# REMEMBERING TO FORGET: THE ROLE OF BRAIN CARBONIC ANHYDRASES ON FEAR MEMORY EXTINCTION

**Scheila Daiane Schmidt[1], Barbara Rani[2], Alessia Costa[2], Fabrizio Carta[3], Jociane de Carvalho Myskiw[1], Maria Beatrice Passani[2], Ivan Izquierdo[1], Claudiu Trandafir Supuran[3], Patrizio Blandina[3], Gustavo Provensi[3]**

*[1]Memory Center, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre - Brazil, [2]Department of Health Sciences, University of Florence, Florence - Italy, [3]Department of Neurosciences, Psychology, Drug Research and Child Health, University of Florence, Florence - Italy*

**Introduction:** Fear extinction is an active form of learning defined by the attenuation of a learned response following non-reinforced exposure to a previously fearful stimulus which is used as the cognitive therapy for the treatment of several disorders such as phobias, post-traumatic stress disorder, anxiety and obsessive-compulsive disorder. Despite being highly effective, exposure therapy has also some limitations such as the long time required, high costs, and frequently observed relapse episodes. In this context, pharmacotherapy can be used as adjunctive therapy to overcome these fragilities by bolstering the formation and persistence of extinction memories. Therefore, a key goal is to identify new targets to improve the extinction learning process. Recent evidence provided by us and others, indicates a role for brain carbonic anhydrases (CAs) in fear memory acquisition and consolidation, but nothing is know about its role on extinction. Thus, the aim of this study was to investigate the impact of CAs activation on fear memory extinction.

**Materials and methods:** The effects of CAs inhibitors acetazolamide (ACZ) and compound 18(C18) or the CA activator D-phenylalanine (D-PHE) were evaluated using the extinction of contextual fear conditioning paradigm in rats. The protocol consists of 3sessions: acquisition, training and test performed with 24h inter-trial intervals. In the first session, the animals were placed in the conditioning chamber and 3foot shocks (0.5mA, 2s) were delivered at 30s interval. During extinction, animals were placed in the same apparatus for extinction training lasting 15or 30 min depending on the experimental setting, in the absence of punishments. In the third session, rats were placed again in the same chamber for a 3min retention session. Drugs were administered after the extinction training session and the time the rats spent freezing (defined as the complete absence of somatic motility, with the exception of respiratory movement) was manually recorded by a trained researcher unaware of the treatments during all sessions.

**Results:** A dose-dependent effect was observed after systemic administration of acetazolamide: no differences in the time the rats spent freezing were registered between animals receiving vehicle or ACZ at a low dose (10 mg/Kg, i.p.); on the contrary rats treated with the higher dose (30 mg/kg, i.p.) spent more time freezing than controls during the test, indicating a fear extinction impairment. No behavioural alterations were observed after systemic treatment with C18(30 mg/kg, i.p.), a CA inhibitor that does not cross the blood brain barrier, therefore excluding the participation of peripheral CAs in ACZ-induced impairment. Systemic injection of the CA activator D-PHE (300 mg/kg, i.p.) facilitated the learning of fear extinction memory using an experimental design that did not allow the formation of extinction memory per se. Co-treatment with ACZ prevented D-PHE-induced effect. Extinction deficits were also observed following ACZ (10 nmol/side) infusion into the hippocampus, the basolateral amygdala or the ventromedial prefrontal cortex. On the contrary, ACZ was ineffective when infused into the substantia nigra.

**Discussion and conclusions:** In the last decade several research groups demonstrated that fear extinction is mediated by similar neural circuits in rodents and humans, therefore the interest in fear conditioning as model for translating basic research into clinical leads is growing. Here we provided first report of the involvement of central CAs in specific brain areas on fear extinction learning. Therefore, CAs can be considered an innovative target for the development of new compounds for the treatment of disorders characterized by maladaptative fear responses