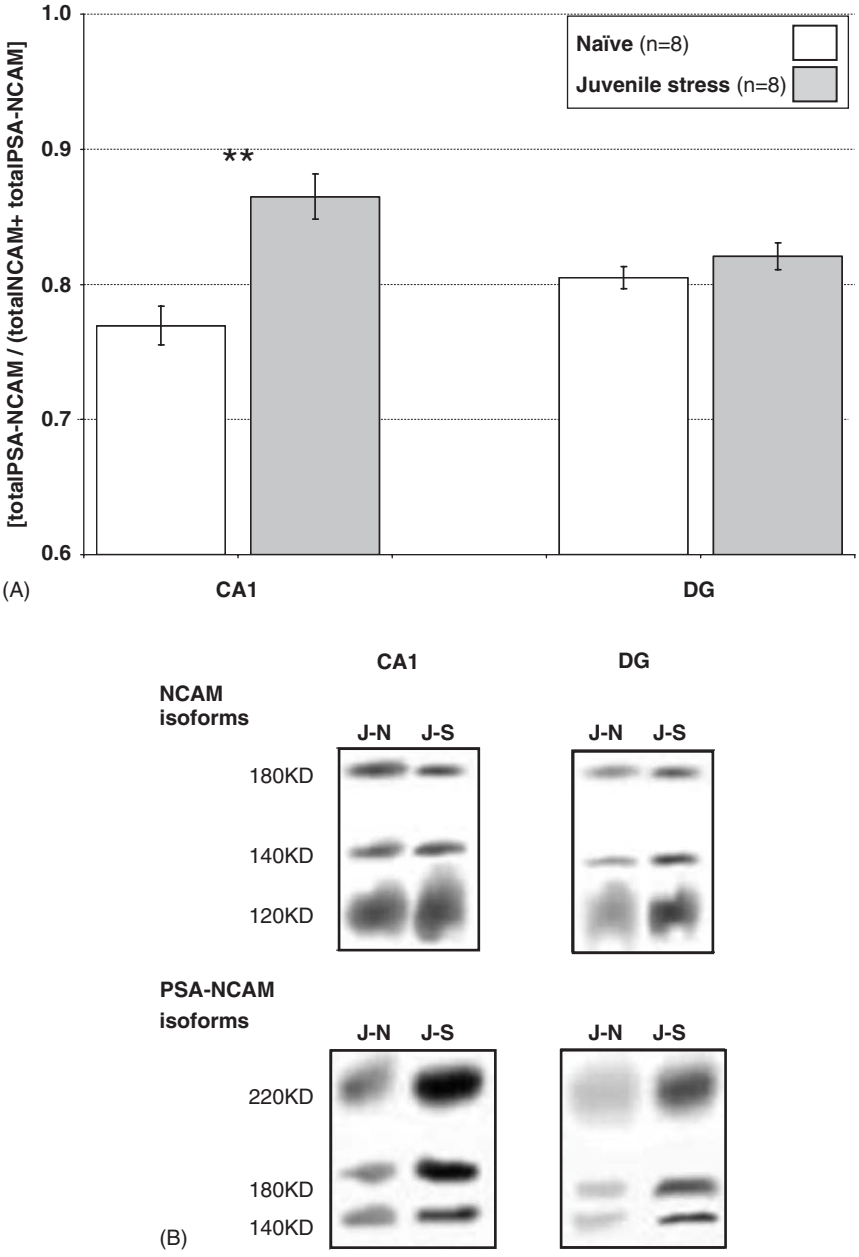

Fig. 5. The effect of exposure to stressors during juvenility on PSA-NCAM to NCAM expression ratio in the CA1 and DG subregions of the hippocampus 4 days after the exposure to "juvenile" stress. (A) Exposure to the "juvenile" stress protocol significantly increased the expression ratio of PSA-NCAM to NCAM in the CA1 but not in the DG ($^{**} = p < 0.01$). (B) The insets depict representative bands of NCAM isoforms (120, 140, 180 kDa); *upper panel*: PSA-NCAM isoforms (140, 180, 220 kDa); *lower panel*: across the J-N vs. J-S groups in the CA1 and DG.

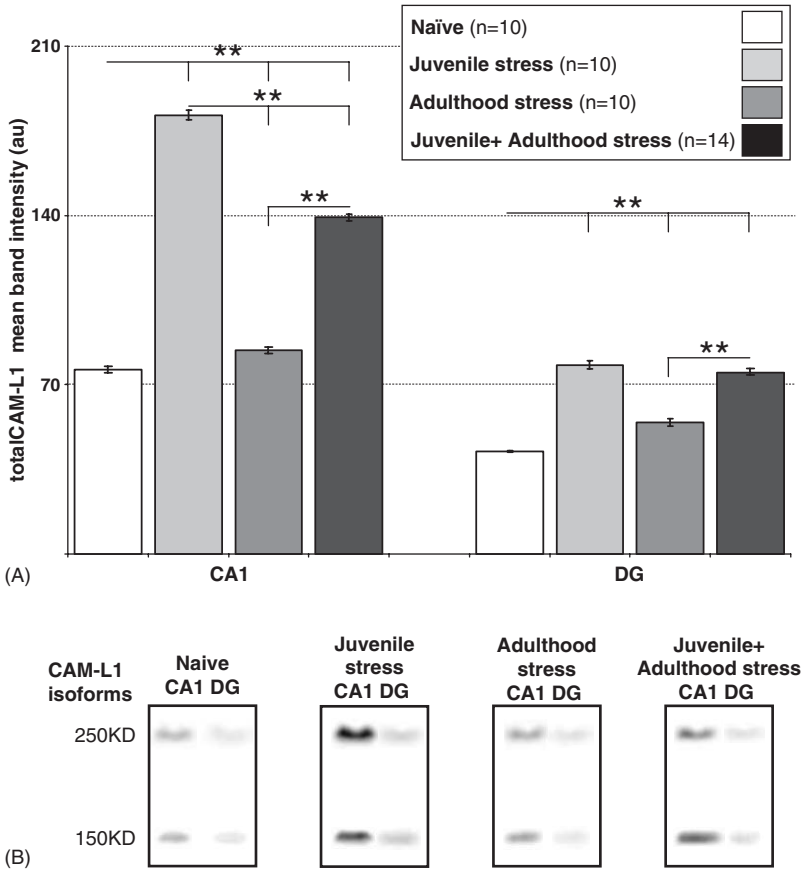

Fig. 6. The effects of exposure to stressors during juvenility, adulthood, or their recurrent combination on CAM-L1 expression in the CA1 and DG subregions of the hippocampus. (A) ANOVA of CAM-L1 expression levels across groups within each brain region indicated a significant main effect for groups in both the CA1 and DG ($p<0.01$). Significant post-hoc differences ($p<0.01$) between naïve rats and rats exposed to stress only in adulthood indicated that acute stress in adulthood significantly increased CAM-L1 expression levels in both CA1 and DG. Significant post-hoc differences between rats exposed only to juvenile stress and rats exposed to both juvenile and adulthood stress indicated that acute adulthood stress that followed a juvenile exposure to stress did not add to the juvenile stress-induced increase in the DG, but attenuated it in the CA1. ($^{**} = p<0.01$). (B) The insets depict representative bands of CAM-L1 isoforms (150, 250 kDa) across groups in the CA1 and DG.

Appendix

Box 1: the "juvenile stress" model

On the basis of the observations in humans indicating early-life stress exposure as a significant risk factor for the emergence and persistence of PTSD (Nemeroff et al., 2006), our "juvenile stress" model consists of: (1) exposure to stressors early in life, during juvenility (~28 days of age) and (2) a subsequent exposure to a stressful challenge in adulthood (60 days of age at the earliest).

Juvenile stress protocols

We utilized either a repeated exposure to the "platform stress" — i.e., at the ages of 26–28 days rats were placed on an elevated platform

for 30 min, three times a day; inter-trial interval (ITI), 60 min in home cage (Avital and Richter-Levin, 2005) — or a variable exposure, i.e., different inescapable stressors at the ages of 27–29 days: DAY1, forced swimming; DAY2, "platform stress;" DAY3, restraint or a short electric foot shock session (Tsoory and Richter-Levin, 2006; Tsoory et al., 2007a, b).

Adulthood stress protocols and behavioral assessments

We utilized in some studies a subsequent exposure to the "platform stress" (at either ~60 and/or 90 days of age), which was followed by the Open Field test, the Morris Water Maze task (spaced or massed training), and Acoustic Startle Response test (Avital and Richter-Levin, 2005). In other studies we employed the "two-way shuttle avoidance task" at 9 weeks of age (10 min free exploration in the apparatus; then one session comprising 100 trace conditioning trials); this task enabled us to simultaneously challenge the rats while assessing their ability to cope with learning under stressful conditions (Tsoory and Richter-Levin, 2006; Tsoory et al., 2007a, b).

The long-term consequences of exposure to "juvenile stress"

Adult rats exposed to stressors during juvenility and to a subsequent challenge in adulthood exhibited reduced exploration and increased avoidance from entering the arena's center in the Open Field, altered learning of the Morris Water Maze, and increased acoustic startle response (Avital and Richter-Levin, 2005). When challenged in the two-way shuttle avoidance task two "profiles" of altered coping with stress in adulthood were evident among adult juvenile stressed rats. (1) Anxious profile: low novel setting exploration, low rates of avoidance shuttles, moderate rates of escape shuttles, and low rates of escape failures; comprising ~40% of these rats. (2) Depressive profile: low novel setting exploration, low rates of avoidance shuttles, moderate rates of escape shuttles, and high rates of escape failures; comprising about a third of these rats. Less than a third of these rats appeared "unaffected" (Tsoory and Richter-Levin, 2006; Tsoory et al., 2007a). It is noteworthy that similar profiles and rates were evident when exposure to a predator scent was used as a stressor during juvenility and adulthood and behavioral profiling was based on altered behaviors in the elevated plus maze and startle responses (Tsoory et al., 2007a).

A substantial increase was observed in the expression ratio of PSA-NCAM to NCAM among adult juvenile stressed rats compared to adult juvenile stress-free rats in the BLA, CA1, DG, and EC (Tsoory et al., 2007b).

Abbreviations

BLA	basolateral amygdala
CA1	cornu ammonis field 1
CAM-L1	cell adhesion molecule L1
CORT	corticosterone
DG	dentate gyrus
DSM-IV	diagnostic and statistical manual of mental disorders, 4th edition
EC	entorhinal cortex
ELS	early-life stress
ERK2	extracellular signal-regulated kinases
HPA	hypothalamic-pituitary-adrenal
ITI	inter-trial interval
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
NCAM	neural cell adhesion molecule
NE	norepinephrine
PP	perforant path
PSA-NCAM	polysialylated neural cell adhesion molecule
PTSD	post-traumatic stress disorder

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Discussion: Chapter 3

OITZL: The developmental pattern of the HPA axis during early-life phase is well established. Can you comment on the characteristics of the HPA axis during the juvenile period?

RICHTER-LEVIN: On the one hand, in the juveniles, the HPA axis is beyond the stress hyporesponsive period and developed already. On the other hand, the stress response lingers when compared to the adult.

BUWALDA: What is the reason that you specifically choose 27–29 days as the juvenile age? Did you ever test at a later period, for instance at 50 days?

RICHTER-LEVIN: Yes, actually thank you for this question. We did test also at a later period. First, we found that in adulthood the juvenile stress population could be divided into two subgroups. A small subgroup showed symptoms of depression rather than of anxiety, but the majority of the animals were anxious. Exposed to stress a week later (around 36 days) the population with depressive symptoms disappeared and stressed rats only showed anxiety symptoms. This finding fits well with the human literature where we find indications that the exact age of exposure to trauma during childhood could make a difference in the potential risk for developing either depression or anxiety.

SCHMIDT: I wonder at what time you are weaning your animals and whether you think the stress of weaning will affect your paradigm.

RICHTER-LEVIN: This is a very good question. We have been dealing with this — because, of course, we want to be as close as possible to the natural weaning condition. We expected to find significant differences between animals that were weaned at age 21 or 28 days, but we did not. So these studies were done all with animals — the ones that I have shown here — that were weaned at age of 21. I believe it will be important to test effects in a population that was allowed weaning in a more natural setting (i.e., weaning as a process over several days).