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Inactivation of the central nucleus of the amygdala blocks classical conditioning but not conditioning-specific reflex modification of rabbit heart rate

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ABSTRACT

Heart rate (HR) conditioning in rabbits is a widely used model of classical conditioning of autonomic responding that is noted for being similar to the development of conditioned heart rate slowing (bradycardia) in humans. We have shown previously that in addition to HR changes to a tone conditioned stimulus (CS), the HR reflex itself can undergo associative change called conditioning-specific reflex modification (CRM) that manifests when tested in the absence of the CS. Because CRM resembles the conditioned bradycardic response to the CS, we sought to determine if HR conditioning and CRM share a common neural substrate. The central nucleus of the amygdala (CeA) is a critical part of the pathway through which conditioned bradycardia is established. To test whether the CeA is also involved in the acquisition and/or expression of CRM, we inactivated the CeA with muscimol during HR conditioning or CRM testing. CeA inactivation blocked HR conditioning without completely preventing CRM acquisition or expression. These results suggest that the CeA may therefore only play a modulatory role in CRM. Theories on the biological significance of conditioned bradycardia suggest that it may represent a state of hypervigilance that facilitates the detection of new and changing contingencies in the environment. We relate these ideas to our results and discuss how they may be relevant to the hypersensitivity observed in fear conditioning disorders like post-traumatic stress.

1. Introduction

Conditioning of autonomic responses such as heart rate (HR) develops rapidly and is thought to reflect an affective or emotional component of classical conditioning that facilitates the acquisition of more slowly acquired adaptive somatomotor or skeletal responses (Buchanan & Powell, 1993; Lennartz & Weinberger, 1992). This early emotionally driven part of the conditioning process may represent an important physiological aspect of the acquisition of abnormal or maladaptive fear responses because clinical research on conditioned fear based disorders such as post traumatic stress disorder (PTSD) and phobias have detailed abnormal cued cardiovascular responses in patients (Bedi & Arora, 2007; Busscher, van Gerwen, Spinhoven, & de Geus, 2010; Ginsberg, Ayers, Burriss, & Powell, 2008; Lang & McTeague, 2009; Peri, Ben-Shakhar, Orr, & Shalev, 2000). Noted for being very similar to the conditioned HR responses observed in humans, conditioned HR deceleration (bradycardia) in rabbits is a widely used model for studying classically conditioned autonomic responding in animals (Ghelarducci & Sebastiani, 1996; Lavond, Lincoln, McCormick, & Thompson, 1984; McCabe et al., 1992b; McEchron, Tseng, & Disterhoft, 2000; Pascoe, Supple, & Kapp, 1991; Powell et al., 1997; Schneiderman, Smith, Smith, & Gormezano, 1966). A thorough understanding of the neural substrates of HR conditioning in rabbits may therefore offer important insight into the mechanisms responsible for altered physiological responding in human fear disorders.

Conditioned HR changes in rabbits were first documented in the 1940s when it was shown that rabbits develop conditioned bradycardia to a bell conditioned stimulus (CS) following pairings of the bell with the unconditioned stimulus (US) ammonia (Kosupkin and Olmsted 1943). Early experiments established that HR conditioning in rabbits is parasympathetically mediated through the vagus and critically dependent on the amygdala (reviewed in McCabe et al., 1992b), a structure well known for its role in the acquisition, expression, modulation, and extinction of a variety of conditioned emotional responses (Davis & Whalen, 2001; Gabriel, Burhans, & Kashef, 2003; Kim & Jung, 2006; LeDoux, 2000; Maren, 2001; McGaugh, McIntyre, & Power, 2002; Shors, 2006). The suggested neural pathway by which the amygdala can influence HR conditioning is through connections of the central nucleus of the amygdala (CeA) to the dorsal motor nucleus of the vagus and nucleus ambiguus which give rise to vagal efferents to the heart and also less directly via connections to the nucleus of the solitary tract that can affect the baroreceptor reflex (McCabe et al., 1992b; Schwaber,

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Kapp, Higgins, & Rapp, 1982). Stimulation of CeA can produce responses that mimic conditioned bradycardia (Applegate, Kapp, Underwood, & McNall, 1983), and damage to or neurochemical manipulation of the CeA can disrupt the acquisition of HR conditioning (Gallagher, Kapp, Frysinger, & Rapp, 1980; Kapp, Frysinger, Gallagher, & Haselton, 1979; McCabe, Gentile, Markgraf, Teich, & Schneiderman, 1992a; Powell et al., 1997) and also retention (Gentile, Jarrell, Teich, McCabe, & Schneiderman, 1986). In addition, studies using extracellular recordings from the CeA during HR conditioning have found correlations between CS-related neural activity and the conditioned bradycardia response (Applegate, Frysinger, Kapp, & Gallagher, 1982; Pascoe & Kapp, 1985).

These previous studies on HR conditioning in rabbits have mainly focused on the neural mechanisms behind the development of conditioned HR changes to a CS such as an auditory tone that is paired with a US, often shock. However, work in our laboratory has shown that the conditioning procedure can also modify unconditioned HR responses to the US when it is presented in the absence of the CS following conditioning (Burhans, Smith-Bell, & Schreurs, 2008, 2010; Schreurs, Crum, Wang, & Smith-Bell, 2005; Schreurs, Smith-Bell, & Burhans, 2011b; Schreurs et al., 2007). These changes are not observed in rabbits receiving explicitly unpaired CS–US presentations (Schreurs et al., 2005) and therefore represent a learning-related cardiovascular change in the unconditioned response to the US that we refer to as conditioning-specific reflex modification (CRM). CRM of rabbit HR is measured in an ABA design in which rabbits are first exposed to varying intensities and durations of a periorbital shock US to obtain a baseline of physiological responsiveness (Pretest) followed by two days of HR conditioning and then another US test identical to the Pretest (Post Test). HR CRM is characterized by a Pre- to Post Test increase in interbeat interval to the US (representative of a decrease in HR) to US intensities that are equal to or approach that of the intensity utilized during conditioning. The observed HR change closely resembles the conditioned bradycardia that develops to the tone CS, suggesting that HR CRM may in part be a generalized conditioned response Burhans et al., 2008). However, we have shown that there are certain conditions that can extinguish HR CRM while leaving the HR conditioned response intact, so there is also evidence for a dichotomy between the two types of responding (Burhans et al., 2010). HR CRM has also been shown to generalize across USs with overlapping features and is enhanced, along with HR conditioning, by dietary cholesterol (Schreurs et al., 2007, 2011b).

PTSD is an example of a fear disorder that involves exaggerated fear responses to discrete stimuli associated with previous trauma *in addition to* abnormal responding to innately stressful stimuli (4th ed.; DSM IV; American Psychiatric Association, 1994). This latter symptom represents a hypersensitivity in reflexive responding that can occur in the absence of any specific trauma-associated cues. CRM of rabbit HR, therefore, may be an important unexplored aspect of conditioned autonomic responding that might be relevant to the physiological reactivity found in fear disorders. To our knowledge, we are the only laboratory to explicitly document this phenomenon in rabbit HR, although we and others have more extensively reported CRM in a different system, the rabbit nictitating membrane response (Burhans et al., 2008; Gruart & Yeo, 1995; Wikgren, Ruusuvirta, & Korhonen, 2002). Our next step is to study the neural substrates of HR CRM and how they relate to those already established for HR conditioning to a discrete CS. Because of the well established critical role of the amygdala in conditioned bradycardia, the CeA is a potential neural substrate for the development of HR CRM, which consists of a similar bradycardic response. In addition, it has been shown that during HR conditioning, some CeA neurons are responsive to both the CS and US (McEchron, McCabe, Green, Llabre, & Schneiderman, 1995), suggesting that the CeA receives converging CS and US information and thus may be in a position to mediate responding to both types of stimuli. Taken together, these findings suggest that the CeA may also play a critical role in HR CRM.

To test whether CeA plays a role in HR CRM acquisition or expression, the current study used reversible inactivation of the CeA using muscimol during HR conditioning or CRM testing. In agreement with previous studies using permanent electrolytic or chemical lesions, it was expected that temporary inactivation of the CeA during HR conditioning would block the development of conditioned bradycardia to the CS. Because HR CRM closely resembles conditioned bradycardia to the CS and does not occur in rabbits receiving explicitly unpaired training, one hypothesis being tested was whether blocking the acquisition of HR conditioning would also block the acquisition of HR CRM. Since the CeA is known to be involved in the expression of many fear behaviors, it was also important to test whether the CeA inactivation would interfere with CRM expression.

2. Methods

2.1. Subjects

The subjects were 35 male, New Zealand White rabbits (*Oryctolagus cuniculus*) weighing approximately 2.0–2.2 kg upon delivery from the supplier (Harlan, Indianapolis, IN). The rabbits were housed in individual cages on a 12 h light–dark cycle and given *ad libitum* access to food and water. They were maintained in accordance with guidelines issued by the National Institutes of Health, and the research was approved by the West Virginia University Animal Care and Use Committee.

2.2. Surgical procedure

After a minimum period of one week for adaptation to living conditions, rabbits underwent stereotaxic surgery for bilateral implantation of chronic guide cannulae targeted at the CeA. The rabbits were anesthetized using a subcutaneous injection (0.6 ml/kg) of a solution containing ketamine HCl (83.3 mg/ml) and xylazine (16.7 mg/ml) with additional 0.25 ml injections of ketamine given every 30–60 min to maintain anesthesia. Rabbits also received a single subcutaneous dose (0.1 ml) of the analgesic flunixin meglumine (50 mg/ml) prior to the start of surgery in addition to an injection of 2% lidocaine HCl (0.25 ml) at the incision site, with a second dose administered at the end of surgery prior to suturing. Stainless steel 22 gauge guide cannulae (length: 13.8 mm), designed to custom fit an internal 28 gauge injection cannula with a 1 mm projection (Plastics One Inc. Roanoke, VA), were implanted bilaterally into the amygdala, targeting the central nucleus: AP = +0.5, ML = ±5.5; DV = 11.0. The stereotaxic coordinates were obtained from the atlas of Girgis and Shih-Chang (1981) and are consistent with morphological characterization of amygdalar nuclei in rabbits (Jagalska-Majewska et al., 2001). The guides were also fitted with protective dummy cannulae that were only removed for subsequent intra-amygdalar infusions. Two small stainless steel screws (Small Parts Inc., Miami Lakes, FL) were partially screwed into the skull to anchor the cement (Jet Repair Acrylic, Wheeling, IL) used to secure the cannulae in place.

2.3. Apparatus

The apparatus has been detailed elsewhere (Schreurs & Alkon, 1990; Schreurs, Tomsic, Gusev, & Alkon, 1997) and is modeled after those developed and described by Gormezano (Coleman & Gormezano, 1971; Gormezano, 1966). Briefly, rabbits were restrained in a Plexiglas box placed inside a sound-attenuating, ventilated chamber (Coulborn Instruments, Allentown, PA; Model