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Antagonism of muscarinic acetylcholine receptors in medial prefrontal cortex disrupts the context preexposure facilitation effect

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ABSTRACT

Cholinergic function plays a role in a variant of context fear conditioning known as the context preexposure facilitation effect (CPFE; Robinson-Drummer, Dokovna, Heroux, & Stanton, 2016). In the CPFE, acquisition of a context representation, the context-shock association, and expression of context fear occur across successive phases, usually 24 h apart. Systemic administration of scopolamine, a muscarinic acetylcholine receptor antagonist, prior to each phase (context preexposure, immediate-shock training, and testing) disrupts the CPFE in juvenile rats (Robinson-Drummer et al., 2016). Dorsal hippocampal (dHPC) cholinergic function contributes significantly to this effect, as local infusion of scopolamine into the dHPC prior to any individual phase of the CPFE produces a disruption identical to systemic administration (Robinson-Drummer et al., 2016). The current experiment extended these findings to another forebrain region implicated in the CPFE, the medial prefrontal cortex (mPFC). Adolescent rats received bilateral infusions of scopolamine (35 μg/side) or PBS 10 min before all three phases of the CPFE or only prior to a single phase. Intra-mPFC administration of scopolamine prior to all three phases significantly impaired fear conditioning suggesting that mPFC cholinergic function is necessary for successful CPFE performance. Analyses of the individual infusion days revealed a significant impairment of the CPFE when infusions occurred prior to preexposure or training (i.e. immediate footshock) but not prior to testing. In total, these findings suggests a role of mPFC cholinergic function in the acquisition and/or consolidation of a contextual representation and the context-shock association but not in retrieval or expression of fear memory. Implications for mPFC involvement in contextual fear conditioning and neurological dysfunction following neonatal alcohol exposure are discussed.

1. Introduction

Cholinergic function is crucial for performance of several forms of Pavlovian conditioning. Scopolamine, a muscarinic acetylcholine (mACh) receptor antagonist, administered during training can disrupt standard contextual fear conditioning (sCFC) to a background context (Anagnostaras, Maren, & Fanselow, 1995; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999; Gale, Anagnostaras, & Fanselow, 2001) as well as conditioning to a discrete cue (however see Hunt & Richardson, 2007). In addition, a variant of sCFC known as the context preexposure facilitation effect (CPFE; Fanselow, 1990) has been used to specify the particular psychological processes affected by cholinergic antagonism during fear conditioning (Brown, Kennard, Sherer, Comalli, & Woodruff-Pak, 2011; Chang & Liang, 2012; Robinson-Drummer, Dokovna, Heroux, & Stanton, 2016). During the CPFE, learning about the context (preexposure), context-shock association (training), and retrieval and expression of the context-fear memory (testing) occur across three separate days. Relative to sCFC, the temporal separation of the learning experiences during the CPFE make it well suited to separately analyze the mechanisms of context learning vs. context-shock learning as determinants of conditioned fear performance.

Similar to sCFC, performance of the CPFE is significantly impaired by antagonizing cholinergic receptors. Prior to conditioning on any single phase of the CPFE, both systemic and intrahippocampal scopolamine administration disrupts testing day performance (Brown et al., 2011; Robinson-Drummer et al., 2016). Furthermore, post-shock (but not post-preexposure) intrahippocampal infusions of scopolamine significantly impairs CPFE performance (Chang & Liang, 2012). These results support previous reports that the hippocampus is critical for contextual conditioning during the CPFE (Matus-Amat, Higgins, Barrientos, & Rudy, 2004; Matus-Amat, Higgins, Sprunger, Wright-Hardesty, & Rudy, 2007) and extend those results by suggesting a specific role of the hippocampal cholinergic system in contextual conditioning using

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the CPFE. Although most CPFE research has focused on this region, the hippocampus is not the singular target of cholinergic projections, so other brain regions receiving these projections may also play a role in the CPFE.

The medial prefrontal cortex (mPFC) is involved in the top down control of cognitive function (Dalley, Cardinal, & Robbins, 2004), in systems consolidation, and in behavioral expression of context conditioning (Frankland & Bontempi, 2005; Wiltgen & Tanaka, 2013). However, recently its role has been extended to include the initial acquisition of context memories (for review see Giustino & Maren, 2015). Following the training phase of the CPFE, the mPFC shows learning-related increases in immediate early gene expression in both adult (Chakraborty, Asok, Stanton, & Rosen, 2016) and developing rats (Asok, Schreiber, Jablonski, Rosen, & Stanton, 2013; Schreiber, Asok, Jablonski, Rosen, & Stanton, 2014) and after hippocampal lesions or inactivation, compensatory mechanisms in the mPFC subserve fear conditioning to contextual stimuli (Zelikowsky et al., 2013). Additionally, the mPFC receives rich innervation from the basal forebrain cholinergic system (Henny & Jones, 2008) making it a likely contributor to the disruptive effects of cholinergic antagonism on contextual fear conditioning.

Although many studies have explored the importance of mPFC cholinergic function to attention and working memory (Broersen, Heinsbroek, de Bruin, Uylings, & Olivier, 1995; Chen, Baxter, & Rodefer, 2004; Chudasama, Dalley, Nathwani, Bouger, & Robbins, 2004; McGaughy, Ross, & Eichenbaum, 2008; Newman & McGaughy, 2008), the neuromodulatory role of the mPFC cholinergic system in (contextual) fear conditioning is largely unexplored. The current study investigated the effect of intra-mPFC antagonism of cholinergic function during all three conditioning phases of the CPFE in 31-day-old rats, a period that marks the transition from juvenile to adolescent stages of development (Spear, 2000). In Experiment 1, scopolamine was administered prior to all three phases of the CPFE to broadly implicate the mPFC cholinergic system in the CPFE. Experiments 2–4 each examined cholinergic antagonism on only a single day of the CPFE (i.e. preexposure, training or testing day only) in order to more precisely identify the psychological processes that may be impaired by mPFC scopolamine infusions. Results of the current study support a role for the mPFC cholinergic system in context learning and context-shock association but not retrieval or expression of context fear.

2. General methods

2.1. Subjects

Time-mated females were housed with breeder males overnight and were examined for an ejaculatory plug the following day and, if found, that day was designated as gestational day (GD) 0. Dams were housed in clear polypropylene cages measuring 45 × 24 × 21 cm with standard bedding and access to ad libitum water and rat chow. Animals were maintained on a 12:12 h light/-dark cycle with lights on at 7:00 am. Date of birth (GD22) was designated as postnatal day (PD) 0. Litters were culled on PD3 to eight pups (usually 4 males and 4 females) and were paw-marked with subcutaneous injections of non-toxic black ink for identification. Pups were weaned from their mother on PD21 and housed with same-sex litter mates in 45 × 24 × 17 cm cages. On PD29 animals were individually housed in small white polypropylene cages (24 × 18 × 13 cm) with ad libitum access to water and rat chow for the remainder of the experiment. All subjects were treated in accordance with a protocol approved by the Institutional Animal Care and Use Committee at the University of Delaware following guidelines established by the National Institute of Health.

2.2. Apparatus and stimuli

Fear conditioning occurred in four clear Plexiglas chambers designated as Context A as described previously (Heroux, Robinson-Drummer, Rosen, & Stanton, 2016; Murawski & Stanton, 2010; Robinson-Drummer et al., 2016). The chambers measured 16.5 × 12.1 × 21.6 cm and were arranged in a 2 × 2 formation on a Plexiglas stand within a fume hood which provided ambient light and background noise. Each chamber had a grid floor made of 9 stainless steel bars, 0.5 cm in diameter and spaced 1.25 cm apart. The unconditioned stimulus (US), two 1.5 mA, 2 s foot shocks, was delivered using a shock scrambler (Med Associates, Georgia, VT ENV-414S) connected to the grid floor. Video of each session (preexposure, training, testing) was recorded using FreezeFrame software (Actimetrics, Wilmette IL), which measures change in pixilation, with freezing defined as a bout of 0.75 s or longer without a change in pixels. The FreezeFrame software recorded video from the four chambers simultaneously. Context B consisted of the same Plexiglas chambers used in Context A with modifications, which have been described previously (Asok et al., 2013; Murawski & Stanton, 2010; Robinson-Drummer et al., 2016; Schreiber et al., 2014). Wire mesh inserts, which protruded into the chambers, changed both the texture of the floor and the dimensions of the chamber. In addition, white opaque coverings were added such that only the wall facing the camera remained unobscured.

2.3. Surgery

On PD29, juvenile rats were taken from post-weaning group housing and anesthetized with an i.p. ketamine/xylazine injection and subcutaneous buprenorphine near the incision site to reduce post-operative discomfort. A fused double-guide cannula (Plastics One, Roanoke, VA) was implanted bilaterally to terminate above the prelimbic region of medial prefrontal cortex using the following coordinates: anteroposterior (AP) +9.0 mm and mediolateral (ML) ±0.6 mm relative to interaural midline and dorsoventral (DV) −2.3 mm relative to the top of the skull. Cannula were fixed in placed using dental acrylic and curved "skull hooks" (Schiffino, Murawski, Rosen, & Stanton, 2011; Watson & Stanton, 2009). Following surgery, dummy internals and dust caps were inserted in the guide cannula to reduce occlusion of the guide cannula and rats were allowed to recover in individual white cages with electric heating pads placed under half of the cage floor. Animals were allowed to recover for approximately 24 h until their cannulas were cleared the following day. For each animal, 0.25 μL of the vehicle phosphate buffered saline (PBS; Fisher Scientific, Waltham, MA) was infused per side to ensure that no cannulas were occluded.

2.4. Drug infusion

Depending on their drug condition (see Sections 2.5 and 2.6) rats received microinjections of either PBS or scopolamine hydrobromide (Scop; Sigma Aldrich, St. Louis, MO) dissolved in PBS approximately ten minutes before behavioral training. Animals were hand held while scopolamine (140 μg/μL dissolved in PBS) was infused at a rate of 0.25 μL per minute for a single minute, administering 35 μg of scopolamine per side per animal. This dose has been used previously in our lab (Brito, Davis, Stopp, & Stanton, 1983; Robinson-Drummer et al., 2016) and similar doses of scopolamine have been infused intra-cranially in other labs (Chang & Liang, 2012; Gale et al., 2001; Rogers & Kesner, 2004). Drug injectors were left in place for an additional minute to allow diffusion of drug before removal. PBS control animals were administered the same volume of PBS at the same rate as scopolamine animals. A 0.25 μL infusion diffuses about 1 mm from the cannula tip ensur-