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MEETING ABSTRACT

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Connectivity of CR$^+$ (VIP$^+$) interneurons in the basolateral amygdala

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Background: The amygdala is known to be involved in simple forms of emotional learning, such as fear conditioning and fear extinction. Interneurons (INs) of the basolateral amygdaloid complex (BLA) represent a highly heterogeneous group of cells that tightly regulate principal cell (P cell) excitability. The unambiguous identification of functionally distinct classes of BLA INs requires the precise definition of their dendritic and axonal pattern, firing activity and presynaptic afferents, together with their neurochemical features. The majority of BLA INs contacts glutamatergic P cells at different plasma membrane specializations. On the other hand, some INs preferentially target other types of INs (interneuron-selective interneurons, ISIs). Among them, calretinin (CR$^+$) INs, which also express vasoactive intestinal polypeptide (VIP), were found to contact calbindin (CB$^+$) INs and to establish a reciprocal connection in the rat BLA. In hippocampus and cortex, VIP$^+$ INs contact dendrite-targeting INs, which in turn lead to a disinhibition of P cells. Such disinhibitory mechanisms may be important in the modulation of amygdala-related behaviors. BLA INs are believed to receive mainly glutamatergic inputs from cortex, thalamus and local P cells in different arrangements. However, little is known about the VIP$^+$ (CR$^+$) pre-synaptic partners. One way to investigate their functional role is to characterize the microcircuits in which they are embedded. Our work focuses on the identification of first order pre-synaptic inputs as well as post-synaptic targets of this specific class of INs in order to elucidate their role in fear and extinction behaviors.

Methods: We will take advantage of the mono-trans-synaptic tracing approach using the mutant rabies virus EnvA-ΔG, in order to retrogradely identify first-order afferents. The mutated virus lacks the gene encoding its envelope glycoprotein (RG) necessary for further viral spread and is pseudotyped with the avian sarcoma leucosis envelope glycoprotein (EnvA) so that only the cells that express the EnvA receptor TVA (not constitutively expressed in mammalian cells), can be infected. To reconstitute the infectious RV it is necessary to express the TVA receptor, along with the native RG, in a specific cell population. Through the use of a Cre-dependent TVA mouse line, crossed with a CR-Cre or VIP-Cre mouse line, the TVA receptor is expressed only in CR$^+$ (VIP$^+$) cells. The stereotactic injection in BLA of an adeno-associated viral vector (AAV) that expresses also in a Cre-dependent fashion the native RG along with the pseudotyped rabies virus coupled with a fluorescent reporter molecule, allows the mono-trans-synaptic spreading of the rabies infection only from TVA-CR$^+$ (VIP$^+$) cells.

Results: We injected CR-Cre:TVA mice with a 1:1 mixture of a Cre-dependent AAV carrying the RG and an EnvA-ΔG-RFP, therefore allowing the spreading from CR$^+$ cells. Preliminary immunofluorescence analysis on injected brains revealed direct presynaptic partners in several forebrain areas including: piriform, enthorinal and temporal cortex, zona incerta, and ventral hippocampus (stratum oriens and stratum pyramidale), along with local presynaptic partners in BLA. Interestingly, the shape and position of hippocampal cells would suggest an interneuronal origin. So far, we have investigated several interneuronal markers (somatostatin, calretinin, and calbindin), but none appeared to coexist with the RV.

Discussion: Relating structure to function is a central goal of modern neuroscience. Our preliminary experiments point in the direction of a broad but organized distribution of presynaptic partners to CR$^+$ INs in BLA. Further analysis is needed to elucidate the distinctives of these afferent inputs.

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