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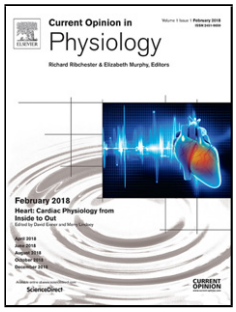

Title: $GABA_A$ receptors and inhibitory neurotransmission in the amygdalar complex

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Highlights

- Inhibitory circuits in the amygdala are complex and play a central role in processing fear, and learning.
- Dysfunction within these circuits, at the cellular and molecular level likely underpin some anxiety related disorders.
- Inhibitory neurotransmission play a central role in fear learning and extinction.
- GABA receptors are differentially distributed in the basolateral and central amygdala.
- γ1 - subunit containing receptors are selectively found in the central amygdala
- In the BLA α1 receptors, most likely in interneurons in the BLA play an important role in fear learning.
- Tonic inhibition mediated by α5 containing extrasynaptic receptor is present in both the basolateral and central amygdala where it plays a role in anxiety-related behaviour.
- Studies of GABA receptors and inhibitory transmission in the amygdala now providing the results that could lead to the development of new targeted anxiolytic agents.

$GABA_A$ receptors and inhibitory neurotransmission in the amygdalar complex

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Introduction

Of the many complex cognitive functions that our brains process, 'emotions' add an ever-changing perceptual contour to our conscious experience. The amygdalar complex (AC), located in the temporal lobe, is a critical centre for integration of sensory inputs to generate appropriate emotional response. The functional role of the AC has largely been studied using an aversive Pavlovian conditioning paradigm (fear conditioning), in which subjects are presented with neutral stimulus, such as a tone or light (the conditioned stimulus, CS) that is contingently paired with an aversive stimulus, typically a foot-shock (the unconditioned stimulus, US) [1]. Following a small number of pairings, subjects form an association between the CS and US, and subsequently respond to the CS with an avoidance 'fear' response. This learnt response, the conditioned response (CR), is rapidly acquired and forms a long lasting 'memory trace' for later recall. Subsequent presentations of the CS, not paired with the US, progressively breaks this association and lead to a gradual reduction of the CR through a process known as fear extinction. Fear extinction is not an erasure of the original memory trace, but a new associative learning event in which subjects learn that the CS is no longer dangerous [2]. Integration of sensory inputs in the AC neural circuits mediate associative learning that underlie 'fear' acquisition and extinction. The neural circuits that underpin fear learning and extinction likely arose to enable animals to learn and react rapidly to dangerous conditions, are crucial for survival.

There are many physiological similarities between the fear response and human anxiety, and a plethora of experimental evidence show that these two behavioural states share common neural circuits [3]. For example, exposure to traumatic events can form deleterious memory traces which lead to aberrant arousal and anxiety in safe environments as in post-traumatic stress. Patients with dysregulated emotional responses show heightened activity in the AC [4]. Treatment strategies for dysregulated anxiety, such as exposure therapy, have their roots in fear extinction while pharmacological agents such as benzodiazepines modulate GABA signalling in the AC to mediate anxiolytic their actions [5]. Therefore, understanding the physiology and functional roles of GABAergic transmission in the AC is not only interesting in terms of understanding basic brain function, but may also lead to the development of targeted and better therapies for the treatment of anxiety disorders. In this review, we focus of GABAergic circuits in the AC and their physiological functions through actions on GABA type-A receptors ($GABA_A$Rs), the ubiquitous inhibitory synaptic receptors in the mammalian CNS.

GABAergic circuits in the AC

The AC is a heterogeneous structure composed of about 13 nuclei with extensive intra- and extra-nuclear connections. Broadly, the AC is classified into three main groups: the basolateral amygdala (BLA), the central amygdala (CeA), and the intercalated cells (ITCs). Sensory inputs enter the AC at the level of the BLA, where they are integrated to provide output to the CeA; downstream projections from the CeA elicit the behavioural responses. The ITCs interposed as lateral and medial clusters form feedforward interfaces for sensory integration in the BLA and its output to the CeA (reviewed in [1])(Figure 1A).

BLA

The BLA is a cortical-like structure and is anatomically subdivided into the lateral (LA) and basal amygdala (BA). Although LA and BA have distinct afferent and efferent connections, they contain a homogenous neural population of glutamatergic principal neurons, and GABAergic interneurons. Principal neurons constitute the majority (80-85%) of the total neuronal population and are the source of the BLA output. Interneurons, although less numerous (15-20%), form a diverse population that tightly control activity of principal neurons by a combination of feedforward and feedback inhibition. As in other cortical-like regions, BLA interneurons are classified based on the expression of the calcium binding proteins, parvalbumin (PV), calbindin (CB) and calretinin (CR), and the neuropeptides, somatostatin (SOM), cholecystokinin (CCK), neuropeptide Y (NPY), and vaso-intestinal peptide (VIP). Nearly half of these interneurons are PV-positive, and nearly half of these also express CB, but are negative for other peptides (reviewed in [6]). The majority of PV interneurons form 'basket' synapses on the soma and proximal apical dendrite of principal neurons and other interneurons. A small proportion of the PV interneurons (≈5%) make characteristic 'cartridge' type synapses on the axon initial segment (AIS) of principal neurons, similar to cortical PV Chandelier (PV-Chn) neurons [7] [8]. Interneurons expressing CCK co-localize with either CR, VIP, or SOM, and make synaptic contacts on the somato-dendritic compartment of principal neurons, but predominantly innervate other interneurons [9]. Finally, ~15% of interneurons express SOM along with CB or NPY, but not PV, and these make synaptic contacts on the distal dendrites of principal neurons and influence dendritic propagation of extrinsic excitatory inputs to the principal neurons [10]. Therefore, distinct population of interneurons influence electrical signal propagation in principal neurons in a compartment specific manner and control their output (Figure 1B). *In vivo,* principal neurons receive synchronized GABAergic inputs which facilitate spike timing and orchestrate BLA output [11].

CeA

In contrast to the BLA, the CeA is a striatal like structure that is entirely GABAergic and anatomically divided into lateral (CeL) and medial (CeM) sectors, with extensive local connections (reviewed in [1]). In the CeL, neurons are divided into a number of different populations based on expression of immunohistochemical markers, electrophysiological properties, and synaptic connections. Of these, the two best understood are those expressing protein kinase C-delta (PKC), and those expressing somatostatin (SOM) but negative for PKC (PKC -ve). PKC +ve neurons that project to the CeM are thought to mediate inhibition of 'fear' output and referred as CeL_{OFF} cells [12] [13]. Both PKC and SOM neurons make extensive local connections that control local activity [14]. Following fear conditioning, CS activates PKC -ve/SOM neurons that are referred as CeL_{ON} cells. These (ON)-cells inhibit PKC +ve expressing neurons, leading to disinhibition of CeM output [12] [13] [15] (Figure 1C). In addition, some CeL neurons express corticotropin releasing hormone (CRH) and are proposed to mediate flight behaviour [16]. Apart from fear learning, the CeA has also been implicated in a range of other behaviours such as feeding and addiction, however, the exact role of different populations and circuits in these behaviours remain to be determined [17] [18,19] [20].

ITCs

The ITCs are clusters of GABAergic interneurons that surround the BLA. Of these, lateral ITC neurons receive sensory inputs and provide feedforward inhibition to LA interneurons [21] [22] [23]. Similarly, medial ITC neurons form feedforward inhibitory projections to the CeL [24]. Thus, the lateral and medial ITCs function as feedforward inhibitory interfaces to gate LA and CeA output, respectively.

Thus, it is clear that GABAergic circuits in the AC control all stages of fear learning from integration of sensory inputs to key outputs that initiate the physiological responses [3]. Functionally, within the BLA, both excitatory input to interneurons, as well as GABAergic synapses undergo plasticity [25], and interference of GABAergic transmission within the CeA or its output impair fear expression. In turn, fear extinction is thought to result from potentiation of inputs to mITC neurons that inhibit the CeA [24] [26], thus reducing fear responses [21] [26].

$GABA_A$Rs in the AC

In the CNS, fast inhibitory signalling is mediated via $GABA_A$Rs which inevitably control the activity of the AC circuits. GABAergic signalling mediates phasic and tonic signalling through synaptic and extra-synaptic ionotropic $GABA_A$Rs, respectively. These receptors are anion-selective channels with high permeability for Cl^- and $HCO3^-$ ions with the direction of ionic transport being determined by their respective electrochemical gradients. In mammalian neurons, the chloride equilibrium potential (E_{GABA}) is regulated by the activity of local cation coupled anion transporters, anion exchangers, pH, and local impermeant anions [27] [28]. In mature neurons, GABA is generally thought to be an inhibitory neurotransmitter, however, excitatory actions of $GABA_A$Rs have also been reported. In the BLA, some PV-Chn interneurons have been suggested to be excitatory at the principal neuron AIS, the key site for action potential initiation [29] [8]. Interestingly, aversive stimuli, delivered during fear learning, recruit PV-Chn interneurons with a short-latency, and this activity is thought to facilitate synchronization of BLA principal neurons [23]. A direct excitatory mechanism at the AIS of principal neurons, and the widely-distributed nature of these synapses [8] provides an obvious mechanism for such a synchronization. However, direct evidence for such GABAergic excitation of BLA principal neurons is still lacking.

The ionotropic $GABA_A$Rs are pentameric oligomers formed from various combinations of the following subunits: α1-6, β1-3, γ1-3, δ, ε, θ, π and ρ1-3. In the adult mammalian CNS, the most abundant synaptic isoform incorporates α1, β2, and γ2 subunits in a 2:2:1 stoichiometry [30]. The subunit composition of $GABA_A$Rs determine their kinetics, their localization within a neuron and their pharmacological modulation. Gene expression studies in adult rats show that all almost $GABA_A$R subunits are expressed in the AC (Table 1), and quantitative analysis of mRNA expression suggests relative expression of $GABA_A$R subunits in the BLA follows α2 > α1, β1, β3 > β2, γ2 >> γ1 [31]. In the BLA, principal neurons predominantly express α2- and α1-containing $GABA_A$Rs that mediate phasic GABA signalling, while α3- and α5- containing $GABA_A$Rs mediate a tonic conductance [32]. Interneurons by contrast, predominantly express α1 subunits [34]. Little is known about the developmental regulation of $GABA_A$R subunits in amygdala. However, in BLA principal neurons, a switch from α1- to α2- containing $GABA_A$Rs is reported to correlate with developmental switch in E_{GABA} [33].

In the CeA, $GABA_A$R subunit expression is largely similar to that in the BLA except that γ1 subunits are expressed at higher levels than γ2 [31]. Here, α2-containing $GABA_A$Rs mediate phasic GABA signalling [35], while α5-, α1-, and δ- containing $GABA_A$Rs mediate

a tonic conductance in PKCδ+ neurons[36]. The selective expression of γ1 subunits in the CeA is unusual as they are not found in most of the mammalian CNS. Receptors containing γ1 subunits can cluster at synapses[37], where they mediate IPSCs with slower kinetics than those containing γ2[38]. Pharmacologically, benzodiazepines exert a potent positive modulating effect on γ2-containing receptors but have little effect on γ1-containing receptors [30]. In the CeA, benzodiazepines have been reported to reduce fast GABA inhibition and this has been speculated to be caused by an as yet undefined variant of γ1−containing $GABA_A$Rs [39]. This unusual pharmacological action implies that it might be possible to develop anxiolytic drugs with high specificity for the $GABA_A$Rs found in this region of the amygdala.

Subunit specific putative functional roles of $GABA_A$Rs in the AC

Gene knockout (KO) experiments in the BLA have shown that α1-containing $GABA_A$Rs are essential for sedative/hypnotic actions by diazepam, whereas α2-containing $GABA_A$Rs mediate their anxiolytic actions [40] [41] [42]. Modulation of α1 subunits in the BLA also has effects on fear learning and synaptic plasticity, while selective genetic ablation of α1-subunits LA principal neurons had no effect. Thus, it has been suggested that α1-containing receptors in interneurons play a role in auditory fear learning [43]. In the CeA, inhibitory inputs from the Bed Nucleus of Stria Terminalis (BNST) and medial intercalated cells have been suggested to activate receptors containing γ2- and γ1- subunits, respectively [31]. Moreover, selective deletion of extra-synaptic α5-containing $GABA_A$Rs, that mediated a tonic conductance, has been found to enhance anxiety-like behaviour [36]. Together, these findings show that $GABA_A$ receptors play a key role in fear processing and that receptors containing different subunits play distinct roles in fear and anxiety.

Looking forward

GABAergic transmission in the amygdala plays a complex role in emotional learning, memory, and expression. A detailed understanding of $GABA_A$Rs in the amygdala will help to unravel these complexities and may pave the way for the development of novel therapeutics for disorders such as ADHD and anxiety. Recent studies using genetic manipulations of

GABA neurons and $GABA_A$Rs have revealed their diverse functional roles, however, these findings have limitations. Firstly, in the amygdala GABA neurons that are functionally different, with distinct synaptic targets express similar molecular markers. Optogenetic manipulations of these neurons inevitably change physiological activity in ways that we do not fully understand. To address this, what is needed are more specific molecular markers to identify, measure and manipulate functionally distinct neurons during physiological activity. Secondly, the subunit combination of particular $GABA_A$Rs influence their location, activity and functional impact on the neuronal activity. The mechanisms that regulate the stoichiometry of particular $GABA_A$Rs and its influence on cellular and sub-cellular localization is little understood. We need molecular tools to target specific subunits in $GABA_A$Rs to elucidate expression and intracellular trafficking patterns. Finally, a combinatorial approach with tools to simultaneously measure and manipulate GABA neurons and specific subunit containing $GABA_A$Rs can provide insights about functional role of GABA transmission at synaptic level.

Figure legends

Figure:1 A simplified schematic of connections within the amygdalar complex (AC)

A. Scheme of intrinsic connections between different regions of the AC: LA, Lateral amygdala; BA, Basal and basomedial amygdala; CeL, latero-capsular division of central

amygdala (CeA); CeM, Medial division of central amygdala; lITc, lateral cluster of intercalated cells (ITc); mITc, medial cluster of intercalated cells. Extrinsic inputs (black arrows) arrive at the LA, BA, lTc, and CeL. Interactions between different regions of the AC provide output for emotional expression. Cortical-like regions LA and BA contain glutamatergic and GABAergic neurons. Striatal-like regions CeA and ITc contain only GABAergic neurons. Glutamatergic projections (red arrows): LA sends glutamatergic outputs to BA, mITc, and CeL; BA sends glutamatergic projections to the CeL and various non-amygdala regions. GABAergic projections (blue): lITc and mITc send GABAergic projection to LA and CeL, respectively; CeL sends GABAergic projections to the CeM; CeM sends GABAergic projection to structures involved in emotional expression; BA somatostatin interneurons send GABAergic projection to basal forebrain.

B. Scheme of microcircuits shows synaptic and electrical connections formed by interneurons in the basolateral amygdala (BLA) that express specific molecular makers: PV, Parvalbumin; CCK, Cholecystokinin; PV-Chn, Parvalbumin expressing Chandelier neuron; SOM, Somatostatin. Distinct interneuron subtypes target different electrical compartments of principal neurons (P) and tightly control BLA output; synaptic and electrical connections are present between interneurons.

C. Simplified schematic showing intrinsic connections within the CeA. In the CeL, neurons that express somatostatin but negative for Protein Kinase C δ (SOM/PKCδ-) are referred as CeL_{ON} cells which synapse on to PKCδ+, referred as $CeL_{OFF.}$ Interaction between CeL_{ON} cells and CeL_{OFF} control CeM output for fear expression. CeL neurons that express Corticotropin releasing hormone (CRH) inhibit CeL-SOM neurons.

Table: 1A $GABA_A$ receptors ($GABA_A$Rs) in the basolateral amygdala (BLA)

Expression of subunit (IHC)	Identified neuronal Subtype	Subcellular localization	Putative pre-synaptic neuron	Electrophysiological evidence for sub-unit expression
α1	Interneurons [34].	Soma, dendrites	PV, CCK	CB1 receptor agonists reduce α1-containing $GABA_A$Rs signalling [35].
	Principal neuron [34]	distal dendrites	SOM	
α2	Principal neuron	Soma, dendrites [44]	PV, CCK, SOM	α2- containing $GABA_A$Rs mediate diazepam sensitive anxiolytic effects [40].
		AIS [44]	PV Chn	
α3	Principal neuron [32]	AIS [44]	PV Chn	α3-containing $GABA_A$Rs carry most of extra-synaptic GABA signalling [32].
		Extra-synaptic [32].		
α4 [45]				
α5 [45]	Principal neuron	Extra-synaptic [32]		
β1, β2, β3	Principal and interneurons. β2/β3 co-localized with α1 mainly in interneurons especially in PV subtype [34].			
γ2, γ3 [45]	Principal neurons			γ2-containing $GABA_A$Rs carry synaptic

Table: 1B $GABA_A$Rs in the Central amygdala (CeA)

Expression of subunit (IHC)	Identified neuronal Subtype	Subcellular localization	Putative pre-synaptic neuron	Electrophysiological evidence for sub-unit expression
α1	CRH type1 receptor expressing (+ve) (PKCε+) [48]			α1-containing $GABA_A$Rs mediate tonic conduction in CRH type1 receptor +ve neurons [48].
α2			Intra-nuclear connections and ITC projections	α2-containing $GABA_A$Rs carry majority of GABA signalling that is influenced by diazepam [35].
α3[45]			Intra-nuclear connections and ITC projections	
α4[45]				
α5	PKC + [36].	Extra-synaptic		α5-containing $GABA_A$Rs carry majority of extras-synaptic GABA signalling in PKC^+ neurons [36].
β2>β3,β1[45]				
γ2, γ1				Putative inputs from bed nucleus activate γ2-containg $GABA_A$Rs [31].
				Putative inputs from ITC activate γ1-containing $GABA_A$Rs [31].
δ	CRH type 1 receptor -ve	Extra-synaptic		δ-containing $GABA_A$Rs contribute to tonic conduction in the CeL CRH type1 receptor –ve neurons [49].

Table: 1C $GABA_A$Rs in the Intercalated cells (ITCs)

Expression of subunit (IHC)	Identified neuronal Subtype	Subcellular localization	Putative pre-synaptic neuron	Electrophysiological evidence for sub-unit expression
α1	PV expressing large ITCs [23].	Soma, proximal dendrite	Intra-nuclear connections	
α2		Soma	Intra-nuclear connections	α2 and α3-containing $GABA_A$Rs mediate signalling of intra-nuclear connections in the medial ITCs [50].
α3			Intra-nuclear connections	
α4[46]		Extra-synaptic		
δ				δ-containing $GABA_A$Rs carry tonic GABA signalling in the ITCs that is modulated by neurosteroids[46].

References:

1. Sah P, Faber ES, Lopez De Armentia M, Power J: **The amygdaloid complex: anatomy and physiology**. *Physiol Rev* 2003, **83**:803-834.
2. Furini C, Myskiw J, Izquierdo I: **The learning of fear extinction**. *Neurosci Biobehav Rev* 2014, **47**:670-683.
3. Tovote P, Fadok JP, Luthi A: **Neuronal circuits for fear and anxiety**. *Nat Rev Neurosci* 2015, **16**:317-331.
4. Rauch SL, Whalen PJ, Shin LM, McInerney SC, Macklin ML, Lasko NB, Orr SP, Pitman RK: **Exaggerated amygdala response to masked facial stimuli in posttraumatic stress disorder: a functional MRI study**. *Biol Psychiatry* 2000, **47**:769-776.
5. Nuss P: **Anxiety disorders and GABA neurotransmission: a disturbance of modulation**. *Neuropsychiatr Dis Treat* 2015, **11**:165-175.
6. Spampanato J, Polepalli J, Sah P: **Interneurons in the basolateral amygdala**. *Neuropharmacology* 2011, **60**:765-773.
7. Veres JM, Nagy GA, Vereczki VK, Andrasi T, Hajos N: **Strategically positioned inhibitory synapses of axo-axonic cells potently control principal neuron spiking in the basolateral amygdala**. *J Neurosci* 2014, **34**:16194-16206.

* 8. Spampanato J, Sullivan RK, Perumal MB, Sah P: **Development and physiology of GABAergic feedback excitation in parvalbumin expressing interneurons of the mouse basolateral amygdala**. *Physiol Rep* 2016, **4**.
    - This study reported GABAergic excitation by PV-Chn in the BLA evoked polysynaptic feedback excitation.
    - The authors showed PVChn evoked feedback excitation in brain slices from adult animals.
    - It suggests GABA could be excitatory in mature circuits.
    - These PVChn made axo-axonic and axo-somatic synapses on principal neurons and interneuron, respectively.

9. Mascagni F, McDonald AJ: **Immunohistochemical characterization of cholecystokinin containing neurons in the rat basolateral amygdala**. *Brain Res* 2003, **976**:171-184.
10. Wolff SB, Grundemann J, Tovote P, Krabbe S, Jacobson GA, Muller C, Herry C, Ehrlich I, Friedrich RW, Letzkus JJ, et al.: **Amygdala interneuron subtypes control fear learning through disinhibition**. *Nature* 2014, **509**:453-458.

11. Windels F, Yan S, Stratton PG, Sullivan R, Crane JW, Sah P: **Auditory Tones and Foot-Shock Recapitulate Spontaneous Sub-Threshold Activity in Basolateral Amygdala Principal Neurons and Interneurons**. *PLoS One* 2016, **11**.

12. Ciocchi S, Herry C, Grenier F, Wolff SB, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, et al.: **Encoding of conditioned fear in central amygdala inhibitory circuits**. *Nature* 2010, **468**:277-282.

13. Haubensak W, Kunwar PS, Cai H, Ciocchi S, Wall NR, Ponnusamy R, Biag J, Dong HW, Deisseroth K, Callaway EM, et al.: **Genetic dissection of an amygdala microcircuit that gates conditioned fear**. *Nature* 2010, **468**:270-276.

14. Hunt S, Sun Y, Kucukdereli H, Klein R, Sah P: **Intrinsic Circuits in the Lateral Central Amygdala**. *eNeuro* 2017, **4**.

15. Yu K, Garcia da Silva P, Albeanu DF, Li B: **Central Amygdala Somatostatin Neurons Gate Passive and Active Defensive Behaviors**. *J Neurosci* 2016, **36**:6488-6496.

16. Fadok JP, Krabbe S, Markovic M, Courtin J, Xu C, Massi L, Botta P, Bylund K, Muller C, Kovacevic A, et al.: **A competitive inhibitory circuit for selection of active and passive fear responses. *Nature* 2017, **542**:96-100.

- This study reported Cortico-tropin releasing factor (CRF+) expressing CeL neurons mediate flight response.
- Optical activation or suppression of CRF+ neurons increased or decreased conditioned flight response, respectively
- Inhibtion of SOM by CRF+ neurons is proposed to underlie flight response.
- It shows different neuronal subtypes could mediate distinct behavior outcomes.

17. Cai H, Haubensak W, Anthony TE, Anderson DJ: **Central amygdala PKC-delta(+) neurons mediate the influence of multiple anorexigenic signals**. *Nat Neurosci* 2014, **17**:1240-1248.

18. Murray JE, Belin-Rauscent A, Simon M, Giuliano C, Benoit-Marand M, Everitt BJ, Belin D: **Basolateral and central amygdala differentially recruit and maintain dorsolateral striatum-dependent cocaine-seeking habits**. *Nat Commun* 2015, **6**:10088.

19. Warlow SM, Robinson MJF, Berridge KC: **Optogenetic Central Amygdala Stimulation Intensifies and Narrows Motivation for Cocaine**. *J Neurosci* 2017, **37**:8330-8348.

20. Douglass AM, Kucukdereli H, Ponserre M, Markovic M, Grundemann J, Strobel C, Alcala Morales PL, Conzelmann KK, Luthi A, Klein R: **Central amygdala circuits

modulate food consumption through a positive-valence mechanism. *Nat Neurosci* 2017, **20**:1384-1394.

- This study showed optical activation of serotonin receptor 2a (Htr2a)- expressing CeA neurons mediate food consumption.
- Cell-type specific ablation or optical suppresion of Htr2a-CeA neurons reduced food consumtion.
- Htr2a-CeA neurons form input specific clusters that might mediate distinct functions.
- Htr2a-CeA neurons that projected to parabrachial nucleus mediated food consumption.
- It demonstrates GABAergic output from the CeA play potential functional roles apart from 'fear' expression.

21. Royer S, Martina M, Pare D: **An inhibitory interface gates impulse traffic between the input and output stations of the amygdala**. *J Neurosci* 1999, **19**:10575-10583.
22. Manko M, Geracitano R, Capogna M: **Functional connectivity of the main intercalated nucleus of the mouse amygdala**. *J Physiol* 2011, **589**:1911-1925.

*23. Bienvenu TCM, Busti D, Micklem BR, Mansouri M, Magill PJ, Ferraguti F, Capogna M: **Large Intercalated Neurons of Amygdala Relay Noxious Sensory Information**. *The Journal of Neuroscience* 2015, **35**:2044-2057.

- This study showed L-ITCs respond to noxious stimuli and provide feedforward input to the BLA.
- L-ITCs axon made synaptic contacts on PV interneurons in the BLA.
- It supports the hypothesis that ITCs could gate fear processing in the BLA through disinhibition.
- Authors also showed L-ITCs express high levels α1-containing $GABA_A$Rs.

24. Strobel C, Marek R, Gooch Helen M, Sullivan Robert KP, Sah P: **Prefrontal and Auditory Input to Intercalated Neurons of the Amygdala**. *Cell Reports* 2015, **10**:1435-1442.
25. Trouche S, Sasaki JM, Tu T, Reijmers LG: **Fear extinction causes target-specific remodeling of perisomatic inhibitory synapses**. *Neuron* 2013, **80**.
26. Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Pare D: **Amygdala intercalated neurons are required for expression of fear extinction**. *Nature* 2008, **454**:642-645.
27. Blaesse P, Airaksinen MS, Rivera C, Kaila K: **Cation-chloride cotransporters and neuronal function**. *Neuron* 2009, **61**:820-838.

28. Glykys J, Dzhala V, Egawa K, Balena T, Saponjian Y, Kuchibhotla KV, Bacskai BJ, Kahle KT, Zeuthen T, Staley KJ: **Local impermeant anions establish the neuronal chloride concentration**. *Science* 2014, **343**:670-675.

29. Woodruff AR, Monyer H, Sah P: **GABAergic excitation in the basolateral amygdala**. *J Neurosci* 2006, **26**:11881-11887.

30. Mohler H: **GABA(A) receptor diversity and pharmacology**. *Cell Tissue Res* 2006, **326**:505-516.

31. Esmaeili A, Lynch JW, Sah P: **GABA_A Receptors Containing Gamma1 Subunits Contribute to Inhibitory Transmission in the Central Amygdala**. *Journal of Neurophysiology* 2009, **101**:341-349.

32. Marowsky A, Rudolph U, Fritschy JM, Arand M: **Tonic inhibition in principal cells of the amygdala: a central role for alpha3 subunit-containing GABAA receptors**. *J Neurosci* 2012, **32**:8611-8619.

33. Ehrlich DE, Ryan SJ, Hazra R, Guo JD, Rainnie DG: **Postnatal maturation of GABAergic transmission in the rat basolateral amygdala**. *J Neurophysiol* 2013, **110**:926-941.

34. McDonald AJ, Mascagni F: **Parvalbumin-containing interneurons in the basolateral amygdala express high levels of the alpha1 subunit of the GABAA receptor**. *J Comp Neurol* 2004, **473**:137-146.

35. Marowsky A, Fritschy JM, Vogt KE: **Functional mapping of GABA A receptor subtypes in the amygdala**. *Eur J Neurosci* 2004, **20**:1281-1289.

36. Botta P, Demmou L, Kasugai Y, Markovic M, Xu C, Fadok JP, Lu T, Poe MM, Xu L, Cook JM, et al.: **Regulating anxiety with extrasynaptic inhibition. *Nat Neurosci* 2015, **18**:1493-1500.

- This study showed extrasynaptic GABA signalling in PKCδ+ neurons regulate anxious behavior
- Conditional knowdown of α5-containing $GABA_A$Rs in PKCδ+ CeL neurons induced excessive anxiety.
- Mice with Conditional knowdown of α5-containing $GABA_A$Rs also showed increased fear generalization.
- It suggests tonic GABA signalling could regulate behavior in cell-type specific fashion.

37. Dixon CL, Sah P, Keramidas A, Lynch JW, Durisic N: **gamma1-Containing GABA-A Receptors Cluster at Synapses Where they Mediate Slower Synaptic Currents than gamma2-Containing GABA-A Receptors**. *Front Mol Neurosci* 2017, **10**:178.

38. Dixon C, Sah P, Lynch JW, Keramidas A: **GABAA receptor alpha and gamma subunits shape synaptic currents via different mechanisms**. *J Biol Chem* 2014, **289**:5399-5411.

39. Delaney AJ, Sah P: **GABA receptors inhibited by benzodiazepines mediate fast inhibitory transmission in the central amygdala**. *J Neurosci* 1999, **19**:9698-9704.

40. Low K, Crestani F, Keist R, Benke D, Brunig I, Benson JA, Fritschy JM, Rulicke T, Bluethmann H, Mohler H, et al.: **Molecular and neuronal substrate for the selective attenuation of anxiety**. *Science* 2000, **290**:131-134.

41. Smith KS, Engin E, Meloni EG, Rudolph U: **Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct GABAA receptor subtypes in mice**. *Neuropharmacology* 2012, **63**:250-258.

42. Heldt SA, Ressler KJ: **Amygdala-specific reduction of alpha1-GABAA receptors disrupts the anticonvulsant, locomotor, and sedative, but not anxiolytic, effects of benzodiazepines in mice**. *J Neurosci* 2010, **30**:7139-7151.

43. Wiltgen BJ, Godsil BP, Peng Z, Saab F, June HL, Linn ML, Cook JM, Houser CR, O'Dell TJ, Homanics GE, et al.: **The alpha1 subunit of the GABA(A) receptor modulates fear learning and plasticity in the lateral amygdala**. *Front Behav Neurosci* 2009, **3**:37.

44. Gao Y, Heldt SA: **Enrichment of GABAA Receptor alpha-Subunits on the Axonal Initial Segment Shows Regional Differences**. *Front Cell Neurosci* 2016, **10**:39.

45. Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G: **GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain**. *Neuroscience* 2000, **101**:815-850.

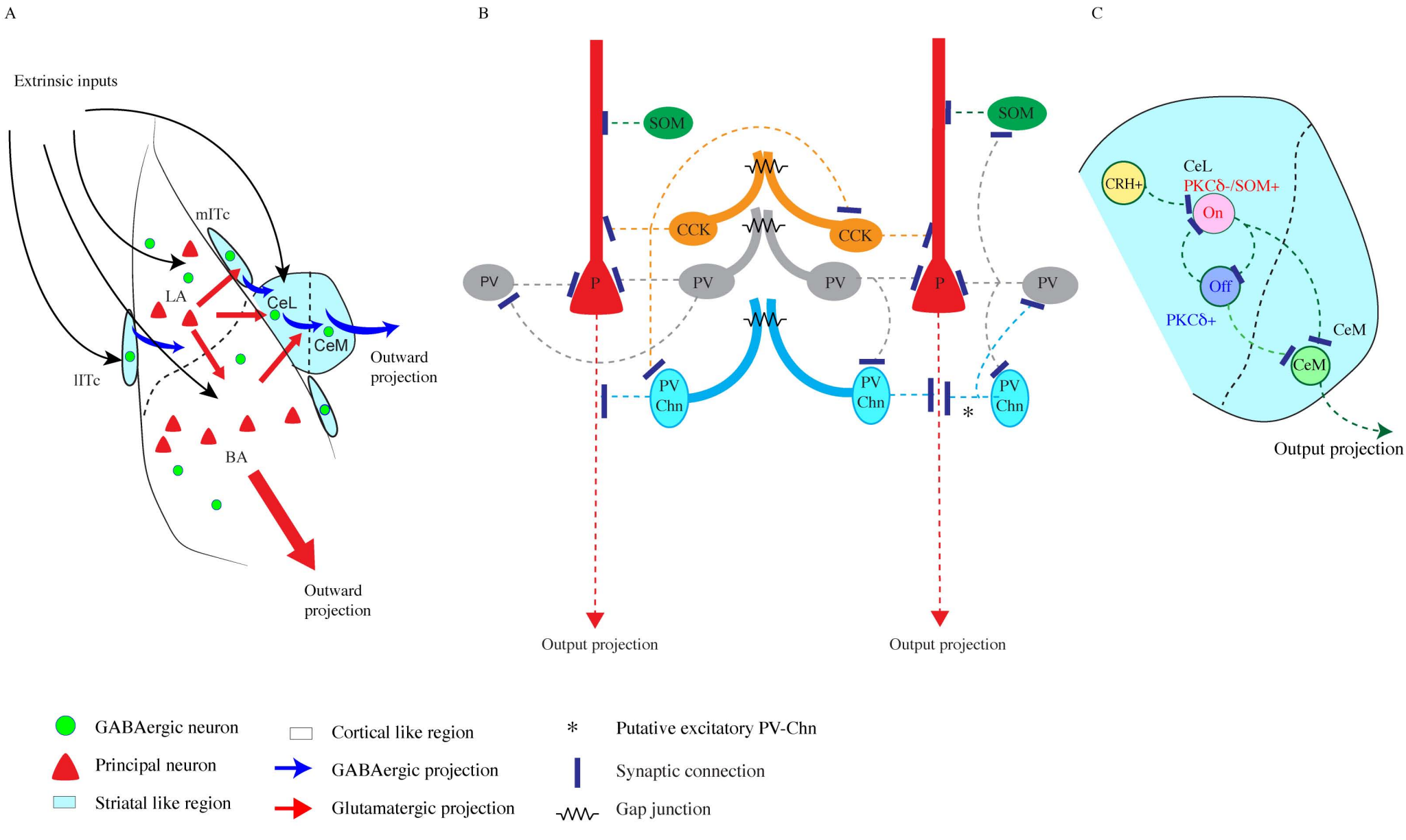
46. Marowsky A, Vogt K: **Delta-subunit-containing GABAA-receptors mediate tonic inhibition in paracapsular cells of the mouse amygdala**. *Frontiers in Neural Circuits* 2014, **8**.

47. Liu ZP, He QH, Pan HQ, Xu XB, Chen WB, He Y, Zhou J, Zhang WH, Zhang JY, Ying XP, et al.: **Delta Subunit-Containing Gamma-Aminobutyric Acid A Receptor Disinhibits Lateral Amygdala and Facilitates Fear Expression in Mice**. *Biol Psychiatry* 2017, **81**:990-1002.

48. Herman MA, Contet C, Justice NJ, Vale W, Roberto M: **Novel subunit-specific tonic GABA currents and differential effects of ethanol in the central amygdala of CRF receptor-1 reporter mice**. *J Neurosci* 2013, **33**:3284-3298.

49. Romo-Parra H, Blaesse P, Sosulina L, Pape HC: **Neurosteroids increase tonic GABAergic inhibition in the lateral section of the central amygdala in mice**. *J Neurophysiol* 2015, **113**:3421-3431.

50. Geracitano R, Fischer D, Kasugai Y, Ferraguti F, Capogna M: **Functional expression of the GABA(A) receptor α2 and α3 subunits at synapses between intercalated medial paracapsular neurons of mouse amygdala**. *Frontiers in Neural Circuits* 2012, **6**:32.


A
Extrinsic inputs
mITc
LA
CeL
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lITc
Outward projection
BA
Outward projection
B
SOM
CCK
CCK
PV
P
PV
PV
P
PV
SOM
PV Chn
PV Chn
PV Chn
*
Output projection
Output projection
C
CRH+
CeL
PKCδ-/SOM+
On
Off
PKCδ+
CeM
CeM
Output projection
GABAergic neuron
Principal neuron
Striatal like region
Cortical like region
GABAergic projection
Glutamatergic projection
* Putative excitatory PV-Chn
Synaptic connection
Gap junction