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Fear potentiated startle increases phospholipase D (PLD) expression/activity and PLD-linked metabotropic glutamate receptor mediated post-tetanic potentiation in rat amygdala

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ABSTRACT

Long-term memory (LTM) of fear stores activity dependent modifications that include changes in amygdala signaling. Previously, we identified an enhanced probability of release of glutamate mediated signaling to be important in rat fear potentiated startle (FPS), a well-established translational behavioral measure of fear. Here, we investigated short- and long-term synaptic plasticity in FPS involving metabotropic glutamate receptors (mGluRs) and associated downstream proteomic changes in the thalamic–lateral amygdala pathway (Th–LA). Aldolase A, an inhibitor of phospholipase D (PLD), expression was reduced, concurrent with significantly elevated PLD protein expression. Blocking the PLD–mGluR signaling significantly reduced PLD activity. While transmitter release probability increased in FPS, PLD–mGluR agonist and antagonist actions were occluded. In the unpaired group (UNP), blocking the PLD–mGluR increased while activating the receptor decreased transmitter release probability, consistent with decreased synaptic potentials during tetanic stimulation. FPS Post-tetanic potentiation (PTP) immediately following long-term potentiation (LTP) induction was significantly increased. Blocking PLD–mGluR signaling prevented PTP and reduced cumulative PTP probability but not LTP maintenance in both groups. These effects are similar to those mediated through mGluR7, which is co-immunoprecipitated with PLD in FPS. Lastly, blocking mGluR–PLD in the rat amygdala was sufficient to prevent behavioral expression of fear memory. Thus, our study in the Th–LA pathway provides the first evidence for PLD as an important target of mGluR signaling in amygdala fear-associated memory. Importantly, the PLD–mGluR provides a novel therapeutic target for treating maladaptive fear memories in posttraumatic stress and anxiety disorders.

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1. Introduction

1.1. Storage of long-term memory (LTM) plays an important role in pathological states of fear and anxiety. However, the mechanisms underlying these conditions are yet to be understood. Fear potentiated startle (FPS) is an ideal translational paradigm for studying memory mechanisms of anxiety and fear in humans (Grillon, Ameli, Woods, Merikangas, & Davis, 1991; Grillon & Davis, 1997; Grillon et al., 2011; Schmitz, Grillon, Avenevoli, Cui, & Merikangas, 2014) and animals (Campeau & Davis, 1995a, 1995b; Kazama, Schauder, McKinnon, Bachevalier, & Davis, 2013; Parsons & Davis, 2011; Sananes & Davis, 1992). In FPS (a classical conditioning paradigm), a neutral conditioned stimulus (CS, tone) paired with an aversive unconditioned stimulus (UCS, a footshock) results in fear associated learning, where subsequent CS alone elicits fear as heightened startle responses (Davis, 1986; Davis, Falls, Campeau, & Kim, 1993). Hence, FPS is an appropriate model to investigate the physiological and neurochemical mechanisms expressed during long-term memory of specific cue-induced fear conditioning.

1.2. The amygdala is an essential brain area that is involved in anxiety, fear, and other forms of emotional learning and memory (Johansen, Cain, Ostroff, & LeDoux, 2011; Rogan, Staubli, &

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LeDoux, 1997). In brain imaging studies, the degree of activation in the amygdala during fear recall correlates with the extent of conditioning (LaBar, Gatenby, Gore, LeDoux, & Phelps, 1998). In addition, lesions in the amygdala result in selective deficits of facial and auditory recognition of fear in humans (Feinstein, Adolphs, Damasio, & Tranel, 2011). The lateral nucleus of the amygdala (LA) receives auditory sensory inputs directly from the thalamus as well as indirectly from the cortex, and serves as a sensory interface (LeDoux, Cicchetti, Xagoraris, & Romanski, 1990; LeDoux, Farb, & Ruggiero, 1990; LeDoux & Farb, 1991; McDonald, 1998; Pitkanen, Savander, & LeDoux, 1997). Functional inactivation (Muller, Corodimas, Fridel, & LeDoux, 1997; Wilensky, Schafe, & LeDoux, 1999) or lesions (Amorapanth, LeDoux, & Nader, 2000; LeDoux, Cicchetti, et al., 1990; Nader, Majidishad, Amorapanth, & LeDoux, 2001) in the LA result in inability to acquire/recall fear memory. We previously demonstrated that synaptic plasticity recorded after FPS involves alterations in N-methyl-D-aspartate receptors (NMDARs) and non-NMDAR neurotransmission in the thalamic–LA (Th–LA) neural circuit (McKernan & Shinnick-Gallagher, 1997; Zinebi, McKernan, & Shinnick-Gallagher, 2002; Zinebi, Russell, McKernan, & Shinnick-Gallagher, 2001; Zinebi et al., 2003).

1.3. Long-term synaptic potentiation (LTP) is one type of synaptic plasticity that underlies the cellular mechanism of learning and memory (Bliss & Collingridge, 2013; Malenka & Bear, 2004; Tsvetkov, Carlezon, Benes, Kandel, & Bolshakov, 2002). Depending on the induction protocol, LTP elicited in the Th–LA neural circuit can be prevented by inhibition of NMDARs, group I metabotropic glutamate receptors (mGluR5, mGluR1) or voltage-gated calcium channels (Bauer, Schafe, & LeDoux, 2002; Shin et al., 2010; Weisskopf, Bauer, & LeDoux, 1999). These results raise the possibility of additional excitatory signaling in FPS LTM in the LA, particularly via mGluRs, well known for their role in synaptic plasticity.

1.4. Metabotropic GluRs make attractive therapeutic targets in a variety of pathological states through modulation of synaptic plasticity (Nicoletti, Bruno, Ngomba, Gradini, & Battaglia, 2015; Nicoletti et al., 2011; Ribeiro, Paquet, Cregan, & Ferguson, 2010). The mGluRs can be divided into three groups (I, II and III) on the basis of sequence similarity, pharmacology, and the preferred signal transduction mechanisms. Group I mGluR (consisting of mGluR1 and mGluR5) antagonists block contextual conditioning (Nielsen, Macphail, & Riedel, 1997), leading to a transient up-regulation of mGluR5 in the hippocampus (Riedel, Casabona, Platt, Macphail, & Nicoletti, 2000) while activation of mGluR5 is necessary for fear memory and LTP in the amygdala (Fendt & Schmid, 2002; Lee, Lee, & Choi, 2002; Rodrigues, Bauer, Farb, Schafe, & LeDoux, 2002). Additionally, within group III mGluRs, (consisting of mGluR4, mGluR6, mGluR7 and mGluR8), mGluR7, which is the most widely distributed of mGluRs (Flor et al., 1997) and closely associated with vesicle release sites (Shigemoto et al., 1996), has a role in acquisition and extinction of fear memory (Fendt et al., 2013; Masugi et al., 1999). Though phospholipase C (PLC) is the canonical pathway associated with group I mGluR activation, group III mGluRs, which are classically linked to inhibition of adenylyl cylase, are similarly known to associate with PLC (Perroy et al., 2000). However, mGluRs are also known to signal via other phospholipases. For example, cysteine sulfinic acid (CSA), an amino acid released in the hippocampus during specific conditions of high frequency stimulation (Klancnik, Cuenod, Gahwiler, Jiang, & Do, 1992), is an agonist at the mGluR isoform linked to another phospholipase, phospholipase D (PLD) (Boss, Nutt, & Conn, 1994). This unique receptor subtype is defined by its exclusive response to a specific antagonist, PCCG-13 (Albani-Torregrossa et al., 1999). Consequently, PLD activation downstream from selective mGluR activation can mediate aspects of glutamate signaling (Frohman, 2015; Klein, 2005) but the mechanisms of PLD–mGluR actions on synaptic transmission are largely unknown (Cuellar, Griffith, & Merlin, 2005; Fuortes, Rico, & Merlin, 2010; Rico & Merlin, 2004).

1.5. In the present study, we investigated neuronal plasticity in the Th–LA pathway during FPS LTM (Duvarci & Nader, 2004). We examined the downstream elements underlying mGluR signaling during FPS LTM, as well as neurochemical changes in the expression of rat amygdala proteins. We identified a decreased expression of aldolase A, the aldolase isoform most abundantly expressed in human brain (Buono, D'Armiento, Terzi, Alfieri, & Salvatore, 2001) which directly inhibits phospholipase D (PLD) (Kim et al., 2002), a downstream signaling target of mGluRs (Boss & Conn, 1992; Klein, Iovino, Vakil, Shinozaki, & Loffelholz, 1997; Pellegrini-Giampietro, Torregrossa, & Moroni, 1996; Shinomura, del Rio, Breen, Downes, & McLaughlin, 2000). We showed that anti-PLD antibodies immunoprecipitate aldolase A in the amygdala and that PLD activity and proteins are increased in FPS LTM. Subsequently, we analyzed changes in synaptic plasticity produced by the PLD–mGluR agonist and antagonist. Finally, we tested whether PLD–mGluR in the LA plays a role in the expression of long-term fear memory.

2. Methods

2.1. Animals

All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (NIH) and approved (Approval ID: 8907176) by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Medical Branch at Galveston (UTMB). Male Sprague-Dawley albino rats (Harlan, Houston, TX, USA) aged three-four weeks and weighing approximately 45 g at arrival, were used as subjects. After three days acclimation, animals were randomly divided into unpaired (UNP) and paired (PA) groups and housed in a temperature-controlled room at 22–24 °C with a 12 h light/dark cycle and fed a standard laboratory chow diet and water *ad* libitum. Animals were housed two per cage and except for routine handling were kept undisturbed in the isolated stress-free animal facility throughout the experimental schedule.

2.2. Apparatus

Acoustic startle is augmented in the presence of a conditioned stimulus (CS) (Brown, Kalish, & Farber, 1951; Cassella & Davis, 1986; Davis & Astrachan, 1978) and this response is termed fear-potentiated startle (FPS) (Davis, 1986). Animals were trained and tested using enclosures equipped with a foot-shock grid fixed atop a stabilimeter/accelerometer (San Diego Instruments, CA, USA). Startle was measured by the accelerometer, converted to voltage, and stored on computer. Startle was defined as the maximum displacement during 200 ms following the onset of the startle stimulus. FPS testing sessions were performed in separate chambers to control for effects of contextual conditioning.

2.3. Fear potentiated startle

Training and testing were performed as described earlier (McKernan & Shinnick-Gallagher, 1997; Scott & Shinnick-Gallagher, 2005). Briefly, the animals were acclimated to the training and testing chambers for 10 min each on the first day (day one). On day two, the animals were habituated to the startle stimulus by exposing them to 20 white noise bursts [95 decibels (dB)]. Following habituation, the animals received either a paired (PA) or