Neuroscience 260 (2014) 1–11

FEAR RESPONSE FAILED TO RETURN IN AAB EXTINCTION PARADIGM ACCOMPANIED WITH INCREASED NR2B AND GLUR1 PER845 IN HIPPOCAMPAL CA1

L. XUE, [†] Z. D. LI [†] Z. X. CHEN X. G. WANG, Y. W. SHI [*] AND H. ZHAO [*]

Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, Guangdong, China

Abstract—Extinction is a well-known and important behavioral phenomenon that allows an organism to adapt its behavior to its environment. Previous studies have shown that the expression of extinction is highly context dependent, meanwhile, conditioning context, as part of fear memory, might have influence on extinction formation. To this end, we have conducted four different extinction paradigms in this study: extinction conducted in the conditioning context but tested in another, novel context (AAB); conditioning in one context and extinction and testing in the second (ABB); conditioning in context A, extinction training in context B, but test back to context A (ABA); and extinction training in a third context, context C (ACB). Additionally, a low dose of the $GABA_A$ agonist muscimol was used to temporarily inactivate CA1 to observe its effect in extinction. Our results showed that rats under the AAB, but not the ACB or ABA condition, showed a similar level of freezing compared with the typical ABB extinction paradigm. Moreover, muscimol infused into CA1 before extinction training resulted in impaired extinction and down-regulation of NR2B activity and phosphorylated GluR1 (at Ser845) in CA1, and the expression levels of NR2B and GluR1 were decreased significantly in the basolateral amygdala (BLA). Thus, CA1 may play an important role in the context-specific expression of fear extinction, and Ser845 may be a phosphorylation site in GluR1 in CA1, triggering the context-specific response of extinction memory.

Key words: extinction, fear memory, renewal, muscimol, phosphorylation.

*Corresponding authors. Address: Faculty of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, 510080 Guangdong, China. Tel: +86-20-8733-0564; fax: +86-20-8733-4353 (Y. Shi). Tel: +86-20-8733-5987; fax: +86-20-8733-4353 (H. Zhao).

E-mail addresses: shiyanwei801102@hotmail.com (Y. W. Shi), hzhao@163.com (H. Zhao).

[†] Li Xue and Zedong Li contributed equally to this work.

Abbreviations: ANOVA, analysis of variance; BLA, basolateral amygdala; CS, conditional stimulus; EXT, extinction; ITI, inter-trial interval; LTD, long term depression; MAAB, muscimol infusion in AAB version; MABA, muscimol infusion in ABA version; MABB, muscimol infusion in ABB version; MACB, muscimol infusion in ACB version; MUS, Muscimol; NMDA, N-methyl-D-aspartic acid; PKA, protein kinase A; PL, prelimbic; PTSD, post-traumatic stress disorder; SAAB, saline infusion in AAB version; SABA, saline infusion in ABA version; SABB, saline infusion in ABB version; SACB, saline infusion in ACB version; SD, standard deviation; US, unconditional stimulus.

INTRODUCTION

Exposure therapy is the most common behavioral treatment for patients with post-traumatic stress disorder (PTSD) (Foa, 2006). Procedurally, it is similar to the fear extinction paradigm in rodents, involving exposure to a series of conditional stimulus (CS). In fact, just like the poor effect of exposure, fear extinction is generally fragile. One interesting aspect is that fear extinction is context dependent; that is, fear responses can still be expressed if the conditioned stimulus (CS, e.g., a tone or a light) is presented in a different context than that in which extinction was acquired (Ehrlich et al., 2009), a phenomenon known as renewal.

The most common version is ''ABA renewal,'' wherein conditioning is conducted in context A and extinction in context B, which differs in tactile, olfactory, and visual respects. When the CS recurs in the original conditioning context (context A), the response to the CS returns (Bouton and Bolles, 1979; Bouton and Peck, 1989). The second version is ''ABC renewal,'' wherein testing in a third ''neutral'' context, C (Bouton and Bolles, 1979; Bouton and Brooks, 1993; Harris et al., 2000). In a final version, conditioning and extinction are both conducted in the same context (context A); when the CS is presented in a second context (context B), the conditioned response also returns (Tamai and Nakajima, 2000).

Recently, Schiller et al. using two different stimuli as CSs with the same US, found that extinction during reconsolidation affect only the reactivated CS. Their findings indicated that every trace is important for successful extinction. Thus, conditioning context may be recognized as one trace, and then affects the formation of extinction memory. Actually, in PTSD patients, a hypermnesia for the core traumatic event associated with a memory deficit for peritraumatic contextual cues usually exist, impairing the capacity of the subjects to identify the correct predictors of the threat and restrict fear to the correct place and/or to the correct cues (Kaouane et al., 2012). In that case, in auditory fear conditioning, subjects may show more fear to the context rather than conditioning tones. Thus conditioning context seems extremely important in fear extinction. In that opinion, extinction training conducted in the conditioning context, for example, the AAA and AAB paradigms may facilitate extinction. In fact, it is difficult to represent the traumatic context (A) exactly in reality, thus a similar context (B) is conducted for exposure, and patients back to the outside world (C)

0306-4522/13 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.12.014

after treatment, so the ABC paradigm is the most common paradigm used in exposure therapy. Although the effect is not satisfied, many patients still recovered after treatment. Taken together, there is a contradiction between exposure therapy reality and context-dependent extinction theory.

Thus, whether extinction training conducted in conditioning context follow the context-specific rule of extinction expression or not remains unclear. To address this question, we designed three contexts in this study, a conditioning context (A), a context which had similar main features with conditioning context (B) and a novelty context (C). Rats were randomly distributed into three paradigms: AAB, ABB, and ACB. Thus all fear-conditioning and -retrieval testing was conducted in the same context (A/B), but the extinction context differed. In one case, the extinction context was the same as the fear-conditioning context (AAB); in the second, the extinction context was similar to context A and as same as the testing context (ABB) which was recognized as the paradigm not shown renewal; and in the third, the extinction context was almost completely novel (ACB). Moreover, to explore whether test in conditioning context affects extinction recall, the fourth paradigm: ABA was used, that is, conditioning conducted in context A, extinction training in context B, testing back to context A.

Given the involvement of the hippocampus in the formation of contextual memory (Kim and Fanselow, 1992; Phillips and LeDoux, 1992), numerous studies have implicated it in the contextual modulation of fear extinction (Corcoran and Maren, 2001, 2004; Corcoran et al., 2005), moreover, different subfields of the hippocampus had different contributions in extinction. For example, both hippocampal areas CA1 and CA3 contribute to the acquisition of context-dependent extinction, but only area CA1 is required for contextual memory retrieval (Ji and Maren, 2008). In the present study, to investigate the role of area CA1 of the dorsal hippocampus in extinction, a low dose of the $GABA_A$ agonist muscimol was micro-infused into the bilateral CA1 before fear extinction training to temporarily inactivate CA1 and then evaluated the probable cellular mechanism.

Numerous studies have demonstrated that fear extinction does not destroy the initial CS–unconditional stimulus (US) pairing but instead creates a new association (CS–No US) that inhibits the expression of conditioned memory (Bouton et al., 2006; Myers and Davis, 2007; Ehrlich et al., 2009). Since that, studies have showed that the formation of extinction memory is N-methyl-D-aspartic acid (NMDA) dependent (Orsini and Maren, 2012). The NR2B subunit of the NMDA receptor in basolateral amygdala (BLA) is especially involved in the acquisition of extinction (Tang et al., 1999; Sotres-Bayon et al., 2007, 2009). Additionally, regulation of AMPA receptors plays a key role in altering excitatory synaptic transmission in the CNS, extinction have been associated with the surface expression of AMPA receptor subunits. Adult GluR1 knockout mice lack LTP in the CA1 region of the hippocampus (Zamanillo et al., 1999; Jensen et al., 2003). Thus, GluR1 may be involved in extinction. Moreover, previous studies have shown that protein kinase C and calcium/calmodulin-dependent protein kinase II catalyze GluR1 phosphorylation at Ser831 (Barria et al., 1997; Mammen et al., 1997), thereby increasing AMPA channel conductance (Derkach et al., 1999). A similar effect also occurs following phosphorylation of GluR1, catalyzed by cAMP-dependent protein kinase A (PKA) at Ser845 (Roche et al., 1996; Banke et al., 2000). To determine whether protein kinase C (PKC) or PKA could activate GluR1, levels of GluR1 phosphoSer831 and phosphoSer845 were examined.

EXPERIMENTAL PROCEDURES

Experiment 1

Subjects. In total, 55 adult male Sprague–Dawley rats (220–250 g), obtained from the Zhongshan School of Medicine, Sun Yat-Sen University, were housed under a 12/12-h light/dark cycle (lights on at 6:00 am) in Plexiglas cages with *ad libitum* access to standard rodent chow. The rats were handled (10–20 s per rat per day) for at least 5 days to habituate them to the experimenter. Animal procedures were approved by the Institutional Animal Care and Use Committee of the Zhongshan School of Medicine, Sun Yat-Sen University, in accordance with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Behavioral apparatus. The apparatus used was modified from previous studies (Corcoran and Maren, 2001, 2004; Corcoran et al., 2005). Behavioral experiments were conducted in observation chambers ($30 \times 24 \times 21$ cm; Coulbourn Instruments, Lehigh Valley, PA, USA), constructed from aluminum and Plexiglas. Each chambers situated in sound-attenuating cabinets located in a brightly lit isolated room. The floor of each chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center to center). Foot shocks were used as USs produced by foot rods wired to a shock source. And the acoustic CSs were delivered by the speaker set in one wall of the chamber. Above each chamber, closed-circuit video cameras were used to videotape the behavior of each rat.

Sensory stimuli were adjusted within the chambers to generate three contexts, differed in transported boxes, illumination of house and chambers, background noises, chambers cleaners, etc. For example, stainless steel pans containing a thin film of the chamber cleaner solution were placed beneath the chamber floors to provide a distinctive odor before the rats were placed inside. For contexts B and C, wide pieces of white or red paper were fixed on each side wall of chamber respectively. Additionally, clear rubber mats were placed over the grids in context C (Table 1).

Procedure. Rats were submitted to one of the four experimental phases: acclimation, fear conditioning,