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Lidocaine attenuates anisomycin-induced amnesia and release of norepinephrine in the amygdala

Renee N. Sadowski[1], **Clint E. Canal**[*,1], and **Paul E. Gold**[1,2]

[1] Neuroscience Program, University of Illinois at Urbana-Champaign, Champaign, IL 61820

[2] Departments of Psychology, Molecular and Integrative Physiology, and Psychiatry, College of Medicine, University of Illinois at Urbana-Champaign, Champaign, IL 61820

Abstract

When administered near the time of training, protein synthesis inhibitors such as anisomycin impair later memory. A common interpretation of these findings is that memory consolidation requires new protein synthesis initiated by training. However, recent findings support an alternative interpretation that abnormally large increases in neurotransmitter release after injections of anisomycin may be responsible for producing amnesia. In the present study, a local anesthetic was administered prior to anisomycin injections in an attempt to mitigate neurotransmitter actions and thereby attenuate the resulting amnesia. Rats received lidocaine and anisomycin injections into the amygdala 130 and 120 min, respectively, prior to inhibitory avoidance training. Memory tests 48 hr later revealed that lidocaine attenuated anisomycin-induced amnesia. In other rats, *in vivo* microdialysis was performed at the site of amygdala infusion of lidocaine and anisomycin. As seen previously, anisomycin injections produced large increases in release of norepinephrine in the amygdala. Lidocaine attenuated the anisomycin-induced increase in release of norepinephrine but did not reverse anisomycin inhibition of protein synthesis, as assessed by c-Fos immunohistochemistry. These findings are consistent with past evidence suggesting that anisomycin causes amnesia by initiating abnormal release of neurotransmitters in response to the inhibition of protein synthesis.

Keywords

norepinephrine; memory consolidation; memory modulation; microdialysis; anisomycin; protein synthesis inhibitors; amygdala; lidocaine

Introduction

The predominant model for the molecular basis of memory is that memory formation occurs in two stages: an early transient stage independent of protein synthesis and a long-term memory phase dependent on training-initiated *de novo* protein synthesis (cf.: Davis & Agranoff, 1966; Davis & Squire, 1984; Schafe & Ledoux, 2000; Kandel, 2001; Dudai, 2002;

Corresponding author: Dr. Paul E. Gold, University of Illinois, 603 E. Daniel Street, Champaign, IL 61820, Office: (217) 244-0673; Department Fax: (217) 244-5876, pgold@illinois.edu.
[*]Now at College of Pharmacy, University of Florida, Gainesville, FL 32611

Izquierdo *et al.*, 2002; Nader, 2003). Support for this model comes from numerous studies in which protein synthesis inhibitors such as anisomycin administered near the time of training have no effect on memory tested during the first few hours after training but impair memory tested at longer time intervals.

However, evidence obtained over the past forty years suggests that drugs that inhibit protein synthesis may lead to secondary dysfunctions in neural activity that might produce amnesia. Several studies have suggested that protein synthesis inhibitors interfere with neurotransmitter synthesis and release. For example, early studies showed that protein synthesis inhibitors reduced the activity of tyrosine hydroxylase, the rate-limiting enzyme involved in the synthesis of norepinephrine and dopamine from tyrosine (Flexner *et al.*, 1973; Flexner & Goodman, 1975; Goodman *et al.*, 1975). Recent studies found that intra-amygdala and intra-hippocampal injections of anisomycin induced abnormally large increases in the release of norepinephrine, dopamine, serotonin, and acetylcholine at the site of the injection (Canal *et al.*, 2007; Qi & Gold, 2009). Related to these findings is evidence that many neurotransmitter-related drugs and hormones reverse amnesias associated with protein synthesis inhibitors and do so without reversing the extent of inhibition of protein synthesis (cf.: Martinez *et al.*, 1981; Davis & Squire, 1984; Gold, 2008).

The present study tested whether a local anesthetic, lidocaine, would attenuate anisomycin-related memory impairments by inhibiting the anisomycin-induced increases in norepinephrine release, though likely not baseline release (Pan *et al.*, 1996). Since the time course for anterograde amnesia observed with lidocaine is shorter than that for anisomycin, we were able to adjust the timing of the injections such that lidocaine had no effect on memory while anisomycin retained its ability to impair memory. Our hypothesis was that lidocaine would reduce the magnitude of anisomycin-induced increases in norepinephrine release and would attenuate anisomycin-induced amnesia.

Methods

Subjects

Male Sprague-Dawley rats (4–5 months old; Harlan Labs, Madison, WI) were individually housed and maintained on a 12:12-h light-dark cycle with free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Illinois at Urbana-Champaign and were in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Surgery

Rats (total N = 51) were anesthetized with isoflurane and placed in a stereotaxic apparatus with skulls arranged in a horizontal position. For the behavioral experiment, 23-gauge guide cannulae (Plastics One, Roanoke, VA) were aimed bilaterally at the basolateral amygdala (coordinates AP = −2.9 mm from bregma, ML = ±4.8 mm, DV = 5.4 mm below dura) and secured to the skull using dental cement and four small screws. Stylets ending flush with the tips of the cannulae were inserted. For the microdialysis experiment, an injection guide cannula was implanted as above on one side and a guide cannula for a combination microdialysis/injection probe was implanted on the contralateral side. The guide cannula for the SciPro probe was lowered until it was 3 mm above the amygdala (coordinates as above, except DV = −4.4 mm below dura). Rats were allowed to recover from surgery for at least one week prior to behavioral and microdialysis procedures. The rats were handled for 5 days for 3 min/day prior to the days of training and microdialysis sampling.

Inhibitory Avoidance Training and Testing

Rats were trained on a one-trial inhibitory avoidance task using a trough-shaped apparatus (91 cm long, 22.9 cm wide at top, 7.6 cm wide at bottom, and 15.2 cm deep) in which a door separated a well-lit start compartment (31 cm) from a dark shock compartment (60 cm). After placement of rats into the start compartment on the training day, the door was opened and the rats were allowed to cross into the dark compartment where they received a footshock (0.5 mA, 1.5 sec). Forty-eight hr later, rats were again placed into the start compartment and the door was opened. The latency to enter the dark compartment on the test day (maximum = 180 sec) was taken as the measure of memory.

Injections

For behavioral testing, anisomycin (Sigma, St. Louis, MO) was dissolved in 1 N HCl and brought to a final pH of 7.2 with 1 N NaOH. Phosphate-buffered saline (1 mM KH_2PO_4, 155 mM NaCl, 3 mM Na_2HPO_4) was added to obtain a final anisomycin concentration of 125 μg/μl; 0.5 μl (62.5 μg) was injected per side into the amygdala of each rat. Lidocaine was dissolved in 0.9% saline for a final concentration of 10 μg/0.5 μl/side. Rats received bilateral injections (0.25 μl/min for 2 min via CMA/100 microinjection pump) of saline or lidocaine 10 min prior to injections of PBS or anisomycin, the second injection coming 2 hr prior to training. The timing of the injections was determined by pilot studies to ensure an absence of anterograde impairments of memory by lidocaine and a presence of memory impairments by anisomycin. We had found that anisomycin impaired 48-hr memory if injected into the amygdala up to 2 hr but not 6 hrs or more before inhibitory avoidance training (Canal *et al.*, 2007). In pilot studies for the present experiment, lidocaine impaired memory if injected within 30 min of training but not if injected at longer pretraining intervals. Four groups were trained and tested in the behavioral experiment: saline + PBS (n=7), lidocaine + PBS (n=4), saline + anisomycin (n=9), lidocaine + anisomycin (n=12). Following injections, cannulae remained in place for an additional 1 min in order to allow diffusion of drugs. Rats were placed back into their home cages until they were trained on the inhibitory avoidance task.

Norepinephrine samples

In the microdialysis experiment, combination microdialysis/injection probes (MAB 9.14, SciPro, Sanborn, NY) were used to inject drugs and to collect microdialysis samples simultaneously. The probes consisted of a 2 mm membrane and an injection port that extended 1 mm below the microdialysis membrane. Combination probes were inserted into the guide cannulae and artificial cerebrospinal brain fluid (128 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$, 2.1 mM $MgCl_2$, 0.9 mM NaH_2PO_4, 2.0 mM Na_2HPO_4, 1.0 mM dextrose; pH= 6.5) was perfused through the brain at a rate of 0.6 μl/min. Each 45-min sample (27 μl) was collected in a tube containing 40 μl of 0.2 N acetic acid for a total volume of 67 μl. Samples collected during the first hour were discarded. Then, three baseline samples were collected before intra-amygdala bilateral injections of anisomycin (n=4), lidocaine (n=3), or both anisomycin and lidocaine (n=4) at concentrations as above. Injection procedures followed those used in behavioral experiments and drugs were injected during the first 4 min of the corresponding microdialysis sample. Five post-injection samples were collected thereafter. Immediately following collection, samples were frozen at −20°C until assayed. Microdialysis samples were analyzed for norepinephrine content using HPLC with electrochemical detection with procedures described previously (Canal *et al.*, 2007). The detection limit for norepinephrine was 0.5 pg.

Histology

The locations of cannula placements were assessed histologically, using 50 μm frozen sections (Leica 1800 cryostat) through the amygdala and cresyl violet staining. Rats with cannulae placements outside the amygdala were excluded from data analyses. While only rats with bilateral cannula placements in the amygdala were included here, the localization of drug effects to the amygdala or to individual nuclei within the amygdala was not specifically determined. Drug diffusion estimates vary with method of assessment, e.g. tissue staining, autoradiography or neurophysiology (Edeline *et al.*, 2002). Lidocaine injections of 1 μl volumes, twice that employed here, exhibit spread of 1.7 mm using autoradiography measures (Martin, 1991), an extent of spread suggesting that the effects in the present experiment were largely confined to amygdala sites of action.

Immunohistochemistry

Separate groups of rats were used to examine c-Fos protein expression in the amygdala, used as a marker of protein synthesis inhibition as employed previously (e.g., Inda *et al.*, 2005; Mamou *et al.*, 2006; Canal *et al.*, 2007, 2008; Canal & Gold, 2007). Three groups received concentrations and times of injections used in inhibitory avoidance testing. These groups included: saline + PBS (N = 4), saline + anisomycin (N = 2), and lidocaine + anisomycin (N = 2). Sixty minutes after footshock training, rats were anesthetized with sodium pentobarbital and perfused with saline followed by freshly prepared 4% paraformaldehyde. The brains were removed and placed in 4% paraformaldehyde overnight. Brains were then transferred to 20% glycerol in 0.1 M phosphate-buffered saline prior to collecting frozen sections (50 μm) approximately 100 μm from the injection site. The sections were washed in 0.05 M PBS, incubated in 1% H_2O_2 and 0.4% Triton X-100, and blocked with 3% goat serum. The sections were then incubated for 48 hr at 4°C in c-Fos primary antibody (1:7500; c-Fos antibody, rabbit polyclonal; sc-253, Santa Cruz Biotechnology, Santa Cruz, CA), and were subsequently washed in PBS followed by 1-hr incubation in secondary antibody (1:400 goat anti-rabbit; Santa Cruz Biotechnology). Sections were immunostained using the ABC Vectastain Elite kit (Vector Laboratory, Burlingame, CA) and diaminobenzidine.

For quantification, pictures were taken at 10x on a Leica CTR6000 microscope attached to a Leica DFC350FX digital camera and analyzed with Image J (NIH). In Image J, the threshold was set at 2.5x background for all pictures. Using the software, particles were counted within a circle (455 μm diameter) positioned in the basolateral nucleus of the amygdala.

Statistical Analyses

Because of the ceiling inhibitory avoidance scores, parametric statistical tests of the behavioral results were not appropriate. Therefore, non-parametric Mann-Whitney U-tests (two-tailed) (Siegel, 1956) were used to analyze inhibitory avoidance scores. Because norepinephrine means and standard deviations for the groups differed greatly, the neurochemical data were analyzed using $\log_{10}$ transformations of the values. Norepinephrine results were assessed with three-way repeated-measures ANOVAs (Statview software). Scheffe's post hoc t-tests were used to compare norepinephrine levels across groups. Student's t-tests were used to compare c-Fos expression levels across groups.

Results

Inhibitory Avoidance Memory

Lidocaine attenuated the magnitude of amnesia produced by anisomycin. Figure 1 shows the latencies to cross into the dark compartment on the memory test day, 48 hr after training and treatments. Rats that received injections of saline + anisomycin into the amygdala exhibited significantly shorter latencies when tested 48 hr after training compared to rats receiving

vehicle injections ($U_{7,9}$=0; p<0.002). Memory scores for rats receiving lidocaine injections into the amygdala did not differ from those of the vehicle controls, with all rats at the maximum (ceiling) latency in both groups. Importantly, lidocaine given 10 min prior to anisomycin injections resulted in test latencies significantly longer than those of the saline + anisomycin group ($U_{12,9}$=19; p<0.02). The attenuation of the effects of anisomycin by lidocaine was partial; the lidocaine-anisomycin group exhibited memory scores that were still significantly shorter than those of vehicle controls ($U_{12,7}$=0; p<0.002).

Release of norepinephrine

Lidocaine attenuated the anisomycin-induced release of norepinephrine in the amygdala. As shown in Figure 2, the samples collected during and immediately after injections of anisomycin exhibited large increases in release of norepinephrine above baselines. Levels of norepinephrine release returned to baseline values and remained stable in later samples. ANOVAS revealed a significant effect of treatment ($F_{1,5}$=10.82; p<0.05), time ($F_{8,40}$=14.30; p<0.0001) and a treatment by time interaction ($F_{8,40}$=8.65; p<0.0001) between the anisomycin vs. lidocaine + anisomycin groups. Post hoc tests revealed a substantial attenuation of anisomycin-induced increases in norepinephrine release in those rats pretreated with lidocaine (anisomycin 1300% vs. anisomycin-lidocaine 242%; p<0.01). Lidocaine did not itself result in a decrease in norepinephrine release compared to baseline values.

Immunohistochemistry

c-Fos immunoreactivity in the amygdala, assessed after inhibitory avoidance training, was readily evident in the vehicle-treated rats (Figure 3). Anisomycin appeared to block fully the training-initiated increase in expression of c-Fos. In the lidocaine + anisomycin condition, lidocaine did not attenuate the anisomycin-induced block of c-Fos expression; the inhibition of c-Fos expression remained apparently complete. These qualitative descriptions were confirmed quantitatively. As shown in Figure 4, the particle counts in saline/PBS controls (N = 4) were significantly higher than in either the saline-anisomycin (N = 2) or lidocaine-anisomycin (N=2) groups (161 ± 23 vs. 8 ± 7 and 4 ± 0.5, respectively; $t's_{df's=4}$ = 4.33 and 4.47, p's<0.02). Counts in the saline-anisomycin and lidocaine-anisomycin groups did not differ significantly from each other ($t_{df=2}$ = 0.64, p>0.5).

Discussion

When lidocaine and anisomycin were administered directly into the amygdala, lidocaine pretreatment attenuated anisomycin-produced amnesia of memory of inhibitory avoidance training. The attenuation of anisomycin-induced amnesia by lidocaine occurred without concomitant attenuation of anisomycin-induced inhibition of local protein synthesis. However, lidocaine effectively blocked the increase in release of norepinephrine at the amygdala site of anisomycin injection.

Lidocaine injections into the amygdala have been used previously to impair memory formation (Vazdarjanova & McGaugh, 1999; Coleman-Mesches *et al.*, 1996; Parent & McGaugh, 1994; Salinas *et al.*, 1993; Keller *et al.*, 2004). In each of these experiments, lidocaine was administered near the time of training. In the present experiment, we took advantage of the finding that anisomycin had anterograde effects on memory lasting at least 2 hr (Canal *et al.*, 2007), while lidocaine had anterograde effects on memory for less than 30 min after injection. This difference in temporal properties permitted us to administer lidocaine soon before anisomycin under conditions in which only anisomycin would be expected to produce amnesia, revealing effects of lidocaine on the efficacy of anisomycin in producing amnesia without direct effects of the local inactivation on memory. The tests of

memory in the lidocaine-alone group confirmed the absence of anterograde effects on memory and the tests in the anisomycin-alone group confirmed the presence of later amnesia.

The finding here that injections of anisomycin produce amnesia for memory of inhibitory avoidance is consistent with the results obtained using many tasks (cf.: Martinez *et al.*,1981; Davis & Squire, 1984; Dudai, 2002; Kandel, 2001; Alberini, 2008; Gold, 2008; Desgranges *et al.*, 2008; De la Cruz, *et al.*, 2008). Previous results show that intra-amygdala injections of anisomycin impair synaptic plasticity within the amygdala (Huang & Kandel, 2007) and impair fear conditioning (Schafe & Ledoux, 2000; Parsons *et al.*, 2006; Maren *et al.*, 2003). Directly related to the present findings is evidence that intra-amygdala injections of anisomycin impair memory for inhibitory avoidance training (Canal *et al.*, 2007; Canal & Gold, 2008; Milekic *et al.*, 2007; Izquierdo *et al.*, 2002). Together with evidence that memory for inhibitory avoidance training is impaired in rats by lesions, electrical stimulation, and by local inactivation and other pharmacological manipulations of the amygdala (Gold *et al.*, 1975, 1978; Izquierdo *et al.*, 1992; Izquierdo & Medina, 1993; Jerusalinsky *et al.*, 1992; Kim & McGaugh, 1992; Lennartz *et al.*, 1996; Liang *et al.*, 1982; Parent & McGaugh, 1994; Walker & Gold, 1994), the findings indicate that, like fear conditioning, memory for inhibitory avoidance training is also dependent on amygdala functions.

The key behavioral result was in the lidocaine + anisomycin group, in which inactivation of the amygdala just prior to the administration of anisomycin blunted the extent of amnesia. There are many treatments that attenuate amnesia produced by protein synthesis inhibitors (cf. Gold, 2006, 2008). Most studies demonstrating attenuation of amnesia produced by protein synthesis inhibitors used stimulant drugs, which themselves can enhance memory, leading to suggestions that pharmacological attenuation of amnesia produced by protein synthesis inhibitors is accomplished by enhancing residual memory that survives the inhibition (Davis & Squire, 1984; Squire, 2006). The present findings are not compatible with this view. Lidocaine itself impairs memory formation under other conditions (e.g., Vazdarjanova & McGaugh, 1999; Coleman-Mesches *et al.*, 1996; Parent & McGaugh, 1994; Salinas *et al.*, 1993; Keller *et al.*, 2004), and to our knowledge does not enhance memory. The results obtained here with lidocaine are therefore consistent with attenuation of amnesia after protein synthesis inhibition by other drugs that similarly do not themselves enhance memory (Canal *et al.*, 2007; Qi & Gold, 2009; Gold & Sternberg, 1978).

The findings of experiments in which protein synthesis inhibitors injected directly into the amygdala impair memory have generally been interpreted as revealing a role for training-initiated protein synthesis important for the formation of new memories (Duvarci *et al.*, 2005; Nader *et al.*, 2000a,b; Milekic *et al.*, 2007; Cammarota *et al.*, 2004; Izquierdo *et al.*, 2002; Schafe & LeDoux, 2000; Chai *et al.*, 2006), an interpretation common to similar tests of other brain areas as well (Rossato *et al.*, 2007; De la Cruz *et al.*, 2008; Desgranges *et al.*, 2008; Izquierdo *et al.*, 1992; Morris *et al.*, 2006). When trained on fear conditioning tasks in particular, the interpretation is often that protein synthesis is necessary at a primary site of neural plasticity essential for memory formation (Duvarci *et al.*, 2005; Maren *et al.*, 2003; Nader, 2003; Nader *et al.*, 2003a,b); Schafe & LeDoux, 2000), although the evidence for the amygdala as the primary site of memory storage for fear conditioning appears to be unconvincing (Weinberger, 2010). Similar interpretations of necessary protein synthesis have also been offered for inhibitory avoidance memory (Milekic *et al.*, 2007; Izquierdo *et al.*, 2002; Izquierdo & Medina, 1995).

While most interpretations of these reports regard the amygdala as an anatomical locus of memory formation, an alternative function for the amygdala is that the amygdala functions

to modulate memories that are formed elsewhere in the brain (McGaugh, 2004; McIntyre *et al.*, 2003). The evidence for this position includes findings that manipulations of the amygdala can interfere with memory formation and its modulation at other brain regions such as the hippocampus (McGaugh *et al.*, 2002). According to this view, the amygdala does not serve as a storage site for memories but rather as a site of modulation. Related to the present findings, norepinephrine action within the amygdala is a major component of the mechanisms mediating modulation of memory formation. Modulation of memory by release of norepinephrine within the amygdala is consistent with the present findings showing that large increases in release of norepinephrine may mediate anisomycin-induced memory impairments.

The neurochemical results presented here replicate past findings showing that injections of anisomycin into the amygdala result in abnormally high levels of release of several neurotransmitters including norepinephrine (Canal *et al.*, 2007). Norepinephrine effects on memory appear to follow an inverted-U relationship with memory formation. High levels of release of norepinephrine or high doses of norepinephrine injected in the amygdala near the time of training impair later memory (Gold & van Buskirk, 1978a,b; Canal *et al.*, 2007; Liang *et al.*, 1990; Decker & McGaugh, 1989). In addition, the β-adrenergic receptor antagonist, propranolol, blocks memory impairments produced by anisomycin with co-injections into the amygdala (Canal *et al.*, 2007) and hippocampus (Qi & Gold, 2009). These findings support the view that the large increases in release of norepinephrine in response to anisomycin injections contribute to the amnesia produced by the protein synthesis inhibitor. This possibility is supported further by the present findings that lidocaine, under conditions in which the treatment attenuates anisomycin-induced amnesia, also blocks the abnormal release of norepinephrine in response to injection of the protein synthesis inhibitor.

Release of norepinephrine within the amygdala modulates memory formation apparently based on processing both within and beyond the amygdala. In addition, release of norepinephrine at many brain sites, such as the hippocampus, can also more directly modulate memory formation and synaptic plasticity (e.g.: Bramham *et al.*, 1997; Dommett *et al.*, 2008; Izquierdo *et al.*, 1992; Izquierdo & Medina, 1995; Izumi & Zorumski, 1999; Lashgari *et al.*, 2008; McGaugh *et al.*, 2002; Miyashita & Williams, 2004; Reid & Harley, 2010; Roosevelt *et al.*, 2006; Schimanski *et al.*, 2007). Anisomycin injections directly into the hippocampus, like injections into the amygdala, impair memory for inhibitory avoidance learning (Milekic *et al.*, 2007; Cammorota *et al.*, 2004; Izquierdo *et al.*, 2002; Canal & Gold, 2007; Qi & Gold, 2009). Importantly, amnesia produced by anisomycin injections directly into the hippocampus is also associated with increased release of norepinephrine at the site of injection (Qi & Gold, 2009). In that experiment, amnesia produced by intra-hippocampal injections of anisomycin was attenuated by the β-adrenergic antagonist, propranolol, as also seen with amygdala injections (Canal *et al.*, 2007). These results are analogous to those reported here using lidocaine to attenuation anisomycin-induced amnesia and show that anisomycin-induced impairments of memory may be mediated by non-physiological levels of release of norepinephrine, and apparently other neurotransmitters (Canal *et al.*, 2007; Qi & Gold, 2009), within multiple neural systems.

In contrast to the demonstration that lidocaine reduced the norepinephrine response to anisomycin, lidocaine did not attenuate the extent of inhibition of training-related increases in immunoreactivity of c-Fos, used here as a marker of protein synthesis. Rats that received lidocaine + anisomycin showed little c-Fos expression, with immunohistochemistry apparently very similar to that seen after anisomycin alone. The present results are consistent with those we have seen before (Canal *et al.*, 2007); c-Fos immunoreactivity decreased after anisomycin injections into the amygdala but propranolol, which attenuated the amnesia, did not block the decrease in c-Fos immunoreactivity. Similar uncoupling of

attenuating effects of neurotransmitter-related drugs on memory and on protein synthesis inhibition has been shown many times before (e.g., Barondes & Cohen, 1968; Serota *et al.*, 1972; Hall *et al.*, 1976; Quartermain *et al.*, 1977; Flood *et al.*, 1977, 1978; Lundgren & Carr, 1978; Sershen *et al.*, 1982). The finding in the lidocaine + anisomycin group of substantial inhibition of expression of c-Fos while memory remains evident is not consistent with the view (Lamprecht & Dudai, 1996) that the transient expression of c-Fos in the amygdala is required for the formation of memory for amygdala-dependent tasks.

Of note, the large pulse of norepinephrine release evident after anisomycin injection had dissipated by the time training occurred. The return of norepinephrine release to baseline, and sometimes below baseline (Canal *et al.*, 2007), may reflect depletion of releasable stores. Interestingly however, high extracellular levels of norepinephrine at the time of training seem unnecessary for the induction of amnesia, suggesting that norepinephrine initiates a cascade of downstream events that interfere with memory formation. This possibility may be related to gene superinduction seen after anisomycin injection (Edwards & Mahadevan, 1992; Radulovic & Tronson, 2008). There is also evidence that central injections of anisomycin elicit changes in motor cortex maps, synapse size, and number (Kleim *et al.*, 2003) and may induce cell death (Morris *et al.*, 2006). Like the changes in neurotransmitter release, these changes too might contribute to the induction of amnesia by inhibition of protein synthesis, adding to the potential specific cellular mechanisms responsible for amnesia produced by protein synthesis inhibitors. Together, such findings have led to the development of alternatives to the idea that training-initiated protein synthesis is necessary for memory formation (cf.: Routtenberg & Rekart, 2005; Rudy *et al.*, 2006; Holahan & Routtenberg, 2007; Gold, 2006, 2008; Routtenberg, 2008). The evidence supporting these recent interpretations of the effects of protein synthesis inhibitors on memory point to a clear need to distinguish the mechanisms of amnesia from the mechanisms of memory formation; these mechanisms need not be reciprocal.

The present findings add to the evidence that anisomycin may impair memory either as a secondary action of the drug on neurotransmitter release or as a secondary consequence of the inhibition of protein synthesis. If the latter proves to be the case, then amnesias obtained with other protein synthesis inhibitors and possibly with other more specific inhibitors of cell signaling processes may also be secondary to similar neurochemical effects. In this regard, injections of CREB antisense into the amygdala produce amnesia and also perturb local release of norepinephrine and acetylcholine (Canal *et al.*, 2008). Such findings suggest that interference with cell transcription and translation mechanisms might result in disruption of normal neuronal functions in a manner inconsistent with optimal memory and neural plasticity mechanisms. These secondary consequences on memory of interference with transcription and translation may be independent of specific drug, constraining the utility of such experiments for providing specific information regarding the mechanisms underlying memory formation.

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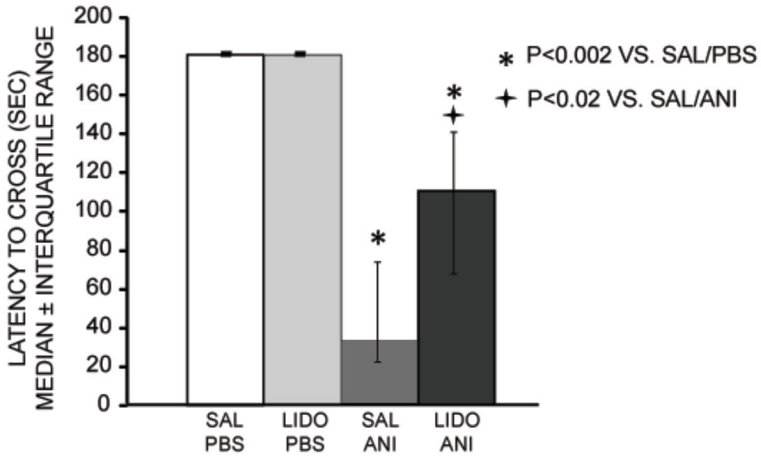

Figure 1.

Effects of intra-amygdala injections of anisomycin (ANI) and lidocaine (LIDO) on inhibitory avoidance memory. Injections of anisomycin into the amygdala 2 hours prior to training impaired memory tested 48 hours after training. Lidocaine injected 10 minutes prior to anisomycin partially attenuated the anisomycin-induced memory impairment. SAL = saline; PBS=phosphate-buffered saline.

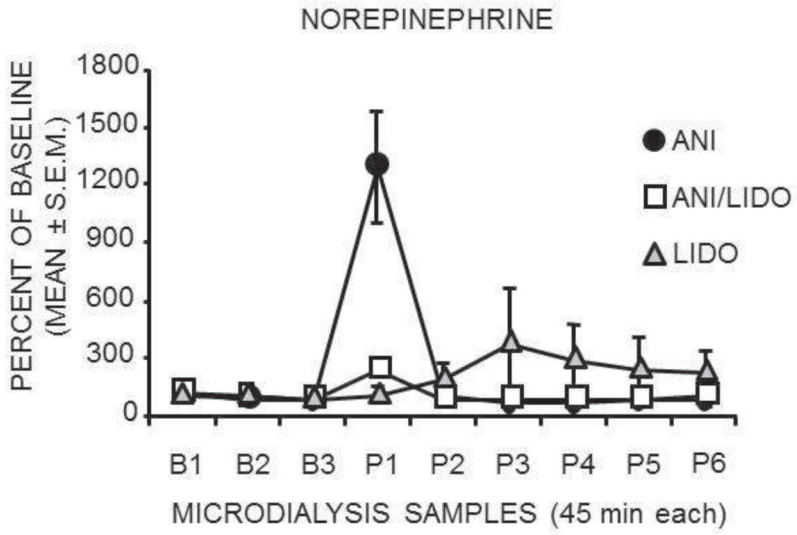

Figure 2.

Effects of intra-amygdala injections on norepinephrine release from the amygdala. Anisomycin (ANI) injections caused a large increase in norepinephrine release. Lidocaine (LIDO) pretreatment substantially and significantly blocked this effect. Lidocaine alone had no effect on norepinephrine release. B= Baseline; P= post-injection.

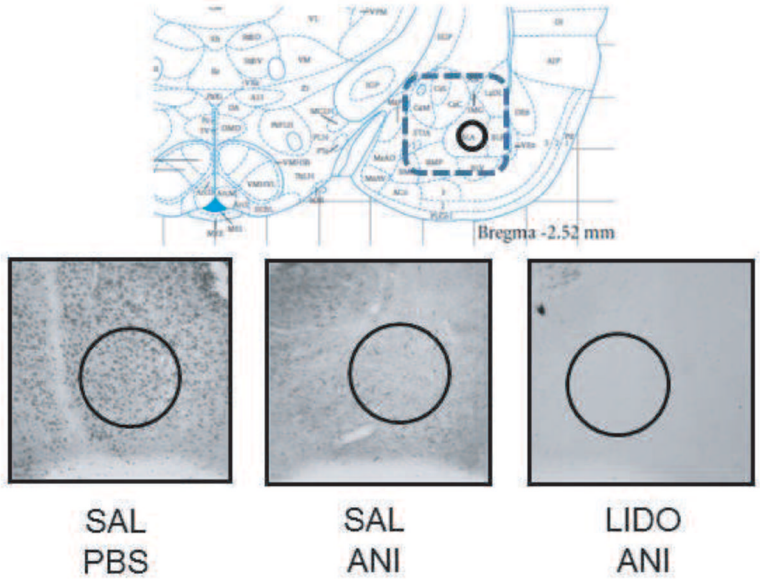

Figure 3.

Top: Schematic showing placement of electrodes and sampling area for c-Fos expression (adapted from Paxinos and Watson, 2005, Figure 54). The rectangular dashed line identifies the area in which cannulae placements were accepted for behavioral and immunohistochemistry analyses. The circle (top and bottom of this figure; 455 μm diameter) represents the area analyzed for immunohistochemistry. *Bottom*: Representative pictures of c-Fos immunohistochemistry. Note that anisomycin reduced c-Fos expression; lidocaine did not attenuate the inhibition of c-Fos immunoreactivity caused by anisomycin. SAL= saline; PBS=phosphate-buffered saline; ANI = anisomycin; LIDO = lidocaine.

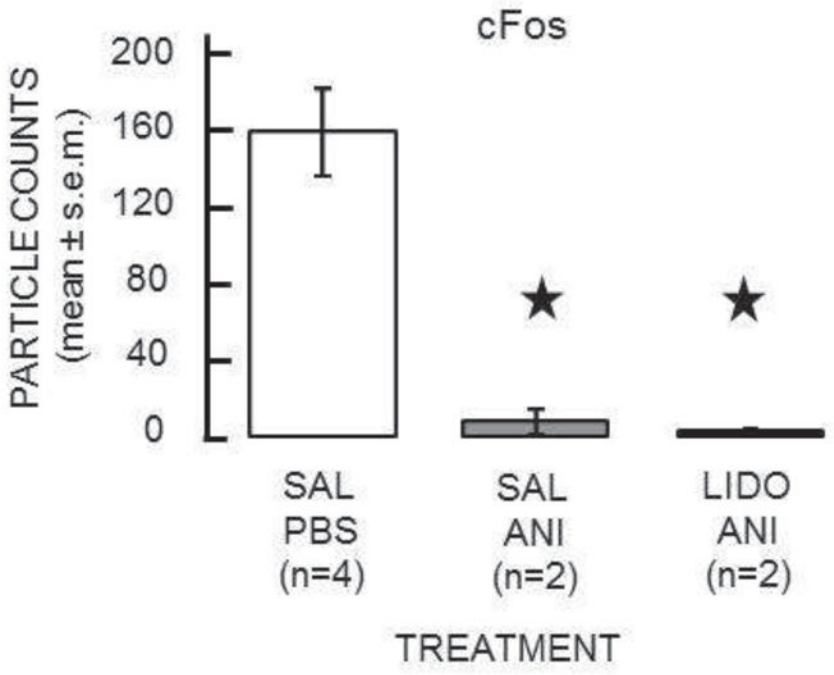

Figure 4.
Quantification of c-Fos results. cFos expression was significantly higher in the sal-PBS condition than in either the sal-anisomycin or lidocaine-anisomycin conditions. (Stars indicate p's < 0.02 vs. sal-PBS.)