Neurobiology of Learning and Memory 155 (2018) 143–156

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Inactivation of the interpositus nucleus blocks the acquisition of conditioned responses and timing changes in conditioning-specific reflex modification of the rabbit eyeblink response

Lauren B. Burhans*, Bernard G. Schreurs

Rockefeller Neuroscience Institute and Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV, USA

ARTICLE INFO

Keywords:
Classical eyeblink conditioning
Reflex modification
Fear conditioning
Stress
Interpositus nucleus
Cerebellum

ABSTRACT

Conditioning-specific reflex modification (CRM) of the rabbit eyeblink response is an associative phenomenon characterized by increases in the frequency, size, and peak latency of the reflexive unconditioned eyeblink response (UR) when the periorbital shock unconditioned stimulus (US) is presented alone following conditioning, particularly to lower intensity USs that produced minimal responding prior to conditioning. Previous work has shown that CRM shares many commonalities with the conditioned eyeblink response (CR) including a similar response topography, suggesting the two may share similar neural substrates. The following study examined the hypothesis that the interpositus nucleus (IP) of the cerebellum, an essential part of the neural circuitry of eyeblink conditioning, is also required for the acquisition of CRM. Tests for CRM occurred following delay conditioning under muscimol inactivation of the IP and also after additional conditioning without IP inactivation. Results showed that IP inactivation blocked acquisition of CRs and the timing aspect of CRM but did not prevent increases in UR amplitude and area. Following the cessation of inactivation, CRs and CRM latency changes developed similarly to controls with intact IP functioning, but with some indication that CRs may have been facilitated in muscimol rabbits. In conclusion, CRM timing and CRs both likely require the development of plasticity in the IP, but other associative UR changes may involve non-cerebellar structures interacting with the eyeblink conditioning circuitry, a strong candidate being the amygdala, which is also likely involved in the facilitation of conditioning. Other candidates worth consideration include the cerebellar cortex, prefrontal and motor cortices.

1. Introduction

Since its first detailed description by Pavlov in the 1920s (Pavlov, 1927), classical conditioning has traditionally been characterized as the emergence of a conditioned response (CR) against the backdrop of an unconditioned response (UR) once considered to be automatic, fixed, and relatively invariant. Almost a century later, classical conditioning studies still tend to focus mainly on the CR; however, there is a much greater understanding and appreciation of the dynamic qualities of the UR, which can be modified by nonassociative phenomena such as sensitization and habituation (Boulis and Sahley, 1988; Gormezano and Kehoe, 1975; Hawkins, Cohen, & Kandel, 2006; Thompson, 2009) and importantly, can also be modified by associative factors during conditioning (Canli, Detmer, & Donegan, 1992; Schreurs, Oh, Hirashima, & Alkon, 1995; Weisz and McInerney, 1990). Studying conditioning-related changes in the UR alongside development of the CR is highly relevant for disorders of abnormal fear conditioning like post-traumatic stress disorder (PTSD) in which symptomology can include not only CRs to cues associated with trauma but also exaggerated reflexive responding to innately stressful stimuli (i.e. URs).

Classical conditioning of the rabbit nictitating membrane response, or eyeblink conditioning, is one paradigm for which associative changes in the UR have been well characterized, starting first with studies documenting changes in the UR in the presence of the conditioned stimulus (CS) and continuing with later and ongoing work detailing changes in the UR when tested in the absence of the CS (*for review, see* Burhans, Smith-Bell, & Schreurs, 2008). Earlier studies examining changes in the UR following presentation of the CS described increases in UR amplitude that rapidly developed at the start of conditioning, termed reflex facilitation (Ison and Leonard, 1971; Weisz and McInerney, 1990; Young, Cegavske, & Thompson, 1976) and decreases in UR amplitude when preceded by the trained CS, known as

* Corresponding author.
E-mail address: lburhans@hsc.wvu.edu (L.B. Burhans).

https://doi.org/10.1016/j.nlm.2018.07.008
Received 4 June 2018; Received in revised form 20 July 2018; Accepted 23 July 2018
Available online 24 July 2018

conditioned diminution (Donegan, 1981; Kimble and Ost, 1961). For the past two decades, ongoing work in our laboratory has extensively detailed changes to the UR that are measured when the unconditioned stimulus (US) is presented alone without the CS, referred to as conditioning-specific reflex modification (CRM) (Burhans et al., 2008; Schreurs and Burhans, 2015; Schreurs, 2003). In a typical experiment, rabbits are presented with varying intensities of a periorbital shock (0.1–2.0 mA) presented alone prior to and following classical delay eyeblink conditioning in which a tone CS is paired with a 2.0 mA periorbital shock US. After conditioning, increases in UR frequency, amplitude, and area, in addition to increases in response latency are observed, particularly to lower intensity shocks that elicited little to no responding prior to conditioning. This exaggerated responding is deemed "conditioning-specific" because the same changes are not observed in rabbits receiving explicitly unpaired presentations of the CS and US (Buck, Seager, & Schreurs, 2001; Schreurs et al., 1995; Schreurs, Shi, Pineda, & Buck, 2000). CRM-like changes during eyeblink conditioning have also been observed by others in rabbits and rodents (Gruart and Yeo, 1995; Servatius, Brennan, Beck, Beldowicz, & Coyle-DiNorica, 2001; Wikgren and Korhonen, 2001), and with conditioning of galvanic skin responses in humans (Morrow, 1966). It has also been reported that during trace eyeblink conditioning, combat veterans with PTSD show increased UR amplitude on US-alone trials, compared to veterans without PTSD (Burriss, Ayers, & Powell, 2007), suggesting CRM-like changes may be part of a PTSD phenotype, which also includes increases in the amplitude of the CR (Handy et al., 2018).

Earlier work delineating the behavioral laws governing CRM in the rabbit eyeblink conditioning paradigm demonstrated commonality with the CR, leading to the theory that CRM may be a CR that has generalized from the CS to the US (Burhans et al., 2008; Schreurs, 2003). The first indication came from observations that the topography of the UR following conditioning closely resembled the CR on paired CS-US trials, with a latency shift to the right and a more complex, sometimes dual peaked profile where the first and second peaks coincided with the timing of CS and US presentations, respectively (Schreurs et al., 1995). Other evidence came from studies demonstrating that the strength of CRM was influenced by the same factors that influenced the strength of conditioning. For example, CRM got stronger with additional days of conditioning, with the greatest changes seen after six, rather than one or three daily sessions (Schreurs et al., 1995). In addition, stronger CRM occurred as the aversiveness of the US utilized during conditioning increased, such as when periorbital shock was used instead of corneal airpuff (Buck et al., 2001) or when shock intensity was amplified (Seager, Smith-Bell, & Schreurs, 2003). However, other findings showed that a dichotomy can exist between CRs and CRM, suggesting that the CR generalization hypothesis does not completely explain the nature of CRM. For example, training rabbits to produce multiphasic CRs by conditioning with a tone paired with two sequential USs did not produce a multiphasic UR that paralleled the CR topography, and it was shown that CRs could be extinguished without CRM and vice versa if rabbits were given CS-alone or US-alone presentations, respectively (Schreurs et al., 2000). It was only when both treatments were combined using explicitly unpaired CS/US presentations that both CRs and CRM were extinguished simultaneously (Burhans, Smith-Bell, & Schreurs, 2015; Schreurs et al., 2000). In addition, CRs and CRM have been shown to differentially respond to systemic serotonergic, glutamatergic, and noradrenergic manipulations (Burhans, Smith-Bell, & Schreurs, 2013, 2017, 2018). Meta analysis studies examining larger pools of behavioral data have also demonstrated that conditioning levels do not strongly predict individual susceptibility to CRM (Smith-Bell and Schreurs, 2017; Smith-Bell, Burhans, & Schreurs, 2012). Overall, these findings suggested that although CRs and CRM may have some commonality, they also have distinctions, suggesting some overlapping but possibly also distinct neural underpinnings. As there is evidence that CRs and URs can be differentiated as far downstream as the eyelid motoneurons (Trigo et al., 1999), there are many places along the eyeblink conditioning pathway where CR and CRM generation may diverge.

Because the positive relationship between CRM strength and aversiveness of the US suggested a fear conditioning component to CRM (Buck et al., 2001), the first study to directly investigate the neural substrates of CRM examined the role of the amygdala (Burhans and Schreurs, 2008), a critical component of the neural circuitry of learned fear (Maren, 2001; Phelps and LeDoux, 2005). The central nucleus of the amygdala (CE) was specifically targeted because of its previously established role in eyeblink reflex facilitation (Choi, Lindquist, & Brown, 2001; Weisz, Harden, & Xiang, 1992; Whalen and Kapp, 1991), characterized as an increase in UR amplitude following CS presentation that is thought to be one of the earliest signs of the development of the CR (Weisz and McInerney, 1990). The main finding of our study was that muscimol inactivation of the CE during eyeblink conditioning slowed the rate of acquisition of CRs, which were eventually acquired to the same level as controls, but did not block the development of CRM. In contrast, inactivation of the CE during testing for CRM specifically blocked the frequency, area, and amplitude changes that are characteristic of CRM. These findings established that the CE appears to be important for the expression of CRM, but left open the question of what neural substrates may be responsible for CRM acquisition.

Because of the overlap between some features of CRs and CRM, a strong neural candidate for CRM acquisition is the interpositus (IP) nucleus of the cerebellum, which is a major site for the integration of CS and US inputs with outputs controlling the generation of the rabbit eyeblink CR (Gonzalez-Joekes and Schreurs, 2012; Gould, Sears, & Steinmetz, 1993; Ostrowska, Zguczynski, & Zimny, 1992; Steinmetz and Sengelaub, 1992; Thompson, 2013). There is general consensus that the IP is necessary for the acquisition of eyeblink conditioning, as indicated by IP lesion and inactivation studies showing severe impairments to CR acquisition and electrophysiological studies demonstrating the development of learning-related neuronal activity in the IP closely associated with the execution of the CR (*for review, see* Christian and Thompson, 2003; Freeman and Steinmetz, 2011; but see also Ammann, Marquez-Ruiz, Gomez-Climent, Delgado-Garcia, & Gruart, 2016; López-Ramos, Houdek, Cendelín, Vožeh, & Delgado-García, 2018). Of particular relevance is work showing that CRM-like increases in the amplitude of the UR in well-trained rabbits is blocked or disfacilitated by IP inactivation, suggesting a role of the IP in CRM expression (Wikgren and Korhonen, 2001).

The goal of the following study was to examine whether the IP is necessary for CRM acquisition by testing whether CRM would develop if the IP was temporarily inactivated with the gamma aminobutyric acid-A ($GABA_A$) agonist muscimol during eyeblink conditioning. As it was expected that IP inactivation would block acquisition of CRs, the current study was also another test of the CR/CRM dichotomy, examining whether CRM could develop in the absence of a learned and performed conditioned eyeblink response. Following conditioning under IP inactivation and initial testing for CRM, conditioning was allowed to proceed without inactivation followed by another test for CRM, in order to confirm that deficits in CR acquisition and any resulting deficits in CRM could be overcome once IP functioning was restored.

2. Methods

2.1. Subjects

The subjects were 20 male, New Zealand White rabbits (*Oryctolagus cuniculus*), 2–3 months of age, weighing approximately 1.8–2.3 kg upon delivery from the supplier (Charles River, Saint-Constant, Canada). 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 the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health, and the research was