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Cholinergic modulation of pavlovian fear conditioning in rats: Differential effects of intrahippocampal infusion of mecamylamine and methyllycaconitine

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Abstract

The cholinergic system has consistently been implicated in Pavlovian fear conditioning. Considerable work has been done to localize specific nicotinic receptor subtypes in the hippocampus and determine their functional importance; however, the specific function of many of these subtypes has yet to be determined. An α7 nicotinic antagonist methyllycaconitine (MLA) (35 μg), and a broad spectrum non-α7 nicotinic antagonist mecamylamine (35 μg) was injected directly into the dorsal hippocampus or overlying cortex either 15 min pre-, 1 min post-, or 6 h post-fear conditioning. One week after conditioning, retention of contextual and cue (tone) conditioning were assessed. A significant impairment in retention of contextual fear was observed when mecamylamine was injected 15 min pre- and 1 min post-conditioning. No significant impairment was observed when mecamylamine was injected 6 h post-conditioning. Likewise, a significant impairment in retention of contextual fear was observed when MLA was injected 1 min post-conditioning; however, in contrast, MLA did not show any significant impairments when injected 15 min pre-conditioning, but did show a significant impairment when injected 6 h post-conditioning. There were no significant impairments observed when either drug was injected into overlying cortex. No significant impairments were observed in cue conditioning for either drug. In general, specific temporal dynamics involved in nicotinic receptor function were found relative to time of receptor dysfunction. The results indicate that the greatest deficits in long-term retention (1 week) of contextual fear are produced by central infusion of MLA minutes to hours post-conditioning or mecamylamine within minutes of conditioning.

Keywords: Hippocampus; Mecamylamine; Methyllycaconitine; MLA; Nicotine; Nicotinic; Acetylcholine; α7; Non-α7

1. Introduction

Classical or Pavlovian fear conditioning involves forming an association between a distinctive cue (i.e., tone) (conditioned stimulus [CS]) and an aversive event (i.e., footshock) (unconditioned stimulus [US]) (see Fanselow, 2000; Maren, 2001 for review). Typically, this CS–US association occurs within a particular context and rats will acquire a conditioned response (CR) (i.e., freezing) to the context associated with the US delivery as well as to the cue. In contrast to the cue-shock association, the context-shock association has been found to be hippocampal dependent (Anagnostaras, Maren, & Fanselow, 1999a; Holland & Bouton, 1999; Maren, Anagnostaras, & Fanselow, 1998). That is, lesions to the dorsal hippocampus have been found to abolish the context-shock association with no effect on the cue-shock association (Anagnostaras et al., 1999a; Fanselow, 2000; Maren, 2001). However, if a long training-to-lesion interval is allowed, then hippocampal lesions have little effect and result in smaller retention deficits (Anagnostaras et al., 1999a; Fanselow, 2000; Kim & Fanselow, 1992; Maren, Aharonov, & Fanselow, 1997; Maren & Fanselow, 1997; Phillips & LeDoux, 1992). This

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temporal gradient suggests that there is a limited amount of time during which the hippocampus is involved in acquisition and consolidation of contextual fear conditioning.

Adult rats also show impairments in contextual fear conditioning, but not in cue conditioning, with disruption of both muscarinic and nicotinic cholinergic neurotransmission (Anagnostaras et al., 1999a; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999b; Caldarone, Duman, & Picciotto, 2000; Davis & Gould, 2006; Gale, Anagnostaras, & Fanselow, 2001; Levin & Rezvani, 2002b; Paylor et al., 1998; Rudy, 1996) or lesions of the fimbria-fornix, which has been shown to block cholinergic innervation of the hippocampus from the ventrolateral diagonal band (Maren & Fanselow, 1997). This is consistent with the growing body of evidence implicating cholinergic neurotransmission in the hippocampus in many forms of context-dependent learning (Anagnostaras, Maren, & Fanselow, 1995; Anagnostaras et al., 1999b; Barros, Ramirez, Dos Reis, & Izquierdo, 2004; Blokland, Honig, & Raaijmakers, 1992; Bovet-Nitti, 1966; Davis & Gould, 2006; Gale et al., 2001; Rudy, 1996; Wallenstein & Vago, 2001). However, the temporal dynamics of muscarinic and nicotinic receptor systems along with functional deficits associated with broad-spectrum nAChR blockade and more specific nAChR subtype dysfunction (i.e., α7) are currently unclear and some results have been mixed.

The nicotinic cholinergic system has been shown to be involved in several cognitive functions with convincing evidence of its involvement in short-term, long-term, and working memory function (Barros et al., 2004; Decker, Brioni, Bannon, & Arneric, 1995; Decker & Majchrzak, 1993; Felix & Levin, 1997; Kim & Levin, 1996; Levin, 2002a; Ohno, Yamamoto, & Watanabe, 1993). Considerable work has been done to localize the alpha and beta subunit receptors involved in hippocampal-dependent memory function (Barros et al., 2004; Levin, 2002a, 2002b; Sargent, 1993). There is now strong evidence for specific nAChR subtypes (i.e., α7, α4β4, α4β2, and α3β4) localized within the hippocampus and associated memory structures (Adams, Stitzel, Collins, & Freedman, 2001; Alkondon & Albuquerque, 1993; Frotscher & Leranth, 1985; Levin & Simon, 1998). However, the role of these receptor subtypes in a variety of cognitive behaviors is currently incomplete (Levin & Rezvani, 2002b; Levin & Simon, 1998) and results from the fear conditioning paradigm have been mixed (Gould & Higgins, 2003; Wehner et al., 2004). Previous fear conditioning studies using systemic administration of selective and non-selective nAChR antagonists, as well as studies involving nAChR subunit null mutant mice have failed to show profound deficits in 24 h retention of contextual fear conditioning, but have shown subunit-specific modulatory effects (Davis & Gould, 2006; Gould & Higgins, 2003; Gould & Wehner, 1999; Wehner et al., 2004). Specifically, the α4β2 nicotinic antagonist, dihydro-β-erythroidine (DHβE), but not the α7-specific antagonist methylycaconitine (MLA) has shown disruptive effects on nicotine enhancement of conditioning (Davis & Gould, 2006). Thus, the present study aimed to examine the time-dependent, functional differences between central blockade of broad-spectrum nAChRs and more specific nAChR blockade (e.g., α7) in long-term retention (i.e., 1 week) of contextual fear conditioning by infusing mecamylamine or MLA directly into the dorsal hippocampus. It has been found that mecamylamine acts as a more broad-spectrum nAChR antagonist (Chavez-Noriega et al., 1997; Debruyne et al., 2003; Martin, Onaivi, & Martin, 1989) making it a good candidate for affecting all nicotinic receptors; whereas, MLA has been found to inhibit α6 subunits at some concentrations, but selectively inhibit the α7 subunit in the hippocampus (Davies et al., 1999; Turek, Kang, Campbell, Arneric, & Sullivan, 1995). The data have implications for general and specific (i.e., α7) nAChR function in long-term memory consolidation and retention in contextual fear conditioning.

2. Materials and methods

2.1. Subjects

Seventy-six male Long-Evans rats (300–365 g) were used in this experiment. All rats were individually housed and located in a colony room maintained on a 12:12 light: dark cycle. Each rat was handled prior to testing and had unrestricted access to food and water. All protocols conformed to the NIH Guide for the Care and Use of Laboratory Animals. The treatment of animals complied with all animal care guidelines that have been approved by the Institutional Animal Care and Use Committee (IACUC) and the University Animal Resource Center (ARC).

2.2. Implantation of guide cannulae

Rats were given an intraperitoneal injection of atropine sulfate (0.4 mg/kg, i.p.) to control for respiratory difficulties. Rats were then anesthetized with an intraperitoneal injection of sodium pentobarbital (65 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was incised and retracted exposing bregma and lambda in the same horizontal plane. Small burr holes (2.0 mm diameter) were drilled bilaterally. Using stereotaxic coordinates, stainless steel guide cannula (28-gauge, stainless steel; Plastics One, Roanoke, VA) were then lowered bilaterally into (1) the dorsal hippocampus ($n = 68$; 3.6 mm posterior to bregma, 2.5 mm lateral to the midsagittal suture, and 2.5 mm ventral from the brain surface) or (2) the overlying cortex ($n = 8$; 3.6 mm posterior to bregma, 2.5 mm lateral to the midsagittal suture, and 1.8 mm ventral from the brain surface). These coordinates were chosen based on the histological analyses of Wallenstein and Vago (2001); however, in order to cover more of the dorsal hippocampus, the coordinates were changed 0.2 mm more lateral and 0.3 mm more ventral (see Fig. 1). The guide cannula were fixed with dental cement for which three small skull screws (1 mm) were previously screwed into the skull as anchors. Stainless steel stylets (33 gauge) with dustcaps were screwed into the top of the cannulae to prevent occlusion. Following surgery, the rats were allowed to recover on a heating pad before returning to their home cage. Rats were given acetaminophen (Children's Tylenol) (~150–200 mg/kg) dissolved in drinking water for 2–3 days post-surgery as an analgesic.

2.3. Behavioral apparatus

The training chamber (used for conditioning and context testing) consisted of two clear Plexiglas walls (rear and front door) and two aluminum side panels (28w × 21h × 22d cm; Colbourn Instruments, Allentown, PA). The floor of the chamber consisted of 18 steel rods (0.2 cm radius situated 1.5 cm apart) connected to a precision shock scrambler (Colbourn Instruments). A speaker was inserted in one of the side panels of the chamber to