Author

Without question, Anna Litmanovich is a "Renaissance woman." Her first four years of school were spent studying piano in Uzbekistan, the country of her birth. Since coming to the United States, Anna has excelled in both the arts and sciences. For the past six years she has performed for Opera Pacific in Costa Mesa, appearing in nine operas and one ballet. While at UCI, she has investigated the effects of drugs and hormones on memory storage and has tutored students ranging from adopted Russian children who cannot speak English to fellow undergraduates in such fields as math, psychology, physics, and biology. If that's not enough, she also plays competitive tennis. Anna hopes to pursue a career in academic medicine.

Key Terms

- Agonists
- Antagonists
- Basolateral Amygdala
- Inhibitory Avoidance
- Muscarinic Cholinergic Agents
- Muscarinic Receptors

Memory Enhancement Involves Activation of Both Type 1 and Type 2 Muscarinic Cholinergic Receptors in the Basolateral Amygdala

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Abstract

The basolateral amygdala (BLA) is the putative site for integration of neuronal and hormonal signals for emotional learning and memory. The present study explored which muscarinic receptor type(s) (M1, M2 or both) mediates the critical cholinergic activation in the BLA during memory-modulating processes. Sprague-Dawley rats were implanted with bilateral cannulae aimed at the BLA and then trained on an inhibitory avoidance (IA) task. To selectively activate each receptor type, selective antagonists, methoctramine or telenzipine (50 nmol per side), were co-infused with a general muscarinic receptor agonist, oxotremorine, to stimulate M1 or M2 receptors, respectively. Oxotremorine (50 nmol per side) was infused alone to stimulate both receptor types. A single trial IA task was used in combination with immediate post-training drug treatments so that the consolidation phase of memory could be selectively manipulated. The mean retention latency of oxotremorine-only group in the 48-hr retention test was significantly higher than the mean retention latencies of the groups that received co-infusion of telenzipine or methoctramine with the oxotremorine. These findings indicate that both muscarinic receptor types need to be activated in order for memory enhancement to occur.

Faculty Mentor

Animals have evolved the amazing ability to store memory better for emotionally significant events. This ability depends on a convergence of hormonal and neurochemical signals in a small region of the medial temporal lobe of the brain called the basolateral amygdala (BLA). The neurotransmitter acetylcholine has been found to play a critical role in BLA modulation of memory storage via activation of muscarinic receptors. This project was the first analysis of the muscarinic receptor subtypes, which mediate this cholinergic activation of the BLA during consolidation. In collaboration with researchers from my laboratory, including Dr. Ann E. Power, who was directly responsible for guiding Anna's research, Anna learned that cholinergic modulation of memory involves activation of both excitatory and inhibitory receptor systems in the BLA. These findings enhance our understanding of the neurobiological mechanisms of modulation of memory storage by emotion and arousal.

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Introduction

Learning and memory are influenced by the emotional and motivational state of an organism. Extensive evidence indicates that the amygdala is a critical site of integration for sensory, neuromodulatory and hormonal influences on memory storage (Liang et al., 1986; McGaugh et al., 1996). Electrical or drug-induced stimulation of the amygdala immediately after training can produce memory enhancement or impairment, depending upon experimental conditions (Gold and van Buskirk, 1978; Gallagher et al., 1981; Cahill and McGaugh, 1991). Specifically, the basolateral region of the amygdala (BLA) plays a central role in influencing the strength of memory storage. Post-training memory modulatory treatments are ineffective if the BLA is lesioned (Roozendaal et al., 1996), and direct selective manipulations of the BLA are sufficient to influence memory strength (Quirarte et al., 1997; Da Cunha et al., 1999; Hatfield et al., 1999; Power et al., 2000).

Behavioral pharmacological studies have revealed a robust influence of muscarinic cholinergic agents in particular on memory modulatory processes. Intra-BLA administration of muscarinic cholinergic agonists facilitate memory storage (Vazdarjanova and McGaugh, 1999; Power and McGaugh, 2002), while intra-BLA administration of muscarinic cholinergic antagonists block memory enhancement induced by intra-BLA or peripheral memory-enhancing treatments (Introini-Collison and McGaugh, 1988; Dalmaz et al., 1993; Introini-Collison et al., 1996; Salinas et al., 1997; Power et al., 2000). These findings indicate that activation of amygdaloid muscarinic cholinergic receptors is critical for enabling modulatory influences on memory consolidation.

An abundance of cholinergic synapses and muscarinic cholinergic receptors have been observed in the BLA (Mash and Potter, 1986; Spencer et al., 1986). The population of these synapses in the BLA is heterogeneous, including both excitatory (asymmetric) and inhibitory (symmetric) synapses (Li et al., 2001; Wainer et al., 1984). In accordance with this heterogeneity of synapses, the BLA contains high densities of both the major excitatory and inhibitory muscarinic receptors types, M1 and M2, respectively (Hammer et al., 1980; Peralta et al., 1988; Brann et al., 1987; Pinkas-Kramarski et al., 1988). Recent findings suggest that the nucleus basalis magnocellularis (NBM), which sends dense cholinergic projections into the BLA (Mesulam, et al., 1983), is the critical source of cholinergic input to the BLA during modulation of memory storage (Power and McGaugh, 2002). However, the neuronal mechanisms underlying the critical role of cholinergic activation in the BLA during memory modulating processes are not well understood. Therefore, this study investigated which muscarinic receptor type(s) influence BLA-mediated memory modulation.

Sprague-Dawley rats (Charles River Laboratories) were surgically implanted with bilateral cannulae aimed at the BLA and one week later trained on a single trial inhibitory avoidance (IA) task. Rats received post-training intra-BLA infusions in order to selectively affect the consolidation phase of memory. To selectively activate each receptor type, a muscarinic receptor type-specific antagonist was co-infused with a general muscarinic receptor agonist to stimulate either M1 or M2 receptors. The general agonist was administered alone to stimulate both receptor types. If either the M1 or M2 receptors are mediating the memory modulating influences of cholinergic activation in the BLA, then the general muscarinic agonist would not affect IA memory in the presence of the antagonist to that receptor.

Methods and Materials

Subjects

Seventy-seven Sprague-Dawley adult male rats, weighing approximately 300 g at the time of surgery, were used in this study. The animals were housed individually in a temperature- and light-controlled environment for 1 wk prior to surgery. The subjects had free access to food and water. Behavioral training and testing were conducted between 8 a.m. and 5 p.m. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of UCI, under protocol #98-1420.

Surgery

The rats were anesthetized with nembutal (50 mg/kg), given atropine sulfate (0.1 mg) to maintain respiration, and injected subcutaneously with 2.5 ml of 0.9% saline to prevent dehydration during surgery. The animals were placed in a stereotaxic apparatus (Kopf Instruments) and given a subcutaneous injection of a local anesthetic (lidocaine) before the scalp was incised. Cannulae (15 mm, 23 gauge) were implanted and aimed bilaterally at the BLA with the following coordinates: 2.8 mm posterior and ±5.0 mm lateral to bregma, and 6.5 mm ventral to the skull surface (Paxinos and Watson, 1997). Two small, stainless steel screws served as anchors. The cannulae and screws were fixed with dental cement. To prevent occlusion, 15 mm stylets were placed in the cannulae. Post-surgically, the rats were placed in a warm incubator until they awoke from anesthesia. During recovery, rats received daily gentle handling to habituate them to

the infusion procedure, and missing stylets were replaced. Behavioral experiments commenced 1 wk after surgery.

Inhibitory Avoidance

The IA apparatus is a narrow, trough-shaped box (91 cm by 20 cm) divided into a light (30 cm) and a dark shock compartment (60 cm). A retractable door (1 cm) separates the light and dark compartments. The behavioral procedures were performed in a light- and sound-proof room. Each rat was placed in the light compartment and was allowed to enter the dark compartment. When the rat stepped (with all four paws) into the dark compartment the retractable door separating the two compartments was closed and the rat was administered a mild footshock (0.5 mA, 1.0 sec). The rat was then taken from the dark compartment, administered the appropriate drug solution into the BLA via the cannulae, and returned to its home cage. Two days later the rats were tested for retention of the IA training. Rats were placed into the light compartment, as in training, and the time elapsed before each rat entered the dark compartment was recorded (retention latency). Acquisition of the shock-context association was confirmed by comparing the entrance latencies (before drug administration) and the retention latencies (48-hr post-drug administration). A retention latency longer than the entrance latency implied avoidance of the dark compartment and therefore memory of the training. Memory in a treated group was considered enhanced if retention latencies were significantly longer than that of the vehicle-treated control group.

Drug Administration

Each rat received bilateral infusions into the BLA of one of the following: saline vehicle (control), oxotremorine (oxo, a nonselective muscarinic receptor agonist; 10 ng in 0.2 μl per side), a mixture of oxo + telenzipine (tel, selective M1 receptor antagonist; 50 nmol per side), or a mixture of oxo + methoctramine (met, selective M2 receptor antagonist; 50 nmol per side). The drug solutions were infused over approximately 25 sec via a 30-gauge needle connected to PE-20 polyethylene tubing pushed by an electronic infusion pump (Sage Instruments). The infusion needle was left in place for an additional 30 sec to allow diffusion of the drug solution into the BLA. Previous work conducted in this laboratory has shown that this infusion volume allows selective infusion into the BLA (Roozendaal and McGaugh, 1997; Da Cunha et al., 1999) and that this dose of oxo is effective for enhancing IA retention (Vazdarjanova and McGaugh, 1999). These doses of tel and met have been shown to be selective in the amygdala (Aslan et al., 1997). All drugs were purchased from RBI.

Histology

Following behavioral testing, the subjects were anesthetized with an overdose of sodium pentobarbital (100 mg/kg, intra-peritoneal) and perfused intracardially with 0.9% saline solution to clear the blood, followed by 4% formaldehyde to fix the brain tissue. After extraction from the skulls, brains were post-fixed for 2 days in 4% formaldehyde solution. The brains were then transferred to 30% sucrose in saline solution for cryoprotection until they sank and were then sliced into 40-μm coronal sections on a freezing microtome. Sections were mounted onto gelatin-treated slides and stained with thionin to visualize the infusion sites. Subsequently, the slides were examined under the microscope to verify that the tips of the infusion needles were in the BLA, and the infusion locations were recorded. Only those rats with the tips of both infusion needle tracks in the BLA were used for behavioral data analysis.

Statistics

As neither ceiling nor floor effects were observed, parametric statistical analysis was conducted. The IA data were subjected to an analysis of variance (ANOVA). If indicated by a significant outcome of the ANOVA, post-hoc comparisons using Fisher's Protected Least Significant Difference test were conducted to determine the sources of a detected significance.

Results

Histology

Thirteen rats had one or both infusion sites located outside the borders of the BLA. Only behavioral data from the 64 remaining rats with proper cannulae placement were used for statistical analysis. A representative photomicrograph of an intra-BLA infusion needle tip is shown in Figure 1. The loci of a representative group with acceptable intra-BLA infusion needle tips are shown in Figure 2.

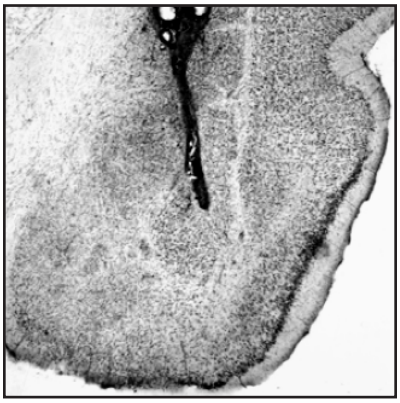

Figure 1
Representative photomicrograph of an infusion needle terminating in the BLA

Inhibitory Avoidance

The mean retention latencies for the vehicle, oxo, oxo + tel, and oxo + met groups were 48 ± 9 sec, 152 ± 32 sec, 31 ± 5 sec, and 51 ± 11 sec, respectively (Figure 3). The overall ANOVA revealed a significant

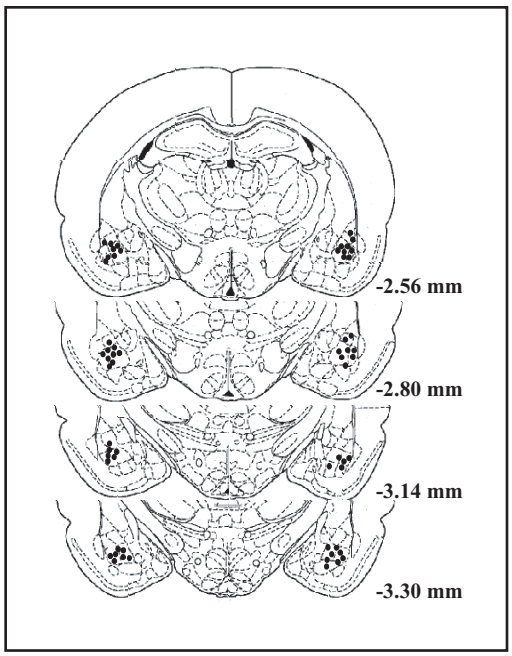

Figure 2
Acceptable sites of intra-BLA infusion (coordinates are relative to bregma)

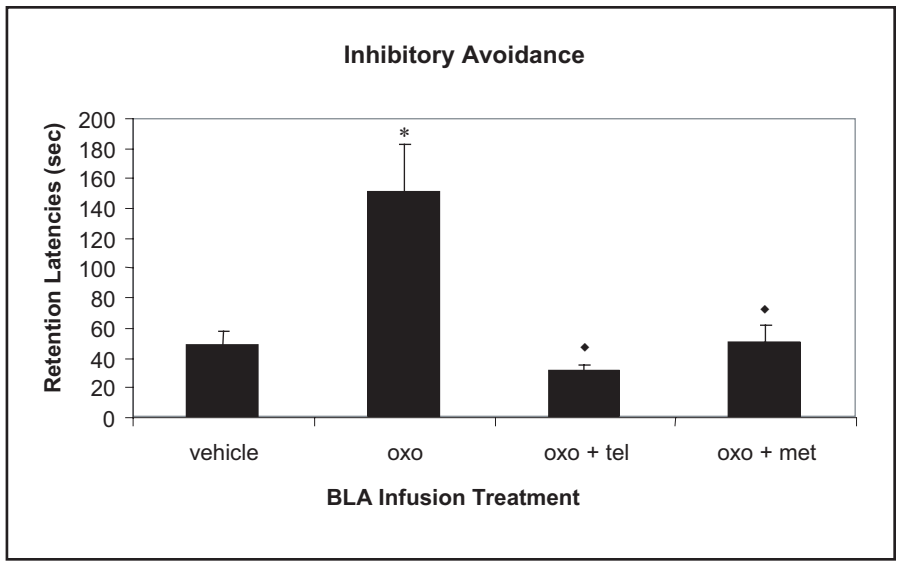

Figure 3
* ($p < 0.0005$) Post-training intra-BLA oxo significantly enhanced memory in a 48-hr retention test.
♦ Subtype specific antagonists (tel or met) were co-infused with a general muscarinic receptor agonist (oxo) to selectively stimulate either M1 or M2 type receptors. Co-infusion of either selective antagonist blocked the oxo-induced memory enhancement ($ps < 0.0005$). The performance for both oxo + tel and oxo + met was not significantly different from the vehicle control group ($ps > 0.05$).

effect of BLA treatment ($F_{3,60} = 9.27$; $p < 0.0005$). The mean retention latency for the oxo group was significantly higher than that of the vehicle controls ($p < 0.0001$). The oxo + tel and oxo + met groups' mean retention latencies were significantly less than the oxo group alone ($p < 0.0001$ and $p < 0.0005$, respectively), but did not differ from that of the vehicle control group ($p > 0.05$). Therefore, the intra-BLA oxo infusion was ineffective when co-infused with either M1- or M2-receptor antagonist.

Discussion

The present study investigated the mechanisms underlying the requirement for muscarinic cholinergic activation in the BLA during modulation of memory consolidation. Specifically, the involvement of M1 and M2 muscarinic receptor types in BLA-mediated memory enhancement was tested. This study was consistent with previous findings in demonstrating enhanced memory, as evidenced by increased retention latencies, with post-training intra-BLA infusion of oxo (Vazdarjanova and McGaugh, 1999). In the present study, intra-BLA oxo-induced memory enhancement was blocked by simultaneous infusion of either tel (selective M1 receptor antagonist) or met (selective M2 receptor antagonist). These results suggest that both M1 and M2 receptor activation in the BLA are required for memory modulation rather than either receptor exclusively mediating cholinergic involvement in memory modulation.

Many possible mechanisms may underlie the required interaction of M1 and M2 receptor types in the BLA during modulation of memory storage. The potent excitatory influence of NBM stimulation or muscarinic cholinergic agonist administration on the BLA (Washburn and Moises, 1992; Yajeya et al., 1997) suggests that perhaps M1 and M2 receptors regulate the excitability of both the inhibitory GABAergic interneurons and the pyramidal projection neurons. For example, acetylcholine may directly increase the excitability of pyramidal projection via M1 receptors while simultaneously inhibiting GABAergic interneurons via the M2 receptors. Such a synergistic mechanism of interaction could regulate BLA responsivity to the glutamatergic inputs from the cortex as well as influences from other neuromodulatory inputs, including the noradrenergic, serotonergic and dopaminergic projections. Electron microscope observations of both excitatory and inhibitory cholinergic synapses on BLA neurons are consistent with this view (Wainer et al., 1984; Li et al., 2001). Testing this hypothesis and gaining a working model of cholinergic circuitry in the BLA will require future studies that examine the expression

of muscarinic receptor types on BLA neuronal populations. In addition, the presence of cholinergic axo-axonic synapses in the BLA (Wainer et al., 1984) suggests that the release of other neurotransmitters may be regulated directly by cholinergic activation. *In vivo* microdialysis studies could determine whether levels of other neurotransmitters in the BLA are influenced by cholinergic agents infused into the BLA.

Studies of dementia due to basal forebrain degeneration, such as Alzheimer's Disease (AD), have concentrated primarily on the degeneration of cholinergic projections to the cortex. However, cholinergic input to the BLA is also compromised in these disorders and the excitotoxic NBM-lesion models for AD (Kesner et al., 1990). Such a decrement in BLA acetylcholine may contribute to the cognitive deficits associated with NBM neuronal loss (Heckers and Mesulam, 1994; Power et al., 2002). Consistent with this hypothesis are findings that selective immunolesions of the NBM-neocortical projections do not produce the robust and reliable memory deficits observed with non-selective excitotoxic lesions of the NBM (Wenk et al., 1994; Waite and Thal, 1996; Wrenn and Wiley, 1998; Power and McGaugh, 2002), which destroy cholinergic efferents to the amygdala as well as to the neocortex (Kesner et al., 1990; Heckers and Mesulam, 1994).

Conclusion

In summary, this study investigated the mechanisms by which cholinergic activation critical to BLA-mediated memory modulatory processes is mediated, specifically, by which muscarinic receptor type(s). These results indicate that activation of both excitatory M1 and inhibitory M2 receptors is necessary for the induction of BLA-mediated memory modulation. We propose that the requirement for both receptor types may be due in part to M2-mediated inhibition of GABAergic interneurons and M1-mediated excitation of pyramidal projection neurons in the BLA. Furthermore, muscarinic cholinergic synapses in the BLA directly regulating the release of other neurotransmitters may be critical. Additional research is needed to test these hypotheses and to elucidate the interaction of the M1 and M2 receptors.

This study provides insight into the mechanisms of memory storage enhancement by analyzing the molecular mechanisms that underlie memory formation. Furthermore, this study and the general goal of similar experiments may provide vital information regarding the mechanisms of memory impairment such as blocking natural, emotionally-enhanced memory in post-traumatic stress syndrome. Overall, this experiment provides crucial elucidation of the brain processes underlying the effects of drugs and stress hormones on memory storage.

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