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Amygdala norepinephrine levels after training predict inhibitory avoidance retention performance in rats

Christa K. McIntyre, Tammy Hatfield and James L. McGaugh
Center for the Neurobiology of Learning and Memory, and Department of Neurobiology and Behaviour, 218 Bonney Research Laboratory, University of California Irvine, Irvine, CA 92697–3800, USA

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Abstract

Previous findings indicate that footshock and several drugs that modulate memory consolidation alter norepinephrine (noradrenaline) release in the amygdala, as assessed by *in vivo* microdialysis and high-performance liquid chromatography. Such findings suggest that norepinephrine release in the amygdala may be critical for regulating memory consolidation. The present study was the first to examine the relationship between norepinephrine release in the amygdala assessed after inhibitory avoidance training and 24-h retention performance within individual animals. Norepinephrine levels increased to >300% of pretraining baseline 30 min after training and remained elevated for 2 h. In individual rats, the increase in norepinephrine levels after training correlated highly with 24-h retention performance. These findings indicate that the degree of activation of the noradrenergic system within the amygdala in response to a novel, emotionally arousing experience predicts the extent of long-term memory for that experience.

Introduction

Extensive research suggests that release of peripheral adrenal hormones in response to a stressor influences memory consolidation through the activation of central adrenoceptors (McGaugh, 2000). The findings of Gold & van Buskirk (1975, 1978), that peripherally administered epinephrine enhances memory and increases brain norepinephrine/noradrenaline (NE), provided the first evidence suggesting that the effects of this stress-released hormone on memory may involve the release of brain NE. Other findings suggest that noradrenergic activity in the amygdala may play a role in memory consolidation. Gallagher *et al*. (1977) and Gallagher & Kapp (1981) reported that β-adrenoceptor antagonists infused into the amygdala immediately after inhibitory avoidance training impaired retention performance and concurrent administration of NE attenuated the impairment. Post-training infusions of the β-adrenoceptor antagonist propranolol into the amygdala also block the memory enhancement induced by systemic administration of epinephrine (Liang *et al.*, 1986) as well as blocking the impairing and enhancing effects of drugs affecting GABA, opiate and glucocorticoid receptors (McGaugh *et al*., 1995). Moreover, post-training, intra-amygdala infusions of NE produce dose-dependent enhancement of memory (Liang *et al.*, 1986; Liang *et al*., 1990; Hatfield & McGaugh, 1999; but see Ellis & Kesner, 1983).

These pharmacological findings strongly suggest that endogenous NE released in the amygdala by training experiences influences memory consolidation. Findings of several studies using *in vivo* microdialysis combined with high-performance liquid chromatography provide additional support for this view. Footshock stimulation similar to that typically used in inhibitory avoidance training increases NE levels within the amygdala (Galvez, Mesches & McGaugh, 1996) and the amount of NE released varies directly with footshock intensity (Quirarte *et al*., 1998). Additionally, systemic injections of epinephrine or drugs that enhance memory consolidation, including the GABAergic antagonist picrotoxin and the opioid peptidergic antagonist naloxone, also increase NE levels in the amygdala (Quirarte *et al*., 1998; Williams *et al*., 1998; Hatfield, Spanis & McGaugh, 1999). Conversely, drugs that impair memory consolidation, including the GABAergic agonist muscimol and the opioid peptidergic agonist β-endorphin, decrease NE release in the amygdala (Hatfield, Spanis & McGaugh, 1999; Quirarte *et al*., 1998).

Considered together, these findings strongly suggest that NE release in the amygdala may play a critical role in mediating the influences of drugs and emotional arousal on memory consolidation (McGaugh *et al*., 2000). However, to date, studies have not investigated the relationship between NE released after training and retention performance. If the release of NE within the amygdala influences memory consolidation, inhibitory avoidance training should release amygdala NE and 24-h retention performance should vary with the amount of NE released. The present experiments examined these implications.

Materials and methods

Subjects and surgery

Forty-five male Sprague–Dawley rats, weighing 300–350 g, were housed individually and maintained on a 12-h light–dark cycle. Animals were treated in accordance with NIH guidelines and procedures were in approved by the IACUC. Rats were anaesthetized for surgery with sodium pentobarbital (50 mg/kg). A plastic guide cannula (CMA/12; Carnagie Medicin, Stockholm, Sweden) was implanted unilaterally 1 mm above the lateral nucleus of the

Correspondence: Dr Christa K. McIntyre, as above.
E-mail: cmcintyr@uci.edu

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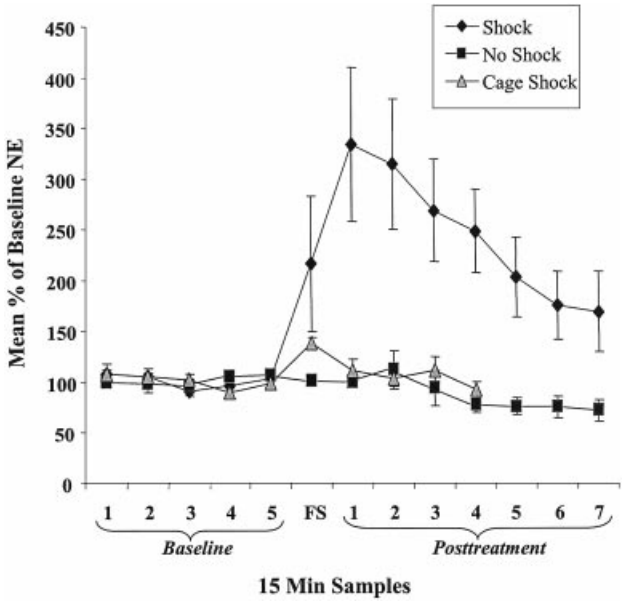

FIG. 1. Effects of inhibitory avoidance training on norepinephrine (NE) levels in the amygdala. Rats in the shock group received a 0.55-mA, 1-s footshock (FS) during training on the inhibitory avoidance task. Rats in the no shock group were given the same inhibitory avoidance training but did not receive a footshock. Rats in the cage shock group received a 0.55-mA, 1-s footshock in the holding cage but were not exposed to the inhibitory avoidance apparatus. Microdiaysis samples were collected every 15 min and automatically injected into an HPLC system optimized for norepinephrine detection. Norepinephrine levels (mean ± SEM) are expressed as a percentage change from average baseline levels. A repeated-measures ANOVA revealed a significant group effect ($P < 0.01$) and a significant effect of sample in both shocked groups ($P < 0.01$).

amygdala (anterior–posterior, –2.8; medial–lateral, +4.8; dorsal–ventral, –6.6 mm), according to the atlas of Paxinos & Watson (1997). After two days of recovery from surgery, rats were handled for 5 days (5 min/day). Microdialysis and behavioural training were conducted 7 days after surgery.

HPLC and microdialysis

Norepinephrine levels were examined using *in vivo* microdialysis combined with high-performance liquid chromatography (HPLC) with coulometric detection (ESA Coulochem II, Chelmsford, MA, USA). After 5 days of handling, a dialysis probe (CMA 12, 1-mm membrane tip; Carnegie Medicin, North Chelmsford, MA, USA) was inserted through the guide cannula at least 1 h before baseline measurements were collected. Rats were allowed to move around freely during collection of baseline samples in a clear Plexiglas holding cage with bedding covering the floor. Artificial cerebrospinal fluid (in mM: NaCl, 128; KCl, 2.5; $CaCl_2$, 1.3; $MgCl_2$, 0.998; Na_2HPO_4, 1.3; and glucose, 1, brought to pH 6.5 with NaOH) was perfused through the probe at a rate of 1 μL/min. The probe recovery rate was determined *in vitro* by inserting the microdialysis probe into a standard solution of NE. Both standard solution and dialysate were injected into the HPLC to determine the percentage of NE taken up by the probe. Samples collected through the microdialysis probe were automatically injected through HPLC equipment using an on-line injector (CMA/160) and analysed every 15 min. The HPLC system was optimized for detection of NE (E1 = –100 mV, E2 = 190 mV, range 10 nA). Mobil phase [ESA Inc. MDTM consisting of: 75 mM monobasic sodium dihydrogen phosphate, 1.7 mM 1-octanesulfonic acid sodium salt, 200 μL triethylamine (TEA), 25 μM EDTA and 10% acetonitrile in 200 mL of distilled, deionized water] was pumped (ESA/580) at a flow rate of 0.5 mL/min.

Inhibitory avoidance apparatus and procedure

After five stable baseline samples were collected, rats were trained on the inhibitory avoidance task. The apparatus was a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top and 6.4 cm wide at the floor) which was divided into two compartments by a retracting door. The lighted compartment (31 cm long) consisted of opaque white plastic walls and floor. The floor and walls of the dark (not illuminated) compartment (60 cm long) were made of metal plates.

On the training trial, rats were placed in the lighted compartment of the inhibitory avoidance box. After a rat entered the dark compartment, the door between the two compartments was closed and the latency to enter the dark compartment was recorded. After reaching the far end of the dark alley and turning around (≈ 5 s), one group of rats received footshock (0.55 mA for 1 s) delivered through the floor of the dark compartment and 10 s later were returned to the holding cage. Control rats did not receive a footshock and were returned to the holding cage 10 s after reaching the far end of the dark compartment and turning around. Microdialysis samples were collected continuously throughout training and for 2 h following the training procedure. A retention test was given 24 h following training. Procedures were the same for testing and training except that no shock was given and microdialysis samples were not collected on the test day. Latency to enter the dark compartment on the test day was used as the retention score.

Cage controls

In a third group of rats, NE levels were monitored for five baseline samples. In place of inhibitory avoidance training, rats remained in the holding cage where a single 0.55-mA, 1-s footshock was delivered from a grid at the base of the holding cage. Following footshock, four post-treatment microdialysis samples were collected at 15-min intervals.

Histology

On completion of the experiment, the rats received a lethal dose of sodium pentobarbital (100 mg/kg; i.p.; Sigma) and were perfused transcardially with 0.9% sodium chloride and 10% formalin. Brains were removed, then sectioned and mounted on slides and stained with Cresyl violet. Brain sections were analysed for probe placement. Rats with probes contained within the amygdala were included in statistical analysis while rats with probe placements outside the amygdala or with extensive damage to the amygdala were excluded.

Data analysis

Eight rats trained on the inhibitory avoidance task with footshock, seven rats from the group trained on the inhibitory avoidance task with no footshock and five rats shocked in the holding cage were used for statistical analysis. Twenty-five rats were excluded, in total, from the experiment. Histology identified six rats that had lesions extending from the probe and 19 rats had probes placed outside the amygdala. Norepinephrine levels in these rats were either lower than detection limits or unstable. NE levels in microdialysis samples from an individual rat were normalized by comparing each sample to the rat's average level of NE in the five samples taken before training (baseline). Differences in the mean percentage of baseline NE between and within groups were analysed using repeated-measures analysis of variance (ANOVA). Significant differences were further

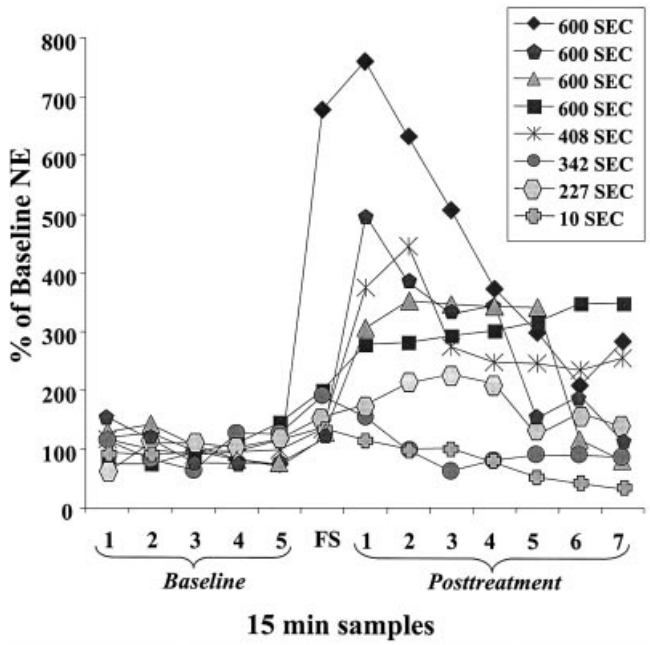

FIG. 2. Effects of inhibitory avoidance training with footshock on levels of norepinephrine in the amygdala. Each line represents norepinephrine levels as a percentage of baseline for an individual rat. Latency to enter the dark compartment on the retention test 24 h after microdialysis and training is identified in the key.

explored using a Fisher's post-test for between-group differences. A two-tailed Student's *t*-test was used to make within-group comparisons. The mean value from each 15-min sample was expressed as a percentage of baseline (100%). A repeated-measures ANOVA was used to assess differences in NE release in animals with retention latencies above and blow the median latency. Additionally, nonparametric Spearman rank correlations were used to evaluate the relationship between NE values and retention latencies in individual rats. Mean values are quoted.

Results

A repeated-measures ANOVA comparing mean percentage of baseline NE ± SEM (0.315 ± 0.03 pg/μL) in 15-min samples taken from rats trained on the inhibitory avoidance task with footshock vs. samples taken from rats trained without shock (Fig. 1) revealed a significant difference between the two groups ($F_{12,156} = 5.4$, $P < 0.001$). A Fisher's post-test indicated that the Shocked vs. Nonshocked comparisons differed significantly in all seven samples following the 15-min sample collected during inhibitory avoidance training ($P < 0.05$ for all comparisons). A repeated-measures ANOVA also revealed a significant effect of Sample for the group of rats given footshock during inhibitory avoidance training ($F_{12,84} = 6.2$, $P < 0.001$). Consistent with previous findings (Galvez, Mesches & McGaugh, 1996; Quirarte *et al*., 1998; Hatfield, Spanis & McGaugh, 1999), a repeated-measures ANOVA revealed a significant within-group difference among NE samples collected from rats given footshock in the holding cage ($F_{9,36} = 4.1$, $P < 0.001$; see Fig. 1). Additionally, the increase in NE levels across the two groups (Trained vs. Cage control) was significantly different following footshock ($F_{9,99} = 4.2$, $P < 0.001$). Fisher's post-test indicated significant differences between rats shocked in the training apparatus vs. those shocked in the holding cage in all samples following the sample collected during the footshock ($P < 0.05$ for each comparison). In rats that were shocked in the holding cage, a two-tailed Student's *t*-test revealed a significant difference between the sample collected during footshock exposure vs. mean percentage of NE during baseline (100%) ($t_{7,5} = 6.3$, $P < 0.001$), but no significant differences were observed in samples following footshock exposure. In rats trained on the inhibitory avoidance task, percentage of baseline NE in the sample collected during inhibitory avoidance training did not differ significantly from baseline while a two-tailed Student's *t*-test indicated that post-treatment samples 1–5 were significantly different from baseline ($P < 0.05$ for each comparison).

Of the rats that received footshock in the inhibitory avoidance apparatus, those with higher retention latencies also had greater increases in NE following training (Fig. 2). An ANOVA indicated that, in NE samples taken after inhibitory avoidance training, the NE levels of rats with retention latencies above the median were significantly higher than those of rats with retention latencies below the median ($F_{12,48} = 2.3$, $P < 0.05$). Fisher's post-test showed significant differences between the groups in mean percentage of baseline NE from post-treatment samples 3, 4 and 5 ($P < 0.05$ for each comparison). In rats trained on the inhibitory avoidance task with footshock, Spearman rank correlations revealed a significant relationship between NE levels in each of the first five post-training samples and retention latencies [post-treatment sample (1) $r = +0.80$, $P < 0.05$; (2) $r = +0.75$, $P < 0.05$; (3) $r = +0.87$, $P < 0.05$; (4) $r = +0.92$, $P < 0.05$; (5) $r = +0.86$, $P < 0.05$] but no significant correlations were seen in the nonshocked group [post-treatment sample (1) $r = -0.24$, $P > 0.5$; (2) $r = -0.06$, $P > 0.5$; (3) $r = +0.13$, $P > 0.5$; (4) $r = -0.12$, $P > 0.5$; (5) $r = +0.01$, $P > 0.5$].

Discussion

The main finding of the present study is that NE levels assessed after inhibitory avoidance training correlated highly with retention latencies on the following day. This finding provides additional support for the hypothesis that NE release in the amygdala plays a key role in mediating the effect of emotional arousal on memory consolidation (McGaugh *et al*., 2000). Previously, this hypothesis was supported by pharmacological findings and evidence from studies using *in vivo* microdialysis and HPLC examining effects of footshock and drugs on NE release in the amygdala. The novel finding of the present study is that the degree of amygdala NE activation following an aversive training experience predicts the long-term memory of the experience in groups of rats as well as in individual rats.

A somewhat surprising finding was that NE levels increased to >300% of baseline and remained elevated for 2 h following training whereas the increase in NE levels following a footshock of the same intensity and duration delivered in the holding cage was smaller (140% of baseline) and transient (lasting only 15 min). The NE response to footshock given in the holding cage is consistent with previous findings (Galvez, Mesches & McGaugh, 1996; Quirarte *et al*., 1998; Hatfield, Spanis & McGaugh, 1999). It is likely that the prolonged increase in NE levels seen in the current study was due to the footshock training, as NE release was not increased in rats that were placed in the inhibitory avoidance apparatus and given no footshock. The change observed in norepinephrine levels may reflect either increased release or reduced reuptake, or both. It is possible that various hormonal and neurotransmitter systems affected by novelty or arousal may influence release and/or reuptake of NE. The prolonged NE increase seen in rats that were shocked during training

closely resembled that seen after administration of the GABAergic antagonist picrotoxin (Hatfield, Spanis & McGaugh, 1999), as well as that induced by the opioid antagonist naloxone, administered immediately after a footshock given in the holding cage (Quirarte *et al*., 1998). Both of these drugs enhance memory when administered after training (Brioni & McGaugh, 1988; McGaugh, Introini-Collison & Nagahara, 1988). These findings suggest that the prolonged increase in NE seen in the current study reflects sustained activity of neurotransmitter systems activated by the concurrent contextual and footshock stimulation received in the inhibitory avoidance training. Additionally, those rats that received a footshock in the holding cage had >2 h to habituate to the context. Therefore, it is possible that latent inhibition to the familiar context contributed to the smaller increase seen in norepinephrine levels in cage-control rats.

The noradrenergic system within the amygdala appears to be important for memory processing immediately, or up to 24 h, after training, but does not influence memory retrieval processes 1 month after training (Bevilaqua *et al*., 1997; Izquierdo *et al*., 1997). Lesions of the stria terminalis, a major output pathway of the amygdala, block memory enhancement induced by post-training systemic injections of epinephrine (Liang & McGaugh, 1983), as well as that induced by intra-amygdala infusions of NE (Liang *et al.*, 1986). This evidence, considered together with evidence of a time-limited role of the amygdala in memory processing (Packard, Cahill & McGaugh, 1994; Parent & McGaugh, 1994; Bevilaqua *et al*., 1997), suggests that consolidation of emotionally arousing memories involves amygdala modulation of plasticity in efferent brain regions. More specifically, the findings of recent studies indicate that noradrenergic activation of the basolateral region of the amygdala is critical in enabling amygdala influences on memory consolidation in other brain regions (Roozendaal *et al*., 1999; McGaugh *et al*., 2000; Roozendaal, Quirarte & McGaugh, 2002).

In summary, the present findings provide strong evidence that the modulation of long-term storage of an emotionally arousing event involves prolonged activation of the noradrenergic system within the amygdala. Moreover, the finding that the degree of activation of the noradrenergic system following training predicts retention performance supports the view that the noradrenergic system within the amygdala plays a central role in memory consolidation.

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Abbreviations

ANOVA, analysis of variance; HPLC, high performance liquid chromatography; NE, norepinephrine/noradrenaline.

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