Gap junction based electrical communication modulates hippocampal contribution to fear learning and memory

529.5

Stephanie Bissiere, Ravikumar Ponnusamy, Sarah R. Sterlace, Michael. S Fanselow

Department of Psychology and Brain Research Institute, University of California Los Angeles, Los Angeles, CA, USA.

INTRODUCTION

- Fear conditioning involves the pairing of a neutral conditional stimulus (CS) like a tone with an aversive unconditional stimulus (US) such as a foot-shock in a specific context. The tone and the context acquire aversive properties and, on subsequent exposure, elicit a fear response [1].

- It is now well established that the basolateral amygdala (BLA), the hippocampus and the prefrontal cortex are among the brain areas necessary for emotional memory formation, maintenance, expression and storage [1].

- Rythmic oscillatory activity has been shown to be important for the integration and the binding of emotional information. Oscillatory activity in the range of theta were previously observed in the BLA during arousing events and, in the BLA and hippocampus during context fear retrieval [2,5].

- The frequency at which population activity oscillate is thought to be tightly controlled by a powerful network of local GABAergic interneurons that communicate via gap junction forming electrical synapses [4,3].

- In the amygdala, hippocampus, and prefrontal cortex only a specific subpopulation of parvalbumin expressing (PV+) interneurons were found to be interconnected via gap junctions formed by the assembly of connexin 36 (Cx36) protein subunits [4,3,7].

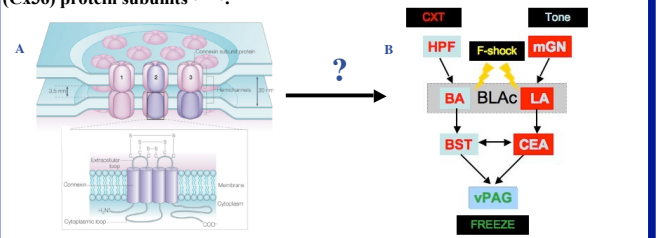


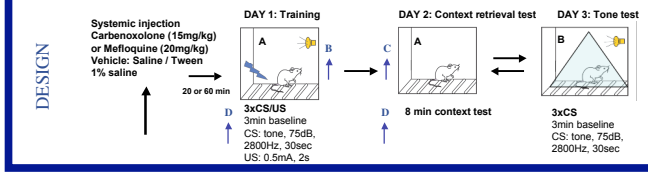
Fig.1 *A: Molecular organization and schematic topology of a gap junctional plaque (Picture taken from Söhl et al, Nature Reviews Neuroscience 6, 191-200 (March 2005). B: Simplified scheme of the neural circuits underlying Pavlovian fear conditioning*

HYPOTHESIS

Using tone / context fear conditioning in adult rats, we tested the hypothesis that pharmacological blocking of neuronal gap junctions would disrupt behaviors associated with fear memory processes.

RESULTS & METHODS

EXPERIMENT 1: Pre-training systemic injections of Carbenoxolone and Mefloquine: Effect on context and tone fear memories



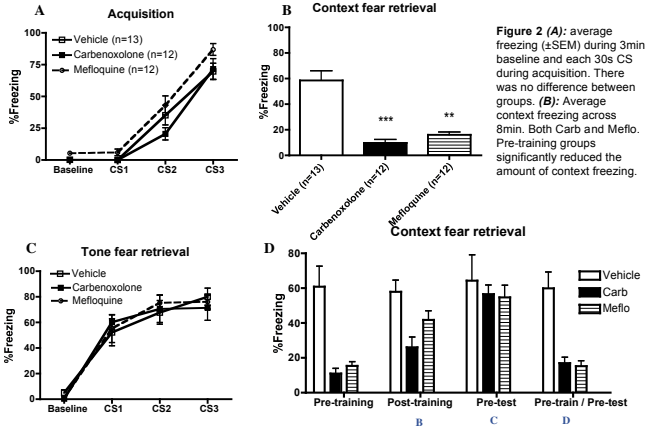


Figure 2 *(A):* average freezing (±SEM) during 3min baseline and each 30s CS during acquisition. There was no difference between groups. ***(B):*** Average context freezing across 8min. Both Carb and Meflo. Pre-training groups significantly reduced the amount of context freezing.

Figure 2 *(C):* Average freezing (±SEM) during 3min baseline and each 30s CS during tone fear retrieval. There was no difference between groups. ***(D):*** Drugs or vehicle were injected at different time points across training and context retrieval test: post-training for consolidation, pre-test for effect on fear expression per se, Pre-train / Pre-test for drug state dependent effect.

EXPERIMENT 2: Pre-training blocking of gap junction: Effect on tone fear extinction.

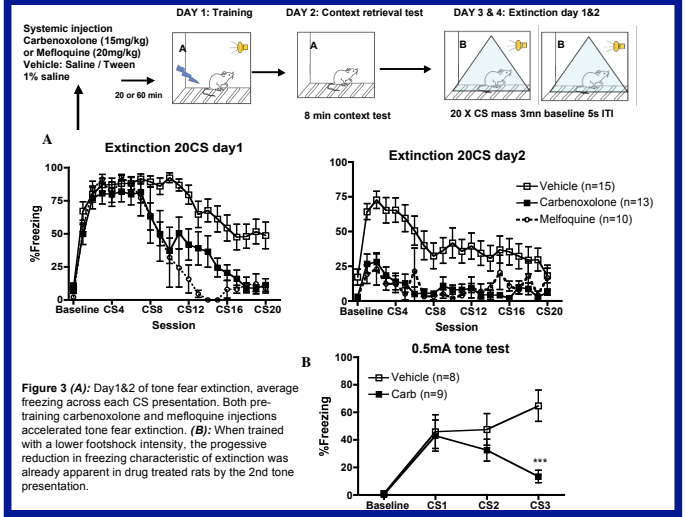


Figure 3 *(A):* Day1&2 of tone fear extinction, average freezing across each CS presentation. Both pre-training carbenoxolone and mefloquine injections accelerated tone fear extinction. ***(B):*** When trained with a lower footshock intensity, the progessive reduction in freezing characteristic of extinction was already apparent in drug treated rats by the 2nd tone presentation.

REFERENCES

1- Fanselow MS, Poulos AM " The neuroscience of mammalian associative learning" *Annu Rev Psychol.* 56:207-34 (2005)
2- Paré D, Collins D.R, Pelletier J.G. "Amygdala oscillations and the consolidation of emotional memories" *TRENDS in Cognitive Sciences*, Vol.6, No.7, 306-314 (2002).
3- Sinfield J.L, Collins D.R. " Induction of synchronous oscillatory activity in the rat lateral amygdala *in vitro* is dependent on gap junction activity " *European Journal of Neuroscience* Vol.24: 3091-3095 (2006).
4- Fuchs EC, Zivkovic AR, Cunningham MO *et al.*" Recruitment of Parvalbumin-Positive Interneurons Determines Hippocampal Function and Associated Behavior" *Neuron*, Vol 53(4): 591-604 (2007).
5-Seindenbecher T, Laxmi TR, Stork O, Pape HC "Amygdalar & hippocampal theta rhythm synchronization during fear memory retrieval. *Science* 301 (5634): 846-50 (2003).
6-Cruikshank SJ, Hopperstad M, Younger M *et al.* "Potent block of Cx36 and Cx50 gap junction channels by mefloquine" *PNAS*, 101(33):12364-9 (2004).
7- Muller JF, Mascagni F, McDonald AJ. "Coupled networks of parvalbumin-immunoreactive interneurons in the rat basolateral amygdala" *J.Neurosci* 25(32):7366-76

EXPERIMENT 3: Immediate shock deficit

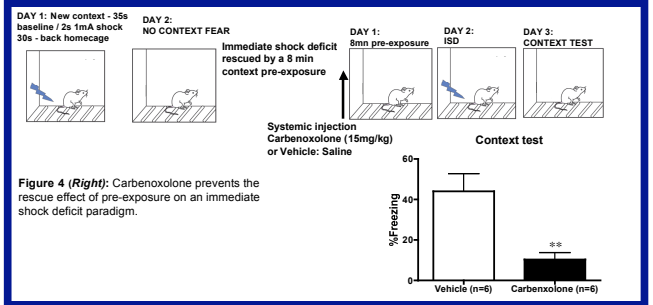


Figure 4 *(Right):* Carbenoxolone prevents the rescue effect of pre-exposure on an immediate shock deficit paradigm.

EXPERIMENT 4: Direct dHippocampal infusion of gap junction blockers: Effect on fear learning and memory.

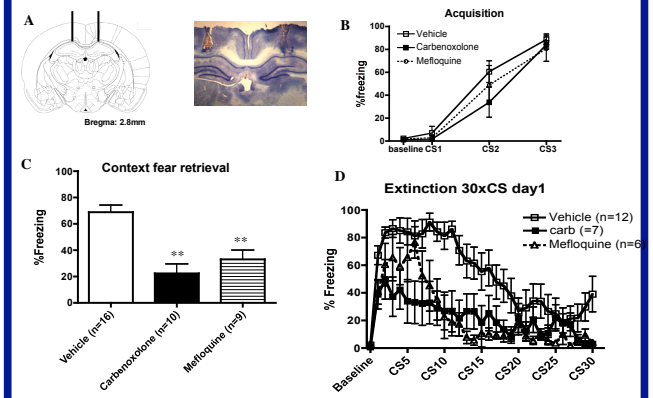


Figure 5: Pre-training dHippocampal infusion of gap junction blockers (A): Phase contrast representative image of localization of cannulae placement aimed at the dHippocampus regions ***(B):*** Fear acquisition, there was no difference between groups in baseline activity or level of freezing. ***(C):*** Context fear retrieval is impaired in the drug groups compared to vehicle.***(D):*** Tone fear extinction was also accelerated in the carb. and mefloquine group.

CONCLUSIONS

➢Pre-training IP injections of the general gap junction blocker carbenoxolone and of the specific Cx36 blocker mefloquine impair the acquisition of context but not tone fear memories. Both drugs have no effect on the expression of fear per *se* and do not show any state dependent effect.
➢Pre-training injections of gap junction blockers accelerate tone fear extinction.
➢Gap junction blockers prevent the benefits of the context pre-exposure in an immediate shock deficit procedure pointing towards an hippocampal effect.
➢Direct dHippocampal infusion of gap junction blockers produced similar results on context fear memories and tone extinction to the systemic infusions. These results suggest that gap junctions, specifically the neuronal gap junctions containing Cx36 can modulate hippocampal function and its involvement within the fear circuitry. This is the first behavioral evidence for a role of gap junction in the behavior of fear learning and memory.

This work is supported by the NINDS grant POI N535985 and the Swiss National Fund Grant PA00A-115367