**Generation of model sharp wave oscillations in BLA**

**INTRODUCTION**

Add in bullet form all the key points, including lit review

**METHODS**

**Single cell models of the basolateral amygdala (BLA)**

To reproduce the diversity of spike frequency adaptation seen in principal LA neurons (reviewed in Sah et al., 2003), our group has modeled three types of regular spiking principal cells in the past, with high (type-A), intermediate (type-B), or low (type-C) spike frequency adaptation, due to the differential expression of a Ca2+-dependent K+ current. We will use the type C model for the sharp-wave model study. LA also contains local GABAergic interneurons that exhibit various firing patterns, even among neurochemically-homogeneous subgroups (Lang and Paré, 1998; Rainnie et al., 2006; Sosulina et al., 2006; Woodruff and Sah, 2007; Jasnow et al., 2009). However, the majority displays a fast-spiking pattern, which was reproduced in the PV model.

The principal cell model has five compartments. The first three representing a soma (diameter 24.75 µm; length 25 µm), an apical dendrite (dia 2.5 µm; length 296 µm) on which synapses were placed, and another dendrite (dia 5 µm; length 400 µm) that helped match passive properties. Values of specific membrane resistance, membrane capacity and cytoplasmic (axial) resistivity were, respectively, Rm = 55 KΩ-cm2, Cm= 1.2-2.4 µF/cm2, and Ra= 150-200 Ω-cm. Leakage reversal potential (EL) was set to -67 mV. The resulting Vrest was – 69.5 mV, input resistance (RIN) was 128 MΩ, and τm was 34 ms, all of which were within the ranges reported in previous physiological studies (Washburn and Moises, 1992; Faber et al., 2001). All three compartments had the following currents: leak (IL), voltage-gated persistent muscarinic (IM), high-voltage activated Ca2+ (ICa), spike-generating sodium (INa), potassium delayed rectiﬁer (IDR) and A-type potassium (IA) (Li et al., 2009; Power et al., 2011). In addition, the dendrites had a hyperpolarization-activated nonspecific cation (Ih) currents and a slow apamin-insensitive, voltage-independent afterhyperpolarization current (IsAHP) (Power et al., 2011).

The other two compartments represents Axon initial segment (length 5 µm; diameter 1.22 µm; nseg 10) and axon (length 100 µm; diameter 1 µm). The first two segments of AIS has variable diameter (Segment 1: 1.7 to 1.5 µm; Segment 2: 1.5 to 1.22 µm), while the rest of the segment has constant diameter of 1.22 µm. Values of Axial resistivity, membrane capacity, conductance of the

Passive channel, are respectively, Ra = 150 Ω-cm, cm = 0.5 µF/cm2, g\_pas = 1/30000 S/cm2. The reversal potential of leak channel is set to -70 mV. The Axon initial segment has following currents: High threshold Sodium (Nav 1.2), Low threshold Sodium (Nav 1.6), Potassium, and leak currents. The distribution of Nav 1.2 channels is highest at the proximal AIS and least at the distal AIS while for Nav 1.6 the distribution is vice versa (Hu et al., 2009). The axon has all the currents same as the AIS except it does not have Nav 1.2 current. Table 1a shows ion channels and their conductances in the Principal Cell.

**Figure 1A** shows the voltage response of the three principal cell models to depolarizing (two left panels) and hyperpolarizing (right panel) current injection. The three model cells could reproduce previous experimental observations (Sah et al., 2003) including the temporal dynamics of repetitive firing produced by membrane depolarization as well as their responses to membrane hyperpolarization.

The interneuron model had two compartments, a soma (dia 15 µm; length 15 µm) and a dendrite (dia 10 µm; length 150 µm). Each compartment contained a fast Na+ (INa) and a delayed rectiﬁer K+ (IDR) currents with kinetics (Durstewitz et al., 2000) that reproduced the much shorter spike duration that is characteristic of fast-spiking cells. The passive membrane properties were as follows: Rm = 20 KΩ-cm2, Cm=1.0 µF/cm2, Ra= 150 Ω-cm, and EL = -70 mV. As shown in **figure 1B**, the interneuron model could reproduce the non-adapting repetitive firing behavior of fast spiking cells, as observed experimentally (Lang and Paré, 1998; Woodruff and Sah, 2007).

**Single cell models of Chandelier cell (Chn)**

The cell model has 17 compartments representing a soma, 10 apical dendrites and 6 basal dendrites. Values of specific membrane resistance, membrane capacity and cytoplasmic (axial) resistivity were, respectively, Rm = 5555 Ω-cm2, CmSoma= 1.4 µF/cm2, CmDend= 1.4 µF/cm2, RaSoma = 100 Ω-cm, and RaDend= 100 Ω-cm. Leakage reversal potential (EL) was set to -65 mV. The resulting Vrest was – 65 mV, input resistance (RIN) was ~52 MΩ, and τm was 7 ms, all of which were within the ranges reported in previous physiological studies (Bezaire et al. 2016). All compartments had the following currents: A-type K+ (IKvA), N-type Ca2+ (ICavN), ICavL , IKCaS , IKvCaB. In addition, the soma and dendrites has INav , IKdrfast , Ileak currents. (Bezaire et al. 2016). See Table 2a & Table 2b for ion channels and their conductances at highest density location in cell and gating variables of ion channels.

Chn-BLA synapse (onto axon distal segment of BLA). The reversal potential was -60 mV. At a holding potential of -50 mV, the EPSC amplitude was 36.45 pA, rise time was 0.85 ms and decay time was 11.57 ms (Bezaire et al., 2016).

**Network structure and connectivity (**from another manuscript in progress)

Rodent BLA was estimated to have 43,000~50,000 neurons (Tuunanen and Pitkanen, 2000; Mozhui et al., 2007; Yang et al., 2008). We developed a scaled down (~2: 1) model of this region with 27,000 neurons randomly distributed in a cuboid geometry with sides 1.4 x 1.4 x 1.4 mm. The model included 24,300 PNs (type-A: type-C in the ratio 64:26), and 2,430 PV Basket Cells, and 270 Chandelier cells with a PN:ITN ratio of 9:1 (Headley et al., unpublished data), ensuring an inter-soma distance > 25 µm.

*Intrinsic connections*. Except for ITN-ITN connectivity that had both synaptic and electrical components, all other connections were via chemical synapses; hereafter, unless qualified by ‘electrical’, the connections are assumed to be synaptic. Connection probabilities have been found to be distance-dependent for PN-PN contacts in BLA, and we used 3%, 2% and 1% probabilities for inter-PN distances of 50, 100 and 200 µm, respectively (Stoop Lab, personal communications). For connections from 200-600 µm, a 0.5% connectivity was used. This resulted in the convergence found in Table 3. For all the other connection types, we used data from *in vitro* BLA reports (Woodruff and Sah, 2007), limiting connectivity from/to ITNs to within ~300 µm. Using such data, probabilities in the model for unidirectional ITN-to-PN and PN-to-ITN synaptic connections, and for ITN-to-ITN electrical connections were, respectively, 34%, 12%, and 8%. Also, reciprocal connections between PNs and ITNs was set to 16%. In BLA, electrically coupled ITNs have 50% and 25% probability, respectively, of unidirectional and bidirectional synaptic connectivity; the corresponding numbers for ITNs not electrically coupled were 19% and 3% (Woodruff and Sah, 2007). These connectivity numbers in our model resulted in an overall synaptic ITN-ITN connectivity of 26% of which 20% was unidirectional and 3% bi-directional. These probabilities resulted in the following intrinsic connectivity in the model: each PN received 24.98+/-9.5 excitatory connections from other PNs, and 42.6± 12.9 inhibitory connections from ITNs; each ITN received 214.8±58 excitatory connections from PNs, and 21.6± 7.4 inhibitory connections from other ITNs. Next, we consider extrinsic connectivity.

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Info./Data from Madhu: Each principal neuron receives input from 2-3 Chandeliers while Chandelier innervate onto 50-100 principal neurons. Principal neurons make large monosynaptic connections on Chns and Interneurons. A single spike in a principal neuron could drive interneurons (including Chn) to threshold. There is about 40% reciprocal connections ( 9/22 Chn- Pyr pairs). 2 PV neurons may converge on a Chandelier.

Slice Information:

* Principal neurons synchronized their feedback within ~ 20 ms from the initial spike in all Chn, regardless the size of the slice they are sampled from.
* Samples are usually within 50 um radius from the Chn. But axons of the Chn extend to 200-300 um in the BLA.
* BLA slices and LFP electrode was placed 100-200 um from the intracellular electrode.
* Recordings were done from pyramidal neurons that are 20-30 um away from the Chn.
* Each neuron spreads spread over 100-200 um and axonal branches spread over 300 um. Table 3a shows the synaptic properties of AMPA/NMDA, GABA and Chandelier – Pyramidal synapses.

**Inputs**

The only input to the network is the current clamp given to one chandelier cell enough to generate one spike in it.

**Conditioning protocol used in simulations**

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**Model tuning and validation**.

**Questions**

1. Do you think a coupled Pyr and Chn should be able to oscillate at the ripple frequency? Or is the idea that the two Chns in the ‘elementary module’ fire 180 degrees out of phase so that the pyramidal cell fires at the ripple frequency? Do you think the pyramidal cells are firing at every peak of the ripple or only some?
2. Do you have recordings from the axon initial segment? I found that this is critical for fast firing of the Pyramidal cell. If there is backpropagation, firing is constrained because the soma provides a short current injection that increases the width of the AP in the axon.

**Results (Preliminary)**

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**TABLES**

Table 1a. Gating variables for ion channels used in the BLA single cell models.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Current Type** | | **Gating Variable** | **α** | **β** |  | **τx (ms)** |
|  | | *p=3* |  |  |  |  |
| *q=1* |  |  |  |  |
|  | | *p=1* | exp[−0.1144(𝑉−13)] |  |  |  |
|  | | *p=1* | exp[0.08316(𝑉+75)] |  |  |  |
| *IM* | | *p=2* |  |  |  |  |
|  | | *p=2* |  |  |  |  |
| *q=1* | ― | ― |  | ― |
| *IA* | **soma** | *p=1* |  |  |  |  |
| *q=1* | exp[0.1144𝑉+56] | ― |  |  |
| **dend** | *p=1* |  |  |  |  |
| *q=1* |  | exp[0.1144𝑉+56] |  |  |
|  | | *p=1* |  |  |  | 1000-2000 |
| INav 1.2 | | *m = 3* |  |  |  |  |
| *h = 1* |  |  |  |  |
| INav 1.6 | | *m = 3* |  |  |  |  |
| *h = 1* |  |  |  |  |
| IKv | | *n = 1* |  |  |  |  |

Table 1b. Maximal conductance densities of ion channels.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Conductance (mS/cm2)** | | ***I*Na** | ***I*DR** | ***I*M** | ***I*H** | ***I*Ca** | ***I*A** | ***I*sAHP** | ***NaV 1.2*** | ***Nav***  ***1.6*** | ***Kv*** | ***τ*Ca** |
| **Axon Initial Segment** | | - | - | - | - | - | - | - | 3200 | 3200 | 1000 | - |
| **Axon** | | - | - | - | - | - | - | - | - | 3200 | 1000 | - |
| **Principal cell -Type C** | Soma | 54 | 3 | 0.4 | - | 0.2 | 1.43 | - | - | - | - | - |
| Dend | 27 | 3 | 0.4 | 0.0286 | 0.2 | 0.32 | 0.36 | - | - | - | 1000 |
| **Interneuron** | Soma | 35 | 8 | - | - | - |  | - | - | - | - | - |
| Dend | 10 | 3 | - | - | - |  | - | - | - | - | - |

Density ranges of Nav (1.2), Nav (1.6) and Kv current channels from Hu et al. (2009); \*Nav (1.2): 3200 \* ( 0.05) to 3200 \* (0.96) from soma to distal end of AIS; \*\*Nav (1.6) : 3200 \* (0.04) to 3200 \* (0.6) from soma to distal end of AIS; \*\*\*Kv : 1000 \* (0.2) – 1000 \* (1). Values are in (mS/cm2) for all except those in AIS which are in pS/um2

Table 2a. Gating variables for ion channels in Chn model – Refer to Appendix in Bezaire et al. (2016)

Table 2b. Maximal conductance densities of ion channels in chandelier cell model.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Conductance**  **(mS/cm2)** | ***I*KvA** | ***I*CavN** | ***I*CavL** | ***I*KCas** | ***I*KvCaB** | ***I*NaV** | ***I*Kdrfast** | ***Ileak*** |
| **Soma** | 0.00015 | 0.0008 | 0.005 | 0.000002 | 0.0000002 | 0.15 | 0.013 | 1/0.00018 |
| **Dendrites (n=16)** | 0.00015 | 0.0008 | 0.005 | 0.000002 | 0.0000002 | 0.15 | 0.013 | 1/0.00018 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pre-Post** | **AMPA** reversal potential (mV) and rise/decay time constant (ms) and conductance (uS) | **NMDA** potential (mV), rise/decay time constant (ms) and conductance (uS) | **GABA** reversal potential (mV) and rise/decay time constant (ms) and conductance (uS) | **Strength** |
| **Chn - Pyr** | -- |  | -50 mV; Rise: 1 ms; Decay: 5 ms | 10 |
| **Itn – Pyr** | -- | -- | -75 mV; Rise: 0.132 ms; Decay: 3.74 ms; 0.6e-3 | 2 |
| **Pyr- Pyr** | -- | -- | -- | -- |
| **Pyr- Chn** | 0 mV; Rise: 3.65 ms; Decay: 125 ms; 1e-3 | 0 mV; Rise: 0.2527 ms; Decay: 7 ms; .5e-3 | -- | 20 |
| **Itn- Chn** | -- | -- | -75 mV; Rise: 0.132 ms; Decay: 3.74 ms; 0.6e-3 | 5 |
| **Chn – Chn** | -- | -- | -- | -- |
| **Itn - Itn** | ***--*** | ***--*** | -- | -- |
| **Chn - Itn** | -- | ***--*** | ***--*** | -- |
| **Pyr- Itn** | 0 mV; Rise: 3.65 ms; Decay: 125 ms; 1e-3 | 0 mV; Rise: .02527 ms; Decay: 7 ms; .5e-3 | ***--*** | 5 |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Pre-Post** | 0-50 µm | 50-100µm | 100-200 µm | 200-300 µm | 300-400 µm | 400-500 µm | 500-600 µm |
| **Pyr-Pyr (Connections ± std)** | 4.64 cells  4.3 **± 2.00** | **32.5 cells**  ***28.9*±6.8** | 259.7 cells  214.9**±50.7** | 704.8 cells  512.6**±152.8** | 1372.5 cells  907.0**±303.9** | 2262.8 cells  1305.4**±476.1** | 3375.6 cells  1690.7**±637.5** |
| **PV-PV** | 87.9 **± 25.2** | 87.9 **± 25.2** | 87.9 **± 25.2** | 87.9 **± 25.2** | -- | -- | -- |
| **Pyr-Chn** | 1.0 **± 0.0** | 1.0 **± 0.0** | 1.0 **± 0.0** | 1.0 **± 0.0** | -- | -- | -- |
| **Chn-Pyr** | 3.0 **± 1.0** | 3.0 **± 1.0** | 3.0 **± 1.0** | 3.0 **± 1.0** | -- | -- | -- |
| **PV-Pyr** | 42.0 **± 10.0** | 42.0 **± 10.0** | 42.0 **± 10.0** | 42.0 **± 10.0** | -- | -- | -- |
| **Pyr-PV** | 215.0 **± 36.2** | 215.0 **± 36.2** | 215.0 **± 36.2** | 215.0 **± 36.2** | -- | -- | -- |